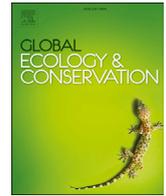




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Original Research Article

Freezing temperatures restrict woody plant recruitment and restoration efforts in abandoned montane pastures

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ARTICLE INFO

Article history:

Received 24 September 2020

Received in revised form 1 December 2020

Accepted 11 January 2021

Keywords:

Acacia koa

Climate change

Restoration corridors

Freezing death

Failed restoration

ABSTRACT

Tropical montane forests are being lost at an alarming rate but harbor some of the globe's most unique biodiversity. The Hawaiian archipelago is a prime example of the importance of high elevation forests to species conservation and persistence as they serve as the last refugia for Hawaiian birds. Yet these forests have been converted to invasive dominated pastures, and efforts to restore them have been met with limited success. Unsuccessful forest restoration may be due to freezing temperatures acting as a demographic bottleneck by killing seedlings recruiting into pastures. We determined freezing tolerances of eight common native woody plants at a high-elevation forest on Hawai'i Island and compared these freezing tolerances to two years of site-specific winter temperatures and 17 years of regional temperature records. Low temperature extremes were more severe and common in pastures than under nearby 30-year-old canopy trees. Freezing temperatures over two years were severe enough to damage leaf tissues of six of eight species tested. Those species that displayed the greatest freezing tolerance were also those found naturally recruiting into open pastures. Temperature trends over the past 17 years show monthly minimum temperatures are not increasing as predicted by climate change. Persistent severe freezing events may limit seedling recruitment in the pasture, slowing native woody plant expansion into these abandoned pastures. The species-level differences in freezing tolerance show that current management actions are using species that are at high risk to freezing damage outside of the forest canopy and that alternative species may warrant consideration.

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1. Introduction

Tropical montane forests are some of the most imperiled ecosystems in the world yet harbor unique and diverse ecological communities (Mulligan, 2010; Myers et al., 2000). Tropical montane forests have experienced large-scale conversions to other land uses like ungulate grazing and agriculture, especially at their highest elevation extent (Bruijnzeel et al., 2011). In addition, climate change is expected to have an increasingly large impact on these forests as species migrate to higher elevations seeking out climate refugia (Duque et al., 2015; Feeley et al., 2011, 2013). To maintain these unique communities, tropical

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forests need to recolonize their previous extent and potentially invade new areas at higher elevations (Rehm and Feeley, 2015a).

The loss of tropical montane forest and the resulting collapse of native biodiversity may be no more pronounced than in the Hawaiian archipelago. The highest elevational extent of these tropical montane forest serve as refugia for the many of the endangered and threatened Hawaii's birds because avian malaria is only seasonally present due to cold climatic conditions (Benning et al., 2002; Paxton et al., 2016). Yet many high elevation forests were cleared over the past century, removing this cooler temperature forest refugia for native bird populations (Mounce et al., 2018; Paxton et al., 2018).

While a warming climate may allow avian malaria to persist at these higher elevation forest refugia, warmer temperatures may also benefit landscape-scale regeneration of high-elevation forests by ameliorating harsh climate conditions. It is also possible that discordant shifts (e.g. differing rates of change in mean versus extreme temperatures) in climate restrict forest regeneration and lead to negative effects on forest regeneration or restoration (Hagger et al., 2018). For example, annual mean temperatures have risen throughout Hawai'i with warming more pronounced at higher elevations (Diaz et al., 2011; Giambelluca et al., 2008; McKenzie et al., 2019) but cloud cover and precipitation appear to be decreasing at mid to high elevations (Cao et al., 2007; Giambelluca et al., 2013). Reductions in cloud cover, especially during winter months, could result in more extreme cold temperatures even as the overall climate warms, because clouds act as an insulating cover for land-surface temperatures.

Cold temperature extremes are known to partially limit recruitment of woody plant species in open grasslands in several tropical locations (Cavieres et al., 2000; Hoffmann et al., 2019; Holdo, 2006; Rehm and Feeley, 2015b; Scowcroft and Jeffrey, 1999). Grasslands tend to have lower minimum temperatures than forests due to the lack of the buffering effects of the forest canopy. Cold temperatures are most extreme near the soil surface where seedlings reside and seedlings do not maintain a large enough canopy or thermal mass to buffer against temperature extremes (Leuning et al., 1989; Melcher et al., 2000). In many of these systems, plants maintain photosynthetically active tissues year-round as daytime temperatures are well above freezing, allowing for photosynthesis despite subzero temperatures at night. Freezing events can therefore damage active tissues, especially in open grassland areas, ultimately leading to seedling death and reinforcing the forest-grassland ecotone. Extreme freezing events may therefore impose a demographic bottleneck in succession or restoration of open grassland systems to forests by preventing woody plants from establishing or reaching adult size (Whitecross et al., 2012). Indeed, freezing temperatures have been implicated in restricting forest restoration success in Hawai'i before (Scowcroft and Jeffrey, 1999).

Understanding the role that freezing temperatures play in woody plant seedling establishment can be particularly important to forest restoration practices. Throughout the tropics, many native forests areas that were cleared for agricultural use (e.g. ungulate grazing) are now abandoned (Chazdon, 2014). Active restoration can accelerate successional processes (Zahawi et al., 2013) and the planting of woody seedlings may be improved by considering species-specific temperature tolerances. In Hawai'i, freezing temperatures are thought to kill outplanted individuals at restoration sites higher than 1600 m.a.s.l but quantitative evidence backing this claim is limited (Melcher et al., 2000; Scowcroft et al., 2000; Scowcroft and Jeffrey, 1999). It is also unclear if restoration efforts result in warmer conditions for understory seedling recruitment as has been shown in other systems (Curran et al., 2010), and whether winter temperature minimums are being altered under current climate change.

Our goals were to identify the freezing tolerances of the majority of common tree and shrub species occurring in high elevation forests on Hawai'i island and used in restoration to see if their tolerances correlate with natural recruitment patterns. In doing so, we hope to provide information to forest managers conducting active restoration on the islands and tropical montane systems elsewhere. We also examined a 17-year temperature record at the site to use as a baseline for winter extremes and to document any changes in winter temperatures over time due to local warming. Previous work on two canopy dominant tree species, *Acacia koa* and *Metrosideros polymorpha*, that showed these species are often intolerant of the cold temperature extremes experienced above 1600 m.a.s.l. (Melcher et al., 2000; Scowcroft et al., 2000; Scowcroft and Jeffrey, 1999). One of these two, *A. koa*, has been planted extensively at the site to create a canopy under which native shrub and seedlings can be planted. In this paper, we build on previous research by asking the following specific questions: (1) Which native woody species may be tolerant of extreme cold temperatures and does this correlate with natural seedling occurrences within open pasture? (2) Are there differences in low temperature extremes in the pasture compared to those under the *A. koa* forest restoration corridors and how do these differences compare to species freezing tolerances? and (3) Is there a trend in temperature minimum and maximum over the 17-year record for this site that can inform forest restoration planting? Our overarching aim is to inform restoration outplanting efforts to promote forest recovery in avian malaria-free zones to boost forest bird populations.

2. Methods

2.1. Study site

This study took place at Hakalau Forest National Wildlife Refuge (NWR) on the Island of Hawai'i, Hawai'i, USA (Figure S1) which has been identified as an area important for endangered forest bird persistence (Benning et al., 2002). The refuge consists of relatively intact forest dominated by *M. polymorpha* at lower elevations (<1600 m.a.s.l) and open pasture dominated by invasive grasses that extend from approximately 1600 m.a.s.l. to the upper refuge boundary at 1900–2000 m.a.s.l.

Open pastures were created when native forests were removed and introduced grasses planted to support cattle. Grazing ended around 1980 and the United States Fish and Wildlife Service has since been actively planting native forest canopy trees into pastures. This has largely involved planting strips of the native tree, *A. koa*, into pasture assuming that these corridors would expand over time. These corridors largely consist of *A. koa* overstory with an invasive grass understory with few native understory plants within corridors. Efforts over the last ~10 years have begun to plant understory seedlings into corridors, with the bulk of the plantings focused around the refuge station (~1800 m.a.s.l.) and between 1600 and 1700 m.a.s.l.

2.2. Freezing tolerance

Freezing tolerance of leaves was determined for the two overstory species, *M. polymorpha* and *A. koa* that are dominant in intact forest and five understory woody species that are also common in intact forest (*Cheirodendron trigynum*, *Coprosma rhynchocarpa*, *Ilex anomala*, *Myrsine lessertiana*, *Vaccinium calycinum*). We also included *Vaccinium reticulatum*, a shrub species typically found in open habitat including the pasture matrix and because it is found in open pasture we assumed it would be the most tolerant of freezing temperature. Ideally all individuals sampled for freezing tolerance would occur in pastures as we were interested in understanding recruitment outside of the restoration corridors. However, observations indicated that only *V. reticulatum*, *A. koa*, and *M. polymorpha* can be found in the pastures (>10 m away from nearest forest edge). Therefore, for *C. trigynum*, *C. rhynchocarpa*, *I. anomala*, *M. lessertiana*, and *V. calycinum* we sampled 30-month old seedlings (<2 cm diameter at base height, < 100 cm tall) that were part of a concurrent study investigating survival of manually outplanted seedlings in pasture and forest habitats along five transects (Transects 1–5 all within 500 m of each other and between 1640 and 1660 m.a.s.l.; Figure S1). When possible, individuals were sampled from pastures, but when sufficient individuals were not available they were sampled within restoration corridors that were <100 m from the nearest open grassland site. A full summary of the seedling status (natural or planted) and the number of seedlings sampled in habitats (pasture or restoration corridor) can be found in the supplement (Table S1). All sampling occurred in the same area where we measured temperatures (see below) so to match microclimate as closely as possible to realized leaf freezing tolerances.

We focused on leaf sampling as leaves represent a significant biomass component for small seedlings and is commonly used to assess freezing tolerance in small woody individuals (Lenz et al., 2013; Rehm and Feeley, 2015b; Vitasse et al., 2014). In addition, stem or whole seedling sampling requires destructive seedlings which was not an option for our seedlings as they are part of an ongoing concurrent study and are part of restoration efforts throughout the refuge. For each species we took six healthy, intact leaves from six individuals to test for freezing tolerance from 23 January and February 27, 2019, the coldest time period. Leaves were harvested between 06:30–07:30, immediately placed in polyethylene bags to minimize water loss, and transported to Hilo, Hawai'i in an insulated cooler kept at 3–6 °C. Leaves were placed into freezing treatments by 11:00 the same morning they were harvested. One leaf from each individual was assigned to one of six temperature treatments: 4, –2, –5, –8, –11, –80 °C. Leaves within the same temperature treatment were packaged together in brown paper bags. Leaves in the 4 °C treatment were placed in a refrigerator kept at 4 °C and leaves in the –80 °C treatment were placed in a –80 °C freezer for 24 h. Leaves in the 4 and –80 °C treatments serve as live and dead reference samples, respectively.

Leaves in the –2, –5, –8, and –11 °C were placed inside a polystyrene box inside a commercially available freezer with a circulating fan to ensure even freezing. In addition, the freezer was filled with bricks to create a large thermal mass that buffers against temperature fluctuations such as when the freezer was opened. An inline programmable thermostat (Inkbird technology, model ITC-310T-B) was used to control temperature during the freezing procedure. The freezer was 4 °C when the experiment began and lowered at a rate of 1–3 °C/h until reaching the first target temperature of –2 °C. Once the temperature reached –2 °C it was held at this temperature for 1 h, after which the leaves in the –2 °C treatment were removed from the freezer (freezer opened < 1 min) and placed inside the 4 °C refrigerator. The freezer temperature was then lowered to the next target temperature of –5 °C and the process repeated for each target temperature. Freezer temperatures were monitored in real time and fluctuated by an average of ± 0.5 °C around each target temperature.

After freezing, leaves were held at 4 °C for 72 h to allow leaf necrosis to develop. Leaves were then placed in individual test tubes and submersed in distilled water for 72 h at 4 °C. We next measured electrolyte leakage (Flint et al., 1967) using an electric conductivity meter (Greisinger GMH 3430, Greisinger Electronic). Test tubes were then placed inside an autoclave to kill leaf samples and stored for 72 h at room temperature before taking a second electrolyte leakage measurement.

2.3. Natural seedling recruitment

To document which species are currently recruiting into open grassland, we conducted a refuge-wide survey of woody plant seedling occurrence in the pastures. Using aerial imagery and GIS, we first randomly selected 100 points throughout the grasslands (>10 m from the nearest tree crown) that occur between restoration corridors throughout the entire Hakalau Forest NWR. At each point we established a 5-m radius circular plot, counted all woody seedlings, and classified seedlings into diameter at basal height size classes (0–1, 1–2, 2–5, 5–10, or 10+ cm diameter at basal height).

2.4. Short and long-term temperature data

To understand cold temperature extremes plants are exposed to in different habitat types (open grassland versus *A. koa* corridors), we deployed temperature sensors in and around the locations where we collected leaf samples. Our main goals

with these sensors were to (1) determine the coldest temperatures which plants in the open grassland may be exposed to and (2) determine the difference in cold temperature extremes between pastures and restoration corridors. From December 12, 2017, to January 24, 2018, we placed temperature sensors (U10-003, Onset computer corporation) at three locations; two of which were at the same locations where we sampled individual seedlings (Transects 1 and 2; Fig. S1) and a third location at a site around 1900 m.a.s.l. (hereafter, High site 1). At each location, we placed a sensor in the open grassland (>10 m from the forest edge) and a paired sensor under the canopy of an *A. koa* within a restoration corridor that was within 100 m of the open plot. Each sensor was covered with a solar shield and placed 10 cm above ground level, roughly the same height from which leaves were sampled. Similarly, from December 12, 2018, to April 15, 2019, we placed sensors again at Transect 1, High site 1, and a second high elevation site (High site 2) at a location at approximately 1800 m.a.s.l. midway between Transect 1 and High site 1. Temperature was recorded every 10 min.

To investigate longer-term trends in mean and temperature extremes we used monthly minimum, maximum and mean temperature data from April 12, 2002, to March 30, 2019, at a climate station (data can be downloaded from <https://www.ncdc.noaa.gov/cdo-web/>, station ID: GHCND:USR000HHAK) located 3.2 km from where seedlings were sampled at the upper boundary of Hakalau Forest NWR (1950 m.a.s.l.). The temperature sensor at this station is located 2 m above ground level so minimum temperature records will be warmer than those temperatures measured at ground level due to radiative cooling at ground level (Melcher et al., 2000). Despite these differences in temperatures, the 17-year climatic record allowed us to investigate longer temporal patterns in temperature change in our study system that overlapped with restoration efforts at our site. This station is part of the Remote Automated Weather Stations Climate archive and has been in operation since April 2002.

2.5. Statistical analysis

All analyses were conducted in R 3.3.1 (R Development Core Team, 2016). We define freezing tolerance as the threshold temperature at which 50% of leaf tissues were killed (LT_{50} ; Sakai and Larcher 1987). We calculated LT_{50} for each individual using nonlinear Gompertz models (following Lim et al., 1998 and Lenz et al., 2013) calculated with the `nlsLM` function from the `minpack.lm` package (Elzhov et al., 2016). Individual model outputs can be found in Figure S2. We then used a generalized linear model with a post-hoc Tukey Honestly Significant Difference (HSD) test to test for differences ($p < 0.05$) in species freezing tolerance using the `multcomp` package (Hothorn et al., 2008).

We did not statistically analyze natural seedling occurrence data as there were a disproportionate number of zeros in the dataset at the plot and species level. Therefore, we use these data to inform which species can occur in the open grasslands and not necessarily about their abundances or frequency of occurrence.

We compared daily minimum and mean minimum temperatures between sensors in open grassland and within restoration corridors using a paired *t*-test for each of the three pairs of sensors for each year (six total pairs tested). We also compared the number of freezing days between grassland and *A. koa* corridors by first computing the number of freezing days for each sensor and then conducting a paired *t*-test comparing grassland site to their *A. koa* corridor pair. Long-term trends in temperature from the climate station were assessed using a Seasonal Mann-Kendall test (`csmk.test` from the 'trend' package (Pohler, 2018)); for correlated data on the monthly mean, minimum and maximum temperatures. We then determined slope of trends using a Theil-Sen estimator when the Seasonal Mann-Kendall test was found significant ($p < 0.05$).

3. Results

3.1. Freezing tolerance

Leaf freezing tolerance was higher (i.e. a lower LT_{50}) in *V. reticulatum* ($LT_{50} = -13.4 \pm 1.3^\circ\text{C}$ standard error (SE); Table 1, Fig. 1, Figure S2) than all other species ($p < 0.001$ for all pair-wise comparisons). This LT_{50} estimate for *V. reticulatum* may actually be a conservative estimate as our lowest experimental temperature besides the -80°C control treatment was -11°C .

Table 1

Description of species growth form, freezing tolerance and occurrence in open surveys. Freezing tolerance is the mean and standard error (LT_{50} ; $^\circ\text{C}$) from six individuals for each species. Range represents range of 6 individuals measured for each species. Number of open plots is the number of plots out of 100 in which the species was encountered.

Species	Growth form	Mean LT_{50} (Standard Error)	Freezing tolerance Range	Number of open plots
<i>V. reticulatum</i>	Shrub	-13.4 ± 1.3	-11.1 to -19.7	2
<i>M. polymorpha</i>	Canopy dominant	-8.8 ± 0.9	-6.5 to -11.9	1
<i>V. calycinum</i>	Understory shrub	-7.8 ± 0.4	-6.7 to -9.5	4
<i>C. rhynchosarpa</i>	Understory Tree	-7.5 ± 0.5	-5.8 to -9.5	0
<i>C. trigynum</i>	Understory Tree	-7.1 ± 0.6	-5.6 to -8.4	0
<i>M. lessertiana</i>	Understory Tree	-6.0 ± 0.5	-4.4 to -7.2	0
<i>A. koa</i>	Canopy dominant	-5.7 ± 0.5	-4.3 to -7.1	26
<i>I. anomala</i>	Understory Tree	-5.6 ± 0.9	-3.5 to -7.9	0

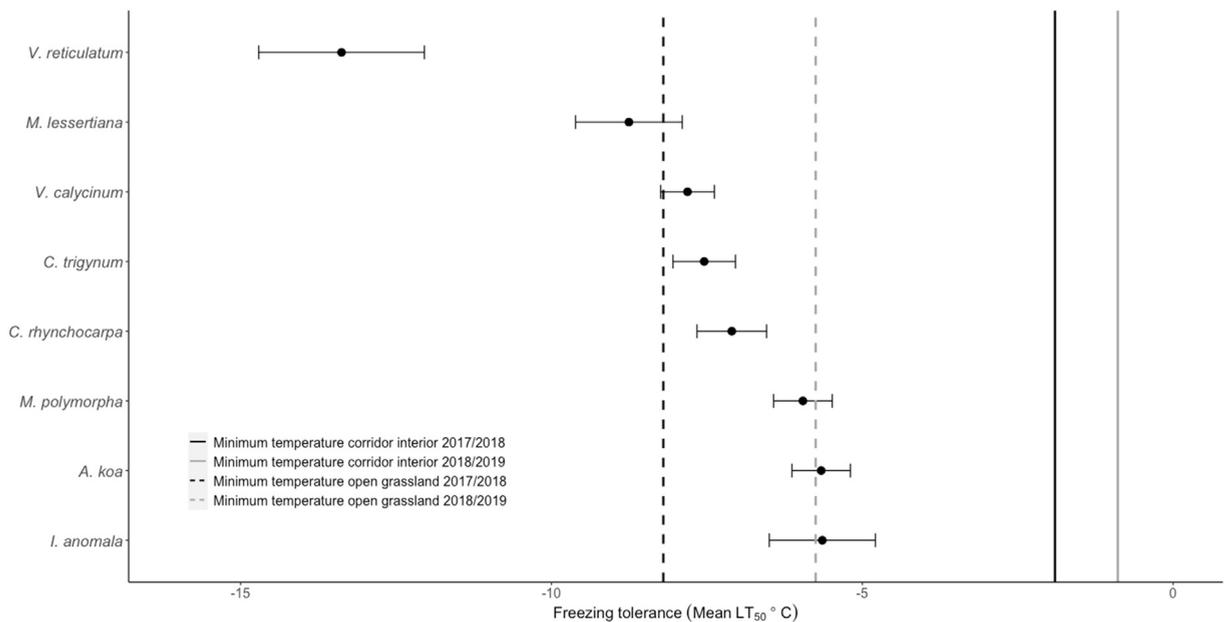


Fig. 1. Freezing tolerances (mean LT_{50} in $^{\circ}C \pm$ standard error) of native woody plant species at Hakalau Forest National Wildlife Refuge, Hawai'i, USA. For reference, vertical lines represent coldest temperatures recorded in open grassland (dashed) and koa corridors (solid) in the winter of 2017/2018 (black) and 2018/2019 (gray).

Therefore the linear gompertz model was estimating LT_{50} from a large temperature range (between -11 and -80 $^{\circ}C$). Regardless, all individuals of *V. reticulatum* resisted freezing down to at least -11 $^{\circ}C$.

The common and ecologically important tree *M. polymorpha* had a trend towards higher freezing tolerance ($LT_{50} = -8.8 \pm 0.9^{\circ}C$ SE) than *A. koa* ($LT_{50} = -5.7 \pm 0.5^{\circ}C$ SE, $p = 0.066$), the native tree used for restoration. *M. polymorpha* also trended to higher freezing tolerance than *I. anomala* ($LT_{50} = -5.6 \pm 0.9^{\circ}C$ SE, $p = 0.063$), a common understory tree in mesic and wet forest. There were no statistical differences ($p > 0.05$) between LT_{50} for all other species pairs.

3.2. Natural seedling recruitment

Surveys for woody seedlings in open grassland revealed very little natural seedling recruitment for most species with 71 of 100 plots having no seedlings at all. The three species with the highest freezing tolerance, *V. reticulatum*, *M. polymorpha*, *V. calycinum*, occurred in 2, 1, and 4 plots respectively. *A. koa*, a species whose true leaves showed poor freezing tolerance, was the most common species occurring in the pastures occurring in 26 of 100 sites. However, the vast majority of plants encountered were root suckers from nearby planted trees as the stems did not have true leaves (a sign the plant was grown from seed) and were often found in dense thickets. Such thickets can be found at restoration corridor edges where suckers grow away from the *A. koa* canopy edge. The remaining species (*C. trigynum*, *C. rhyhocarpa*, *M. lessertiana*, and *I. anomala*) were never found in the open plots.

3.3. Short and long-term temperature data

The coldest temperature recorded at any ground-level sensor was -8.2 $^{\circ}C$ on the morning of January 3, 2018, at Transect 1, which was colder than the LT_{50} for 6 of 8 species (Fig. 1). By comparison, the coldest temperature recorded at any restoration corridor site under *A. koa* canopy was -1.9 $^{\circ}C$ and was not colder than the LT_{50} for any species. Pair-wise tests showed that, in both years, all open grassland sites had colder daily minimum and mean minimum temperatures than their paired restoration corridor ($p < 0.001$ for all comparisons). Pastures also experienced more days below freezing than restoration corridors (Table S2).

Long-term temperature trends from April 2002 to March 2018 showed contrasting patterns. Monthly low temperatures decreased since 2002 with an overall trend of -0.1 $^{\circ}C$ per year (Fig. 2, $Z = -2.82$, $p = 0.005$ Seasonal Mann-Kendall Test). Conversely, monthly mean showed no trend ($Z = -0.83$, $p = 0.406$) and high temperatures showed a marginally significant increasing trend ($Z = 1.74$, $p = 0.093$) but the magnitude of this trend was only 0.8×10^{-5} $^{\circ}C$ per year.

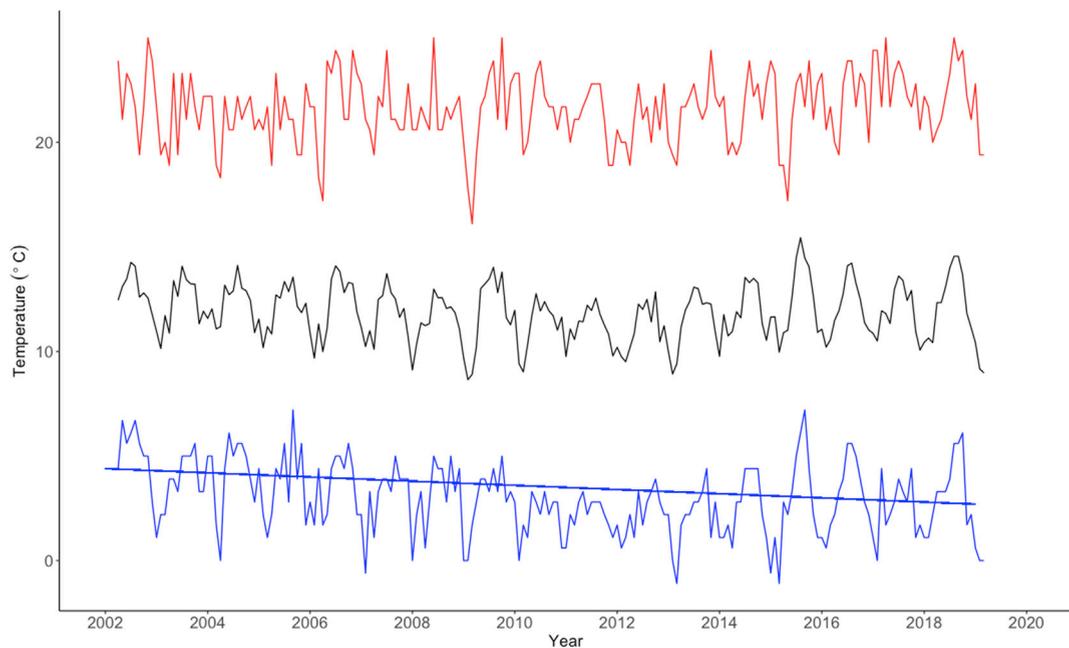


Fig. 2. Monthly maximum (red), mean (black), and minimum (blue) temperatures taken from a climate station at Hakalau Forest National Wildlife Refuge from April 2002–March 2019. The only significant trend from the Seasonal Mann-Kendall Test over time existed for minimum temperatures and is represented by the linear trendline in blue represents with a Thiel-Sen slope of -0.1°C per year. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

4.1. Freezing tolerance and natural seedlings recruitment

In several natural and human-modified tropical locations, freezing temperatures act to reinforce grassland-forest ecotones (Cavieres et al., 2000; Childes and Walker, 1987; Hoffmann et al., 2019; Holdo, 2006; Rehm and Feeley, 2015b). Our community-level assessment of freezing tolerances shows that a similar reinforcement of the forest boundary may be occurring in high-elevation Hawaiian forests. We found that only two of eight species we tested exhibited freezing tolerance sufficient to avoid leaf damage based on ground-level temperatures recorded over two years. *V. reticulatum*, a shrub-like species that is common in the pastures could withstand temperatures below -11°C , our lowest experimental freezing treatment. The lowest ground-level temperature recorded was -8.1°C suggesting that *V. reticulatum* could withstand such temperatures and even colder.

M. polymorpha, the second most freezing tolerant species, had a mean freezing tolerance (-8.8°C) in line with the lowest ground-level temperature recorded. When compared to previous work we see that *M. polymorpha* consistently shows a leaf freezing tolerance between -7 and -9°C (Melcher et al., 2000; Scowcroft et al., 2000). Two recent winters (2012/2013 and 2014/2015) were colder than the winters during our study. Therefore, temperatures more extreme than the coldest temperature measured here could be expected at longer time scales resulting in ground-level temperatures below the freezing tolerance of most *M. polymorpha* individuals, and all other species, exerting a strong demographic pressure on seedlings recruiting in the pasture (Whitcross et al., 2012). Furthermore, nighttime leaf temperatures can drop below air temperatures (Jordan and Smith, 1994), meaning that our estimate of minimum temperatures that leaves experience may be conservative. Thus, even mild winters may be too cold for most of our study species to persist in the pasture.

In addition to our leaf-level freezing tolerance data, the natural occurrence of seedlings in pasture seem to support that freezing temperatures restrict plant recruitment. Only four of eight species were ever encountered in the pasture, with three of these species being the most freezing tolerant species we tested (*V. reticulatum*, *M. polymorpha*, and *V. calycinum*). The fourth species, *A. koa*, was found in several plots despite its limited freezing tolerance. *A. koa* is known to send root suckers from adults and most of the individuals encountered in our survey exhibited this suckering growth form (e.g. growing densely, phyllodes instead of true leaves). Indeed, Scowcroft and Yeh (2013) estimated that *A. koa* corridors in Hakalau Forest NWR are expanding into pastures through this suckering, albeit slowly. Our sampled individuals were thus likely connected via roots to nearby adults, making them more capable of regeneration if damaged by freezing temperatures due to their connection to the parent plant. Unlike suckers, tissue death in true seedlings represents a significant biomass loss as they do not have a large carbohydrate pool from a parent plant. Scowcroft and Jeffrey (1999) showed that 95% of outplanted *A. koa* seedlings died when planted in a microsite that experienced ground-level freezing temperatures down to -4°C , which was

much milder than measured during the present study. The sensitivity of *A. koa* seedlings to cold temperatures is further supported by our freezing tolerance estimate for *A. koa* of just -5.7°C as this estimate is for true leaves that occur on juveniles before phyllodes are developed. Therefore, while *A. koa* stands may expand outward over time via vegetative reproduction, recruitment of true seedlings at greater distances from the forest boundary are unlikely due to freezing temperature sensitivity. The remaining four species, *I. anomala*, *C. trigynum*, *M. lessertiana*, or *C. rhynchocarpa*, were never found in the pasture and this is consistent with high mortality of these species in experimental pasture planting (S. Yelenik, U.S. Geological Survey, personal observation). Combined, these data suggest that if freezing temperatures restrict native woody seedling recruitment in the pasture then significant increases in minimum temperatures need to occur before recruitment may be observed.

4.2. Temperature differences among habitats

Temperatures within forest restoration corridors were much milder than in the open pasture showing that potential tissue loss due to freezing is minimal when under the buffering effects of a canopy. Extreme low temperatures in the open pasture were usually $3\text{--}6^{\circ}\text{C}$ colder than their paired restoration corridor site despite being only $10\text{--}50\text{ m}$ from the forest canopy edge. This spatial restriction of temperature buffering effects by the canopy to a small area directly adjacent to the forest edge has been found in other tropical montane studies (Rada et al., 2009; Rehm and Feeley, 2013, 2015a). The upper portions of the refuge, and the majority of the land surrounding the refuge, have very little forest trees present and therefore no temperature buffering effects, limiting woody plant recruitment to spatially restricted microhabitats where low temperatures do not reach extremes (e.g. ridgetops; Scowcroft and Jeffrey, 1999). Therefore, woody plant recruitment into the large expanses of abandoned pastures will be slowed and limited to the forest-canopy edge of corridors as individuals further into the pasture will continue to experience freezing damage and tissue loss.

4.3. Long-term temperature trends

In many regions of the world, the frequency and intensity of freezing events appear to be easing, although this is not true everywhere (Easterling, 2002; Easterling et al., 2000; Klein Tank and Können, 2003). The decoupling of regional and site-specific climates from global trends means that cold temperatures may become more extreme in the near future (Kodra et al., 2011). We are cautious in making broad conclusions about our limited, 17-year temperature record as climate in the Hawaiian islands is strongly linked to longer temporal scale events (McKenzie et al., 2019). However, this 17-year temperature record taken from a climate station within a few kilometers of our site does offer ecologically relevant data on a timeframe that overlaps significantly with restoration efforts (*A. koa* corridor plantings started about 30 years ago with trees becoming mature around 20 years ago). Even with limited climate data, our site may be a good example of the disparity between regional and site-specific locations in a heterogeneous mountain environment. Climates throughout Hawai'i are warming (Diaz et al., 2011; Kagawa-Viviani and Giambelluca, 2020; McKenzie et al., 2019), but our 17-year climatic record shows no trend in mean temperatures while monthly minimum temperatures appear to be becoming more extreme.

At higher elevations in Hawai'i, minimum temperatures have been shown to have upward trends, sometimes significant (Kagawa-Viviani and Giambelluca, 2020) but sometimes not (McKenzie et al., 2019). Minimum temperatures are tightly linked to cloud base and precipitation but models show contradictory predictions of cloud cover, with simulations showing our sites will become drier with less cloud cover (Cao et al., 2007) and others the opposite (Zhang et al., 2016). Even in studies that analyze longer climate data series, the lack of climate stations at higher elevations ($>1600\text{ m.a.s.l.}$) greatly restricts the ability to find patterns in climate and predict future trends. Therefore, while minimum temperatures likely depend on long-term climate fluctuations at our site, low temperature extremes do appear to continue to limit seedling recruitment in the open pasture areas despite overall regional climate warming.

Even as mean temperatures increase, this could have an overall negative effect on seedlings. Tropical montane species can seasonally adjust their freezing tolerances (Rehm and Feeley, 2015b). However, prematurely reducing freezing tolerance due to warmer days during the winter-spring transition may lead to a high risk of freezing damage when late-spring freeze events occur, as has been documented elsewhere (Augspurger, 2009; Hufkens et al., 2012). Such phenological mismatches between seasonally adjusted freezing tolerance and changing climates could lead to even more severe impacts on seedling recruitment in tropical mountains but the seasonality and sensitivity of freezing tolerance to other environmental variables such as photoperiod and daily temperatures are not well understood in tropical plants.

Our study has several limitations that restrict us from drawing stronger inferences on the relationship of freezing temperatures, woody plant recruitment, and forest restoration. Freezing temperatures are just one factor that could impact how and where seedlings recruit in pasture. For example, pastures are expected to experience higher maximum temperatures and light intensities than nearby forest due to the lack of shade from overstory canopies, potentially leading to photoinhibition (Bader et al., 2007; Rehm and Feeley, 2013). Furthermore, temperatures are buffered within the restoration corridors yet native plant recruitment does not occur (Yelenik, 2017), suggesting that additional factors such as competition with invasive grasses may also limit seedling recruitment. Fine-scale microclimate data (e.g. temperature, UV radiation) coupled with further experiments (e.g. photoinhibition of seedlings in pastures, effects of competition with invasive grasses) are needed to see if such conditions exist and how they influence seedlings. In particular, the trade-off between invasive grasses

ameliorating microclimate severity and direct competition with native seedlings planted in pastures warrants investigation to inform management practices.

5. Conclusions

The loss of high elevation tropical montane forests poses a serious threat to global biodiversity. Restoration of such forests can offset some of these losses but our understanding of forest restoration and re-establishment in pastures is limited in tropical mountains. One example of the importance of this restoration is in the high-elevation forests in the Hawaiian Islands that can serve as refugia for threatened native forest birds (Benning et al., 2002). Most of these forests have been cleared for ungulate grazing making active restoration of large forest tracts necessary to help curb the extinction crisis of Hawaii's birds (Paxton et al, 2016, 2018). Here we show that despite global warming, freezing temperatures likely continue to hinder plant regeneration in pastures, perpetuating the current sparse patterns of native plant recruitment in these degraded areas.

If forests are expected to expand into pastures and into even higher elevations, then outplanting efforts into the colder pasture is necessary. Planting configuration can be altered to buffer microclimates and promote plant growth, such as planting in clumps instead of rows or planting at higher densities (Zahawi et al., 2013). This configuration can also work to increase seed dispersal and further recruitment of native plants (Holl et al., 2000). Similarly, species with the best freezing tolerances (i.e. *V. reticulatum* and *M. polymorpha*) can be used as the primary species for forest restoration and, once established, would be expected to facilitate other plant recruitment or restoration plantings through the amelioration of cold temperature extremes. Planting near established canopies can significantly increase survival (Scowcroft et al., 2000; Scowcroft and Jeffrey, 1999) but may need to be paired with labor-intensive grass control. There is a myriad of potential barriers to restoration of high elevation tropical forests. Our work highlights one such barrier for managers to consider when planning restoration efforts.

Author contributions

ER, CD, and SY conceived and designed the experiment. ER performed the experiment and analyzed the data. ER, CD, and SY wrote the manuscript.

Declaration of competing interest

We have no conflicts of interest concerning this manuscript.

Acknowledgements

We want to thank A. Trudgeon, K. Fuhr and D. Bouck for field assistance. Hakalau Forest National Wildlife Refuge allowed for site access, and the Institute for Pacific Island Forestry provided laboratory space. We are grateful to the University of Hawai'i at Hilo for use of their autoclave. The research was funded by the National Science Foundation [DEB 1557177]. We thank the University of California at Santa Barbara Faculty Senate for providing funding to purchase the freezing equipment. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gecco.2021.e01462>.

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