Freezing temperatures as a limit to forest recruitment above tropical Andean treelines

Evan M. Rehm[1,2,3] and Kenneth J. Feeley[1,2]

[1]*International Center for Tropical Botany, Department of Biological Sciences, Florida International University,*
Miami, Florida 33199 USA
[2]*Fairchild Tropical Botanic Garden, Coral Gables, Florida 33156 USA*

Abstract. The elevation of altitudinal treelines is generally believed to occur where low mean temperatures during the growing season limit growth and prevent trees from establishing at higher elevations. Accordingly, treelines should move upslope with increasing global temperatures. Contrary to this prediction, tropical treelines have remained stable over the past several decades despite increasing mean temperatures. The observed stability of tropical treelines, coupled with the drastically different temperature profiles between temperate and tropical treelines, suggests that using mean measures of temperature to predict tropical treeline movements during climate change may be overly simplistic. We hypothesize that frost events at tropical treelines may slow climate driven treeline movement by preventing tree recruitment beyond the established forest canopy. To assess this hypothesis, we measured freezing resistance of four canopy-forming treeline species (*Weinmannia fagaroides*, *Polylepis pauta*, *Clethra cuneata*, and *Gynoxys nitida*) at two life stages (juvenile and adult) and during two seasons (warm-wet and cold-dry). Freezing resistances were then compared to microclimatic data to determine if freezing events in the grassland matrix above treeline are too harsh for these forest species. Freezing resistance varied among species and life stages from −5.7°C for juveniles of *P. pauta* to −11.1°C for juveniles of *W. fagaroides*. Over a four-year period, the lowest temperatures recorded at 10 cm above ground level in the grasslands above treeline and at treeline itself were −8.9°C and −6.8°C, respectively. Juveniles maintained freezing resistances similar to adults during the coldest parts of the year and ontogenetic differences in freezing resistance were only present during the warm season when temperatures did not represent a significant threat to active plant tissue. These findings support the hypothesis that rare extreme freezing events at and above tropical treelines can prevent recruitment outside of closed canopy forest for some tree species and may significantly slow treeline advancement despite warming mean temperatures. Predictions of treeline shifts under climate change should be reevaluated to include species-specific climatic tolerances and measures of climatic variability.

Key words: *climate change; freezing tolerance; frost; Peru; puna; species range edge; timberline.*

Introduction

High-altitude treelines (hereafter referred to just as treelines) represent one of the most distinct and visible terrestrial ecotones, with closed canopy forests giving way to open alpine vegetation over a relatively short distance (Körner 2012). The abrupt ecotone transition at true climatic treelines is generally believed to occur at elevations where mean growing season temperatures directly limit the growth of upright woody trees (Körner 1998, 2008). This "growth limitation" hypothesis suggests that treelines should shift upslope in response to rising global mean temperatures (Grace et al. 2002, Harsch et al. 2009). Contrary to this prediction, approximately one-half of the studied treelines world-wide have remained stable over the last century despite increasing mean temperatures (Harsch et al. 2009). Furthermore, rates of treeline shifts are spatially inconsistent, such that in some regions (e.g., in tropical mountains, New Zealand, and the temperate Andes) there have been relatively fewer documented upslope shifts in treeline (Wardle 2008, Harsch et al. 2009). While mean growing season temperature may be a good predictor of treelines at coarse geographic scales (Körner and Paulsen 2004, Paulsen and Körner 2014), the inconsistent patterns of treeline movements during global warming suggests that there may be additional climatic factors, such as temperature extremes, and non-climatic factors, such as biotic interactions, that modulate treeline movement at more local and regional scales (Rehm and Feeley, *in press*).

Compared to their temperate counterparts, tropical treelines experience a relatively mild year-round growing season with small seasonal temperature fluctuations

but relatively large diurnal temperature swings, with warm days followed by freezing or near-freezing nights throughout the year (Körner and Paulsen 2004, Hoch and Körner 2005, Rehm and Feeley 2013, Kessler et al. 2014). Climatic variability is known to play a disproportionate role in shaping species' current ranges and future responses to climate change (Jentsch et al. 2007, Zimmermann et al. 2009, Thompson et al. 2013). Therefore, extreme temperatures may play a critical role in determining treeline movements during periods of climatic change especially if there are asynchronous rates of change in mean and extreme temperatures. For example, during climatically stable periods the ultimate position of treelines may be determined by mean growing season temperature but during periods of rapid climatic change, episodic extreme temperature events (e.g., freezing temperatures) could slow species' movements upslope or temporarily stabilize treelines.

One reason that low temperature extremes may play a major role in determining the upper distributional limits of tropical treeline-forming species is the fact that high-elevation tropical trees do not enter into a state of dormancy during cold seasons and therefore maintain their physiological activity throughout the entire year. As such, there is a greater potential for tissue damage due to freezing because active tissues are more susceptible to freezing damage than are dormant tissues (Rada et al. 1985, Sakai and Larcher 1987). Tissue damage may consequently occur even during the relatively mild freezing events that occur at tropical treelines. There is limited but growing evidence that the inability to avoid freezing damage may help to partially explain tropical treeline elevations and the pattern of the treeline ecotone, especially in regions where freezing temperatures regularly occur such as at treelines in the tropical Andes (Wesche et al. 2008, Harsch and Bader 2011).

Compared to species from other geographic regions, there is a relative dearth of information about the freezing resistances of treeline species from the tropics. The work that has been done has largely focused on Andean species (for a review, see Körner [2012]). Adults of most tropical Andean treeline species exhibit freezing resistances between −6° to −13°C (Goldstein et al. 1985, Rada et al. 1985, 2009, Cavieres et al. 2000), but one species, *Polylepis tarapacana*, the highest-elevation treeline-forming species in the world, can tolerate temperatures down to −23°C (Rada et al. 2001). Even at the same treeline, there can be large interspecific variation in freezing resistance. For example, Cavieres et al. (2000) found that the altitudinal limit of one Venezuelan treeline-forming species (*Podocarpus oleifolius*) was likely directly limited by freezing resistance while a second species (*Espeletia neriifolia*) could withstand more severe freezing temperatures. From this study, we can see that species forming current tropical treelines exhibit ecologically meaningful differences in freezing resis-

tances, likely reflecting evolutionary and range-specific climatic variation of individual species (Araújo et al. 2013). As such, low temperatures may directly limit some species' current elevational distributions while other species' ranges are limited by other factors (e.g., mean growing season temperature, precipitation, or edaphic conditions). If increases in low-temperature extremes lag behind rising mean temperature during climate change (Kodra et al. 2011), then low temperatures may continue to prevent some treeline-forming species from invading open habitats above treeline while other species that are relatively insensitive to low temperatures move upslope. Such a scenario could result in severe alterations to the structure and function of the treeline forest community and have cascading effects throughout the species rich cloud forest communities occurring below treeline (Feeley and Silman 2010).

Even within a species, low-temperature resistance can vary with life stage and individual growth morphology. For example, it is generally believed that seedlings and saplings are less freezing resistant than conspecific adults (Larcher and Bauer 1981, Sakai and Larcher 1987). In addition, in an open grassland matrix such as that found above treeline, the height of individuals can play an important role in determining the exposure of plant tissue to extreme temperatures (Squeo et al. 1991, Sierra-Almeida and Cavieres 2012). The variation in freezing resistance due to ontogeny and differences in exposure due to plant height can be especially relevant for the upslope movement of treeline species because young plants growing close to the ground outside of the established forest will experience more severe and frequent frost events than taller individuals growing within closed canopy forests (Wesche et al. 2008, Rada et al. 2009, Rehm and Feeley 2013). Yet to our knowledge there has been no previous attempt to compare the freezing resistance of juvenile and adult trees at tropical treelines or to determine if low temperature extremes actually pose a significant threat to seedlings growing above treeline.

Before we can make accurate predictions about the dynamics and location of tropical treelines under climate change, we must first gain a comprehensive knowledge of how freezing events affect treeline species at different life stages and at different times of the year. As such, our goals in this study were to (1) determine if there are ecologically meaningful differences in freezing resistance within a species at different times of year and in different life stages, (2) determine if freezing resistance varies among species after controlling for ontogeny and seasonal effects, and (3) relate species' freezing resistances to climate patterns to determine if low temperature extremes could plausibly limit tree recruitment above treeline for four tropical Andean treeline-forming species (*Weinmannia fagaroides*, *Polylepis pauta*, *Clethra cuneata*, and *Gynoxys nitida*).

METHODS

Study sites

This study was conducted at two high-elevation tropical treelines in the southeastern Peruvian Andes within Manu National Park, Cusco department, Peru (~13°6′18″ S, 71°35′21″ W). Treeline elevation within the Park varies, but the two treeline sites used in this study occur at 3620 m above sea level with a mean annual air temperature of 6.8°C (Rehm and Feeley 2013), which is consistent with the global treeline isotherm (Körner and Paulsen 2004, Paulsen and Körner 2014). As the treelines in this study represent a life form change from closed canopy tropical montane cloud forest to non-woody grassland vegetation (locally known as *puna*), we consider the treeline center as the point closest to the forest where tussock grasses begin to grow. For both treeline sites, this point was clearly visible as tussock grasses do not invade the shaded understory of the forest and the transition from forest to grasslands occurred over a distance of <5 m. At the treeline center, the forest canopy overhangs the grassland vegetation by 1–2 m but then quickly gives way to open sky. Treeline sites were situated on flat ground on the opposite sides of a 100 m wide section of cloud forest; with treeline site 1 facing toward the northnortheast and treeline site 2 toward south-southeast. Temperature and moisture seasonality at treeline in the southeastern Peruvian Andes can be characterized by two distinct seasons, a relatively cold-dry season occurring from May through September and a relatively warm-wet season from November through March (Rapp and Silman 2012). Diurnal temperature fluctuations are much greater than seasonal temperature variation and frosts can occur almost any time of year.

Throughout the tropics, anthropogenic disturbances can have strong effects on local and regional treeline positions and dynamics (Young and León 2007). Human activities such as cattle grazing and fire have certainly played a role in the vegetative dynamics around our study sites in the past. However, our study sites have been protected for at least 40 years and continuing human disturbance in the area is restricted to a few locations where light cattle grazing occurs only during certain times of the year (Gibbon et al. 2010), with no evidence of new fires (e.g., fire scars or charred tree stumps) occurring for at least 25+ years (Manu National Park officials, *personal communication*). Furthermore, mean annual temperatures at our study sites fall very close to the global treeline isotherm suggesting that the selected treelines are indeed true climatic treelines and are not depressed by anthropogenic disturbances (Körner and Paulsen 2004, Paulsen and Körner 2014). The species included in this study are believed to represent true treeline-forming species and, as such, we treat the relationship between speciesspecific freezing resistance and temperature profiles at our treelines as representative of climatic treelines in the tropical Andes.

Freezing resistance

Freezing resistance was determined for adults (≥10 cm dbh and ≥3 m tall) and small juveniles (≤1.0 cm diameter at ground level and ≤50 cm tall) of the four most dominant canopy-forming treeline species in the study region: *Weinmannia fagaroides*, *Polylepis pauta*, *Clethra cuneata*, and *Gynoxys nitida*. Adult trees were sampled from the treeline center (as defined in *Study sites*) and leaves were taken from a height of 2–5 m above the ground. Juveniles were sampled within the grassland matrix within 3–10 m of the treeline center. Juveniles did not grow to heights above the grass canopy. To test for seasonal variation in freezing resistance, sampling occurred at the beginning of the warm season in November 2013 and during the peak of the cold season in July 2014. To determine if freezing resistance varied between life stages, six adults and six juveniles from each species were sampled during each sampling period. From each individual, at least six intact mature leaves were harvested (for juveniles, leaves were collected 5–20 cm height above the ground) and, in order to avoid major alterations to leaf water content, harvested leaves were immediately placed in sealed polyethylene bags and transported to Cusco, Peru, in an insulated container at 3–6°C. Leaves were harvested shortly after sunrise between 06:00 and 08:00 and placed into freezing treatments by mid- to late-afternoon within eight hours of initial harvest. For each sampling period, we sampled 4 species × 2 life stages × 6 individuals × 6 leaves for a total of 288 leaves.

One leaf from each individual was cut at the petiole and randomly assigned to one of six target treatment temperatures: 4°, −3°, −6°, −9°, −12°, and −30°C. Leaves assigned to the same treatment temperature were then packaged together. Leaves designated to the −3°, −6°, −9°, and −12°C treatments were placed inside polystyrene foam boxes inside a single manually controlled commercially available freezer (Electrolux model EFCW152NSKG; Stockholm, Sweden) which had been pre-cooled to 4°C. Temperature was then lowered at a rate of 2–3°C/h until reaching the first target temperature of −3°C. Temperature was held at −3°C for two hours. After two hours those leaves designated to the −3°C temperature treatment were removed. Temperature in the freezer was then lowered to the next target temperature of −6°C and the process was repeated for each target temperature. As leaves were removed from the freezer they were placed inside a separate refrigerator (Electrolux model ERD092UBGS) and thawed at 3–5°C/h until reaching 4°C.

The 4°C and −30°C treatments served as references for live and dead leaf material, respectively. Leaves assigned to the 4°C reference were kept in a separate refrigerator (Electrolux model ERD092UBGS) maintained at 4°C throughout the entire freezing process. In order to create

a dead leaf reference, assigned leaves were rapidly cooled ($>4°C/h$) to $-30°C$ and held at that temperature for 24 h.

Within each package of leaves, temperature was recorded every 10 s and checked in real-time every 15 min (12-bit Temperature Smart Sensor; S-THB-M006; accuracy $±0.2°C$; Onset Computer Corporation, Bourne, Massachusetts, USA) throughout the freeze/thaw process. At each 15-min interval, the freezer thermostat was manually adjusted to ensure consistent cooling rates and to maintain stable temperatures once a target temperature was reached. A circulating fan was placed inside the freezer to ensure homogeneity of air temperature inside the freezer. During freezing treatments, temperature fluctuated around target temperatures by an average of $±0.3°C$.

After treatment, all leaves were held at $4°C$ for 72 h to allow necrosis development in leaf material. Individual leaves were then visually assessed for tissue damage/discoloration at 5% increments (Sakai and Larcher 1987). Following visual observations, samples were placed in distilled water in individual plastic test tubes and kept at $4°C$ for 48 h. Electric conductivity was then measured using a handheld conductivity meter (Greisinger GMH 3430 conductivity meter; Greisinger Electronic, Regenstauf, Germany) as a measure of electrolyte leakage (Flint et al. 1967, Burr et al. 1990). To kill living tissue, samples were then boiled in a hot water bath at $90°C$ for 15 min and allowed to rest for 72 h before taking a second measurement of maximum electrolyte leakage.

Temperature data

In order to gain a general understanding of the low temperatures experienced at and around treeline, an array of seven HOBO ProV2 temperature/relative humidity data loggers (U23-001; accuracy $±0.2°C$; Onset Computer Corporation) were deployed May 2011–August 2013 and May–July 2014 at the two treeline study sites (Rehm and Feeley 2013). Data was unavailable from September 2013 through April 2013 due to logger failure. Data loggers were located approximately 10 cm above the soil surface, which was the same height as the majority of leaves collected from juveniles used in this study, and shielded from direct sunlight with a well-ventilated polystyrene cover. At treeline site 1, two loggers were placed at the treeline center point and two loggers were placed in the open ground between tussock grasses approximately 10 m from the treeline edge. At treeline site 2, two loggers were placed at the treeline center point but only one logger was placed in the open grassland. Temperature for all loggers was recorded at 30-min intervals. Data from these loggers were screened to find the lowest recorded temperatures at the field sites over several years. The extreme lows were used as estimates of the lowest temperatures experienced by individual plants growing near the treeline ecotone. These estimates are conservative since temperatures may have reached lower temperatures over the lifespan of the sampled plants.

In order to quantify patterns of microclimate across treeline in more detail, additional temperature sensors were established along one transect at each of the two treeline sites from 24 July to 4 September 2014. Each of these transects consisted of four HOBO Microstation dataloggers (H21-002; Onset Computer Corporation) each equipped with three Air Temperature/Relative Humidity Smart Sensors (S-THB-M006; accuracy $±0.2°C$; Onset Computer Corporation). Relative to the treeline center and along each transect, a single datalogger was placed at 10 m within cloud forest, directly at the treeline center, 7 m into the open puna and 15 m into the open puna. Temperature sensors attached to each datalogger were placed at 10 cm above the ground, at the top of the grass canopy (~50–70 cm above the ground), and at 2 m above the ground. All temperature sensors were covered with a well-ventilated polystyrene cover to shield sensors from direct sunlight. This design allows for comparison of microclimate variation within each transect horizontally across the treeline ecotone and vertically at each sample point. Temperatures were recorded every 15 min.

Statistical analysis

In this study, freezing resistance is expressed as the threshold temperature at which 50% of sample tissues die (LT_{50}; Sakai and Larcher 1987). LT_{50} was calculated for each individual and then averaged across conspecifics of the same life stage during the same season ($n = 6$ for each life stage, season, species combination). For visual assessments, LT_{50} was calculated using logistic regression and, for electrolyte leakage, LT_{50} was calculated using nonlinear Gompertz models (Lim et al. 1998). LT_{50} values for the two methods were significantly correlated ($R^2 = 0.8$, $P < 0.001$, Pearson's correlation) but values were generally colder for the electrolyte leakage method. For clarity, only results from the electrolyte leakage method are presented as they represent the more conservative estimate (i.e., colder LT_{50} values mean greater freezing resistance) in the context of this discussion.

Since our goals were to determine freezing resistance variation due to the individual effects of life stage, season and species, differences in LT_{50} between species, life stages, and seasons were analyzed using a series of generalized linear models built in R 3.0.3 (R Development Core Team 2013). To test for ontogenetic differences in freezing resistance with life stage within a species, individual models were built for each species and season combination with life stage (juvenile or adult) as the sole predictor variable. A post hoc Tukey Honestly Significant Difference (HSD) test was then run to look for differences in LT_{50} between life stages within season and species using the Multcomp package in R (Hothorn et al. 2008). Similarly, to test for seasonal differences in freezing resistance between individuals of
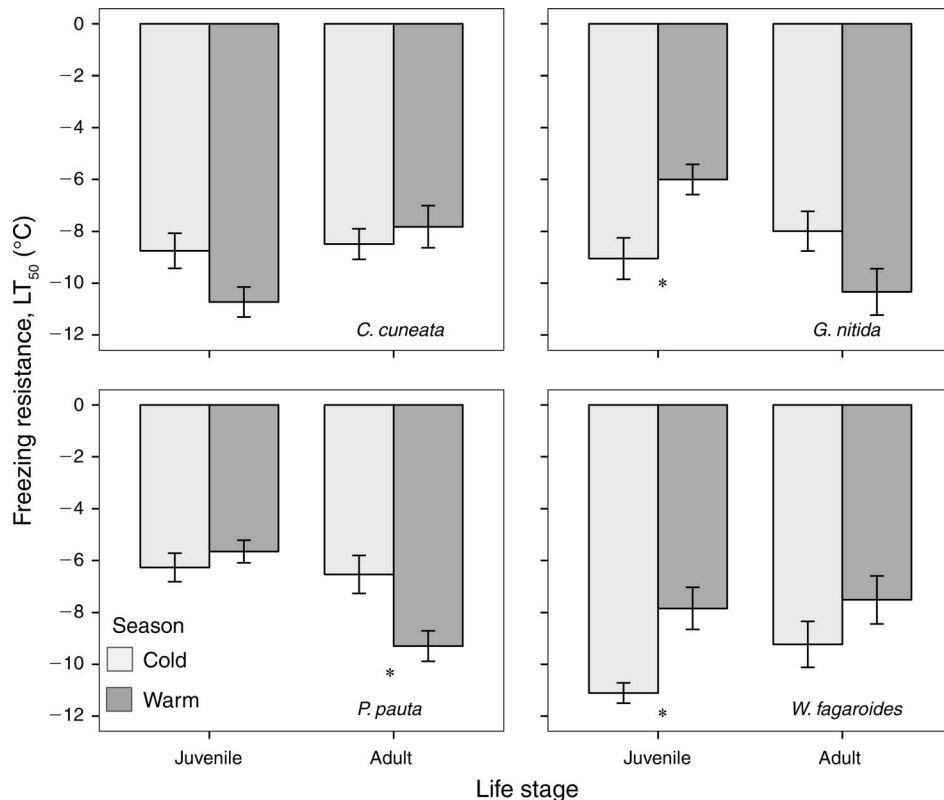
FIG. 1. Variation of freezing resistance (LT_{50}; mean $\pm$ SE) for each species (*Clethra cuneata*, *Gynoxys nitida*, *Polylepis pauta*, and *Weinmannia fagaroides*) at two different life stages during two seasons ($n = 6$ for each species, life stage, and season combination). Asterisks represent significant differences (post hoc Tukey Honestly Significant Difference tests, $P < 0.05$) in freezing resistance within the same species and life stage but during different seasons.

the same life stage within a species, individual models were built for each species and life stage combination with season (cold or warm) as the sole predictor variable followed by HSD tests to determine differences in LT_{50} between seasons within life stage and species. To test for variation in LT_{50} among species, individual models were built for each season and life stage combination with species as the predictor. When a significant effect ($P < 0.05$) of species was present, HSD tests were run to determine which species–life-stage groups exhibited differences in LT_{50}.

RESULTS

Lower or more negative LT_{50} values represent greater freezing resistance than the higher or less negative LT_{50} values. Freezing resistance in leaves varied from a low of $-5.7°C$ in juveniles of *P. pauta* during the warm season to a high of $-11.1°C$ in juveniles of *W. fagaroides* during the cold season. There was no consistent trend in the relationships between leaf freezing resistance, life stage, and season as these relationships varied among and within species.

Freezing resistance of leaves for *C. cuneata* did not vary with season within a life stage (Fig. 1) but there were differences between life stages, with juveniles

maintaining higher freezing resistance than did adults during the warm season (Table 1). For *G. nitida* and *P. pauta*, freezing resistance of leaves varied with both season and life stage. For both of these species, adults had higher freezing resistances than juveniles during the warm season (Table 1). Within life stage, juveniles of *G. nitida* maintained higher freezing resistance during the cold season than the warm season while adults of *P. pauta*, maintained higher freezing resistance during the warm season than the cold season (Fig. 1). For *W. fagaroides*, no difference was found in freezing resistance between leaves of juveniles and adults during either season (Table 1). However, there was a difference across seasons within life stage, with juveniles having higher freezing resistance in the cold season than juveniles during the warm season (Fig. 1).

Freezing resistance varied among species with season but only at the juvenile life stage (Fig. 2). During the warm season, *C. cuneata* maintained higher freezing resistance than all the other species. During the cold season, *P. pauta* maintained lower freezing resistance than all other species. There were no differences in freezing resistance among adults during either season.

Based on the array of seven data loggers deployed since May 2011, we found that absolute minimum

Table 1. Estimated freezing resistances, LT_{50}, of four dominant treeline species (*Clethra cuneata*, *Gynoxys nitida*, *Polylepis pauta*, and *Weinmannia fagaroides*) measured during two different seasons (cold and warm) at two life stages (adult and juvenile).

	Life stage		
Species, season	Adult (°C)	Juvenile (°C)	*P*
C. cuneata			
Cold	−8.5 (0.6)	−8.8 (0.7)	0.778
Warm	**−7.8 (0.8)**	**−10.7 (0.6)**	**0.015**
G. nitida			
Cold	−8.0 (0.8)	−9.1 (0.8)	0.362
Warm	**−10.3 (0.9)**	**−6.0 (0.6)**	**0.004**
P. pauta			
Cold	−6.5 (0.7)	−6.3 (0.6)	0.775
Warm	**−9.3 (0.6)**	**−5.7 (0.4)**	**<0.001**
W. fagaroides			
Cold	−9.2 (0.9)	−11.1 (0.4)	0.082
Warm	−7.5 (0.9)	−7.8 (0.9)	0.818

Notes: Values are means with SE in parentheses. Sample size $n = 6$ for each species, life stage, and season combination. Significant differences (post hoc Tukey Honestly Significant Difference tests, $P < 0.05$) are shown in boldface type and represent difference in freezing resistance within the same season and species but between life stage.

temperature at 10 cm above ground level at our study sites was −8.9°C in the open grassland while the minimum temperature at the treeline center reached −6.8°C, more than 2°C warmer despite being separated by only 10 m. For both the grassland and treeline center, this absolute lowest temperature reading occurred during the same morning on 10 August 2012 at treeline transect 1. Absolute minimum temperatures recorded across the more detailed treeline microclimate transects from 24 July to 4 September 2014 were milder than those recorded from the individual data loggers deployed since May 2011 (likely due to the shorter measurement period). However, there was a clear pattern of more severe nighttime low temperatures at 10 cm above ground level vs. the grass canopy height (50–70 cm) or 2 m above ground at almost all distances from the treeline along both transects (Table 2). Mean nighttime minimum temperatures followed similar patterns, with lowest temperatures occurring at 10 cm above ground level in the open grassland (15 m and 7 m from treeline) and with highest temperatures occurring within the cloud forest and at heights above the grass canopy (Table 2). Similarly, mean nighttime minimum temperatures at 10 cm above ground level were significantly lower than temperatures either at the top of the grass canopy or when coupled to the free atmosphere at 2 m above the ground (Table 2). Mean nighttime minimum
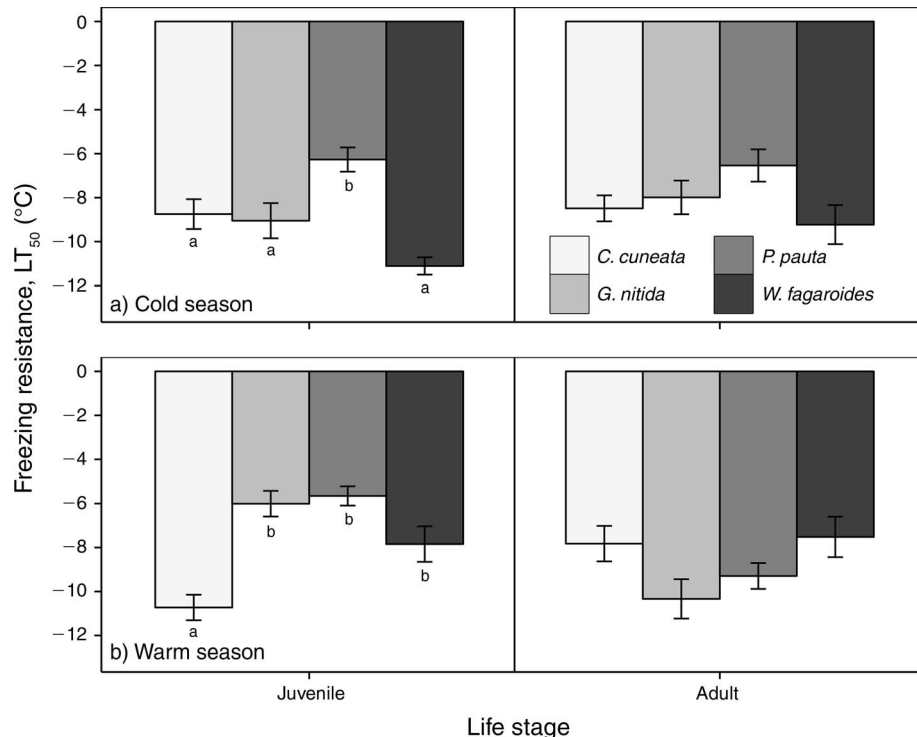

Fig. 2. Variation of freezing resistance (LT_{50}, mean ± SE) among species at two different life stages during two seasons ($n = 6$ for each species, life stage, and season combination). Letters represent significant differences (post hoc Tukey Honestly Significant Difference tests, $P < 0.05$) in freezing resistance within a season and life stage but among different species. For adults, there were no significant differences among species for either season.

TABLE 2. Nighttime minimum temperatures (°C) and absolute minimum temperature (°C; in parenthesis) recorded between 24 July and 4 September 2014 at different heights from the ground and different distances from the treeline along two different microclimate treeline transects.

	Distance from treeline (m)			
	Interior	0	7	15
Transect 1				
Height from ground (cm)				
5	1.8 ± 1.5 (−1.6)	0.0 ± 2.2 (−6.1)	−1.0 ± 2.4 (−7.1)	−0.4 ± 2.5 (−6.3)
50	1.9 ± 1.4 (−1.6)	0.1 ± 2.2 (−5.5)	−0.4 ± 2.5 (−5.9)	−0.3 ± 2.1 (−5.6)
200	2.1 ± 1.4 (−1.5)	1.7 ± 1.5 (−2.1)	1.3 ± 1.4 (−1.9)	1.8 ± 1.2 (−0.9)
Transect 2				
Height from ground (cm)				
5	2.3 ± 1.5 (−1.9)	−0.9 ± 2.6 (−6.6)	−1.4 ± 2.8 (−7.5)	−1.0 ± 2.9 (−7.8)
50	2.4 ± 1.5 (−1.7)	−1.0 ± 2.5 (−7.3)	0.0 ± 2.3 (−5.7)	−1.0 ± 2.5 (−7.3)
200	2.4 ± 1.4 (−1.6)	1.1 ± 1.9 (−3.8)	1.5 ± 1.6 (−3.0)	1.7 ± 1.6 (−2.8)

Note: Values are mean ± SD.

temperatures were generally lowest in the open grasslands and highest in the forest interior when measured at the same height.

DISCUSSION

We found that for several dominant tropical treeline-forming species, the freezing resistances of leaves were insufficient to withstand the low temperature extremes recorded near the ground in the grasslands above treeline. Even when we consider that the LT_{50} of attached leaves in situ may be 1–2°C colder (Taschler and Neuner 2004), all species had, at best, very small safety margins between their maximum freezing resistance and minimum recorded temperatures. What's more, given a longer climatic data set, it is highly likely that absolute minimum temperatures would fall well below the −8.9°C minimum that we recorded over our study period. These findings indicate that juvenile plants growing at or near ground level in the grasslands are exposed to periodic extreme freezing events that can kill their leaves, or potentially entire plants. If this is the case, then our findings strongly suggest that the expansion of some tree species' upper range edges past the current treeline will be inhibited by rare extreme freezing events, even as mean temperatures increase.

Low temperature extremes were not uniform within the grassland habitat, with the most extreme low temperatures occurring at ground level where the juvenile trees maintained the majority of their leaves. These data are consistent with similar microclimatic measurements at other tropical Andean treelines (Hertel and Wesche 2008, Rada et al. 2009). The lower temperatures at ground level can be attributed to nighttime radiative cooling. Once above the grassland canopy, free air-mixing results in milder minimum temperatures. Therefore, as juveniles grow taller they may escape the severe freezing temperatures that occur near the ground. The probability of a plant establishing and reaching heights where exposure to extreme low temperatures is minimized will depend on the species-specific growth rate and the return interval of extreme freezing events (Wakeling et al. 2012). Trees grow relatively slow at tropical treelines suggesting that it may take several decades for individuals to grow to heights above the dangerous cold air layer close to the ground (Hoch and Körner 2005). Our study supports this "escape hypothesis" and demonstrates the high likelihood that, in addition to mean temperature, low temperature extremes play a significant role in determining seedling survival near the ground in the grasslands above treeline.

This study represents the first attempt to quantify freezing resistance of different life stages within tropical treeline-forming tree species. The ontogenetic patterns in leaf freezing resistance that we found indicate that juveniles of the studied species maintain freezing resistances comparable to adults. Similarly, Vitasse et al. (2014) found that juveniles and adults in temperate European forests had the same freezing resistances during similar periods of leaf developmental stage. The lack of ontogenetic differences in leaf freezing resistance during the coldest portion of the year in our study likely represents a critical need for all individuals to maintain high freezing resistance during periods of low temperature extremes regardless of life stage or position relative to the closed-canopy treeline forest (Körner 2003).

Converse to the cold season, we did find ontogenetic differences in leaf freezing resistance for three of the four species during the warm season. However, these statistically different freezing resistances may be ecologically irrelevant because freezing events likely do not reach critical thresholds during the warm season. For two species, *G. nitida* and *P. pauta*, the reduction in leaf freezing resistance of juveniles relative to adults during the warm season may represent a physiological adjustment in juveniles to maximize growth during periods when freezing temperatures pose a minimal threat to active plant tissues. For *C. cuneata*, the high leaf freezing resistance of juveniles relative to adults during the warm season is difficult to explain, but may represent

disparities in the phenological cycles within species. Actively growing tissue tends to be less freezing resistant than dormant tissue (Öquist and Huner 2003) so the patterns of leaf freezing resistance found in our study may reflect differences in the timing of growth between life stages (Vitasse 2013, Vitasse et al. 2014). A more detailed knowledge of phenological patterns (e.g., timing of growth or flowering) at tropical treelines would allow us to disentangle the intricacies of freezing resistance patterns within species.

Species exhibited ecologically meaningful differences in the ability of their leaves to withstand the microclimatic conditions above treeline, suggesting that not all of the current treeline-forming species will be able to establish in the grasslands above current treeline unless extreme low temperatures increase or become less frequent. For example, we found that low temperature extremes have the potential to directly limit the ability of juveniles of *P. pauta* to grow beyond treeline. Conversely, freezing temperatures may be insufficient in explaining the upper elevational distribution limit for *W. fagaroides*, as this species' freezing resistance was slightly colder than the observed minimum temperatures. These species-specific freezing resistances may be reflected in the variable patterns of recruitment occurring at the treeline ecotone, with *W. fagaroides* being relatively more abundant and *P. pauta* being relatively scarce (E. M. Rehm and K. J. Feeley, *unpublished data*).

Understanding which species are most likely to establish beyond the current treeline during climate change is important when making predictions about future treeline shifts. One of the main pathways for rapid upslope treeline shifts during contemporary climate change will be through the establishment of individuals or groups of seedlings at relatively large distances (>20 m) beyond the current treeline. These seedlings can then alter microclimate, creating areas conducive to additional seedling recruitment and act as nucleating foci for further forest expansion throughout the grassland. Of our four study species, only *W. fagaroides* seems capable of withstanding the relatively harsh freezing regimes of the open grasslands. However, in our study area the number of juveniles of all species, including *W. fagaroides*, decreases sharply with distance into the grassland indicating that seedling recruitment in the grassland is most likely limited to just a short distance (<10 m) from the treeline itself (Rehm and Feeley 2013). Therefore, for some species, freezing temperatures may directly inhibit seedling recruitment above treeline while other species that are better able to withstand freezing temperatures may ultimately be limited by additional factors (e.g., low temperature limitations on growth).

The freezing resistance and microclimate data presented here coupled with current recruitment patterns at our study sites suggest that treeline shifts in response to current climate change are occurring in a relatively slow and diffuse manner, possibly hindered by periodic freezing events. Indeed, documented cases of treeline shifts in and around our study sites are rare, and even when treelines are moving upslope they do so at an extremely slow pace, lagging well behind current shifts in mean temperature (Lutz et al. 2013). Similarly, slow rates of treeline shifts were documented in some non-tropical areas where recruitment above treelines may be limited by freezing events (Wardle and Coleman 1992, Wardle 2008). Slow rates of tropical treeline shifts could have major impacts on biodiversity in the species-rich cloud forest community, as many species occurring below tropical treelines have already begun to shift their distributions upslope (Freeman and Class Freeman 2014, Rehm 2014). If current treeline forming species do not also shift upslope into areas currently under grasslands, then cloud forest species will continue to lose habitat at their lower, warm range edge while failing to gain habitat at their upper, cold-range edge, leading to severe range contractions and elevated extinction risks in this biodiversity hotspot (Feeley and Silman 2010). Even when treeline shifts do occur, it is likely that treeline species will shift at discordant rates depending on species-specific factors which limit recruitment above treeline, such as freezing resistance or growth sensitivity to low mean temperatures. In this scenario, uneven shift rates in treeline species will likely result in altered species assemblages and forest composition, which may have cascading effects on the function and dynamics of cloud forest systems.

The freezing resistance of tropical treeline species in the Andes could become even more relevant in the future because the severity and duration of low temperature extremes may stay relatively constant during current climate change despite rising mean temperatures (Vavrus et al. 2006, Kodra et al. 2011). In addition, future increases in mean temperature and CO_2 concentrations can alter phenology and reduce overall freezing resistance of trees (Woldendorp et al. 2008, Martin et al. 2010, Augspurger 2013). Paradoxically, this means that tropical treeline-forming trees may become even more susceptible to extreme freezing events in the future despite mean temperature increases. We show that the upper distributional limits of several tropical treeline species already occur at elevations where freezing temperatures may directly limit survival and recruitment of juveniles in the open terrain above the current treeline. Any reductions in freezing resistance capacities caused by climate change could result in further recruitment limitations at tropical Andean treelines unless there is a concurrent increase in absolute minimum temperatures. As such, based on our findings and the relative stability of treelines in the tropics despite significant climate warming, we may need to reevaluate predictions of upward tropical treeline shifts during climate change, at least in regions where freezing temperatures regularly occur. Furthermore, additional factors beyond just temperature (e.g., water stress, edaphic conditions, and solar radiation) may also play

important roles in determining cloud forest expansion into the grasslands above treelines (Rehm and Feeley, *in press*). Yet to date, most of these other factors have received relatively little attention in tropical treeline systems, making predictions about tropical treeline shifts and the fate of tropical cloud forests even more uncertain.

LITERATURE CITED

Araújo, M., F. Ferri-Yáñez, F. Bozinovic, P. A. Marquet, F. Valladares, and S. L. Chown. 2013. Heat freezes niche evolution. Ecology Letters 16:1206–1209.

Augspurger, C. 2013. Reconstructing patterns of temperature, phenology, and frost damage over 124 years: spring damage risk is increasing. Ecology 94:41–50.

Burr, K., R. Tinus, S. Wallner, and R. King. 1990. Comparison of three cold hardiness tests for conifer seedlings. Tree Physiology 6:351–369.

Cavieres, L. A., F. Rada, A. Azócar, C. García-Núñez, and H. M. Cabrera. 2000. Gas exchange and low temperature resistance in two tropical high mountain tree species from the Venezuelan Andes. Acta Oecologica 21:203–211.

Feeley, K. J., and M. R. Silman. 2010. Land-use and climate change effects on population size and extinction risk of Andean plants. Global Change Biology 16:3215–3222.

Flint, H., B. Boyce, and D. Beattie. 1967. Index of injury—a useful expression of freezing injury to plant tissues as determined by the electrolytic method. Canadian Journal of Plant Science 47:229–230.

Freeman, B. G., and A. M. Class Freeman. 2014. Rapid upslope shifts in New Guinean birds illustrate strong distributional responses of tropical montane species to global warming. Proceedings of the National Academy of Sciences USA 111:4490–4494.

Gibbon, A., M. Silman, Y. Malhi, J. Fisher, P. Meir, M. Zimmermann, G. Dargie, W. Farfan, and K. Garcia. 2010. Ecosystem carbon storage across the grassland–forest transition in the high Andes of Manu National Park, Peru. Ecosystems 13:1097–1111.

Goldstein, G., F. Rada, and A. Azocar. 1985. Cold hardiness and supercooling along an altitudinal gradient in Andean giant rosette species. Oecologia 68:147–152.

Grace, J., F. Berninger, and L. Nagy. 2002. Impacts of climate change on the tree line. Annals of Botany 90:537–544.

Harsch, M. A., and M. Y. Bader. 2011. Treeline form—a potential key to understanding treeline dynamics. Global Ecology and Biogeography 20:582–596.

Harsch, M. A., P. E. Hulme, M. S. McGlone, and R. P. Duncan. 2009. Are treelines advancing? A global meta-analysis of treeline response to climate warming. Ecology Letters 12:1040–1049.

Hertel, D., and K. Wesche. 2008. Tropical moist *Polylepis* stands at the treeline in East Bolivia: the effect of elevation on stand microclimate, above- and below-ground structure, and regeneration. Trees 22:303–315.

Hoch, G., and C. Körner. 2005. Growth, demography and carbon relations of *Polylepis* trees at the world's highest treeline. Functional Ecology 19:941–951.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical Journal 50:346–363.

Jentsch, A., J. Kreyling, and C. Beierkuhnlein. 2007. A new generation of climate-change experiments: events, not trends. Frontiers in Ecology and the Environment 5:365–374.

Kessler, M., J. Toivonen, S. P. Sylvester, J. Kluge, and D. Hertel. 2014. Elevational patterns of *Polylepis* tree height (Rosaceae) in the high Andes of Peru: role of human impact and climatic conditions. Frontiers in Plant Science 5:194.

Kodra, E., K. Steinhaeuser, and A. R. Ganguly. 2011. Persisting cold extremes under 21st-century warming scenarios. Geophysical Research Letters 38:L08705.

Körner, C. 1998. A re-assessment of high elevation treeline positions and their explanation. Oecologia 115:445–459.

Körner, C. 2003. Alpine plant life. Second edition. Springer, Berlin, Germany.

Körner, C. 2008. Winter crop growth at low temperature may hold the answer for alpine treeline formation. Plant Ecology & Diversity 1:3–11.

Körner, C. 2012. Alpine treelines: functional ecology of the global high elevation tree limits. Springer, Basel, Switzerland.

Körner, C., and J. Paulsen. 2004. A world-wide study of high altitude treeline temperatures. Journal of Biogeography 31: 713–732.

Larcher, W., and H. Bauer. 1981. Ecological significance of resistance to low temperature. Pages 403–437 *in* O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, editors. Physiological plant ecology I. Springer, Berlin, Germany.

Lim, C., R. Arora, and E. Townsend. 1998. Comparing Gompertz and Richards functions to estimate freezing injury in *Rhododendron* using electrolyte leakage. Journal of the American Society for Horticultural Science 123:246–252.

Lutz, D. A., R. L. Powell, and M. R. Silman. 2013. Four decades of Andean timberline migration and implications for biodiversity loss with climate change. PLoS ONE 8:e74496.

Martin, M., K. Gavazov, C. Körner, S. Hättenschwiler, and C. Rixen. 2010. Reduced early growing season freezing resistance in alpine treeline plants under elevated atmospheric CO_2. Global Change Biology 16:1057–1070.

Öquist, G., and N. Huner. 2003. Photosynthesis of overwintering evergreen plants. Annual Review of Plant Biology 54: 329–355.

Paulsen, J., and C. Körner. 2014. A climate-based model to predict potential treeline position around the globe. Alpine Botany 124:1–12.

R Development Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org

Rada, F., C. García-Núñez, C. Boero, M. Gallardo, M. Hilal, J. González, F. Prado, M. Liberman-Cruz, and A. Azócar. 2001. Low-temperature resistance in *Polylepis tarapacana*, a tree growing at the highest altitudes in the world. Plant, Cell & Environment 24:377–381.

Rada, F., C. Garcia-Núñez, and S. Rangel. 2009. Low temperature resistance in saplings and ramets of *Polylepis sericea* in the Venezuelan Andes. Acta Oecologica 35:610–613.

Rada, F., G. Goldstein, A. Azocar, and F. Meinzer. 1985. Daily and seasonal osmotic changes in a tropical treeline species. Journal of Experimental Botany 36:989–1000.

Rapp, J. M., and M. R. Silman. 2012. Diurnal, seasonal, and altitudinal trends in microclimate across a tropical montane cloud forest. Climate Research 55:17–32.

Rehm, E. M. 2014. Rates of upslope shifts for tropical species depend on life history and dispersal mode. Proceedings of the National Academy of Sciences USA 111:E1676.

Rehm, E. M., and K. J. Feeley. 2013. Forest patches and the upward migration of timberline in the southern Peruvian Andes. Forest Ecology and Management 305:204–211.

Rehm, E. M., and K. J. Feeley. In press. The inability of tropical cloud forest species to invade grasslands above treeline during climate change: potential explanations and consequences. Ecography.

Sakai, A., and W. Larcher. 1987. Frost survival of plants. Page 321 in W. Billings, F. Golley, O. Lange, J. Olson, and H. Remmert, editors. Ecological studies 62. Springer-Verlag, Berlin, Germany.

Sierra-Almeida, A., and L. A. Cavieres. 2012. Summer freezing resistance of high-elevation plant species changes with ontogeny. Environmental and Experimental Botany 80:10–15.

Squeo, F. A., F. Rada, A. Azocar, and G. Goldstein. 1991. Freezing tolerance and avoidance in high tropical Andean plants: Is it equally represented in species with different plant height? Oecologia 86:378–382.

Taschler, D., and G. Neuner. 2004. Summer frost resistance and freezing patterns measured in situ in leaves of major alpine plant growth forms in relation to their upper distribution boundary. Plant, Cell & Environment 27:737–746.

Thompson, R. M., J. Beardall, J. Beringer, M. Grace, and P. Sardina. 2013. Means and extremes: building variability into community-level climate change experiments. Ecology Letters 16:799–806.

Vavrus, S., J. E. Walsh, W. L. Chapman, and D. Portis. 2006. The behavior of extreme cold air outbreaks under greenhouse warming. International Journal of Climatology 26:1133–1147.

Vitasse, Y. 2013. Ontogenic changes rather than difference in temperature cause understory trees to leaf out earlier. New Phytologist 198:149–155.

Vitasse, Y., A. Lenz, G. Hoch, and C. Körner. 2014. Earlier leaf-out rather than difference in freezing resistance puts juvenile trees at greater risk of damage than adult trees. Journal of Ecology 102:981–988.

Wakeling, J. L., M. D. Cramer, and W. J. Bond. 2012. The savanna–grassland "treeline": Why don't savanna trees occur in upland grasslands? Journal of Ecology 100:381–391.

Wardle, P. 2008. New Zealand forest to alpine transitions in global context. Arctic, Antarctic, and Alpine Research 40:240–249.

Wardle, P., and M. Coleman. 1992. Evidence for rising upper limits of four native New Zealand forest trees. New Zealand Journal of Botany 30:303–314.

Wesche, K., A. Cierjacks, Y. Assefa, S. Wagner, M. Fetene, and I. Hensen. 2008. Recruitment of trees at tropical alpine treelines: Erica in Africa versus Polylepis in South America. Plant Ecology and Diversity 1:35–46.

Woldendorp, G., M. J. Hill, R. Doran, and M. C. Ball. 2008. Frost in a future climate: modelling interactive effects of warmer temperatures and rising atmospheric $[CO_2]$ on the incidence and severity of frost damage in a temperate evergreen (Eucalyptus pauciflora). Global Change Biology 14:294–308.

Young, K. R., and B. León. 2007. Tree-line changes along the Andes: implications of spatial patterns and dynamics. Philosophical Transactions of the Royal Society B 362:263–272.

Zimmermann, N. E., N. G. Yoccoz, T. C. Edwards, E. S. Meier, W. Thuiller, A. Guisan, D. R. Schmatz, and P. B. Pearman. 2009. Climatic extremes improve predictions of spatial patterns of tree species. Proceedings of the National Academy of Sciences USA 106:19723–19728.