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**Short Communication**

# High-temperature tolerance by the endangered Mexican Mayflower orchid, *Laelia speciosa*

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**Abstract**

The Mayflower orchid, *Laelia speciosa,* is an endangered orchid endemic to oak forests of central Mexico. Because of extractive pressure on remaining natural populations, *in vitro* propagation has been proposed as an alternative for the massive propagation of this plant for conservation and commercial purposes. However, it is unknown whether this orchid will be able to tolerate the increased air temperature that is projected to occur during the present century, especially for *in vitro* propagated individuals at early developmental stages. A laboratory assay that measured electrolyte leakage, a common indicator of cell membrane integrity, was utilized to determine the high-temperature tolerance for 8-year-old individuals rescued from a wild population and for 2-year-old micropropagated individuals of the Mayflower orchid. The plants were incubated under day/night air temperatures of 25/15, 30/20, or 35/25 °C. Chlorophyll fluorescence measurements of the quantum yield of photosystem II (Fv/Fm) averaged 0.74 ± 0.01, except for the micropropagated individuals incubated under 35/25 °C, whose quantum yield of 0.64 ± 0.02 was indicative of stress. Electrolyte leakage also responded to incubation temperature. An observed increase of temperature tolerance of 0.6–1.0 °C per increased degree of incubation temperature indicates an ability to acclimate to rising air temperatures. However, the $LT_{50}$ (the temperature that causes half of the maximum electrolyte leakage to occur) dramatically decreased (by 6.7-10.9 °C) for plants kept under 35/25 °C. In this case, the *in vitro* propagated individuals were less able to resist high air temperatures. It appears that the Mayflower will be able to survive climate change, provided that *in vitro* propagated individuals are sufficiently hardened.

Keywords: Conservation physiology, ecological niche, tissue culture, assisted migration, global warming

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## Introduction

Illegal harvesting from their native habitat is a leading cause for diversity loss among tropical epiphytes [1]. This is the case for the Mayflower, *Laelia speciosa*, an endemic orchid from central Mexico that has great cultural significance in the state of Michoacán (Fig. 1) [2,3].The plant produces attractive flowers during the spring (hence its common name of *flor de mayo*; translation: Mayflower) that are illegally harvested and sold in streets and markets of the state capital. In addition, a mucilaginous juice is extracted from its pseudobulbs (the water- and nutrient-storing organs that many epiphytic orchids produce during their development) and mixed into a paste with the pith of dry maize stalks to craft religious art that is displayed in numerous Roman Catholic temples throughout Mexico. Because extensive harvesting has reduced its distribution range by 11% over the past two decades, the Mayflower has been classified as endangered by the Mexican environmental authority [4,5].

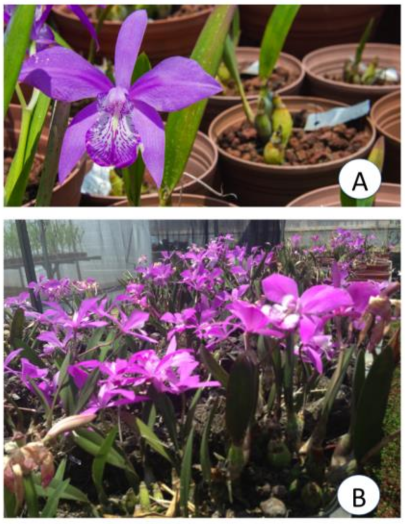


**Fig. 1. The Mayflower orchid (*Laelia speciosa*) in bloom. A) *In vitro* propagated individuals and B) rescued individuals are currently kept in a shadehouse at the Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México. Photograph in panel A kindly provided by Ms. Leonor Solís.**

The conservation of endangered plant species can be aided by *in vitro* propagation, a technique that enables the massive production of plants for reintroduction or commercial purposes, thus relieving pressure on natural populations [6–8]. Species-specific protocols for *in vitro* propagation have been developed for several epiphytic orchids, including the Mayflower [6,7,9]. Numerous individuals resulting from *in vitro* propagation can be contained in relatively small spaces, at relatively low costs, and plant development can be arrested until the regeneration of fully formed plants is desired. However, as with most conservation practices, restricted genetic diversity ensues from use of a limited number of genotypes, although it can be artificially enhanced via artificial mutagenesis, as has been done for *Agave victoria-reginae*; once thought extinct, this species was rescued from a single individual identified in a home garden in west-central Mexico [8,10].

The eventual release of *in vitro* propagated plants requires a relatively long process of acclimation to natural or semi-natural environmental conditions in order to improve the chances of plant survival and establishment. This is especially true for epiphytes, because their environment is highly fluctuating, in stark contrast with the stable conditions prevailing in tissue culture rooms [11,12]. How well young propagated plants might withstand the natural environment needs to be determined so that their release can occur at the youngest age that yields adequate survival rates.

Global climate change poses an additional threat to the persistence of *L. speciosa* and other epiphytic vascular plants. On the one hand, the oak forests of central Mexico, to which *L. speciosa* is restricted, are among the most endangered ecosystems in the country under various climate change scenarios [3,13–16]. On the other hand, epiphytes such as the Mayflower are especially exposed to changing environmental factors, such as insolation, changes in temperature, severe and frequent droughts, and even atmospheric pollution [11,12,17–19]. Higher exposure leads to increased risk unless a species is tolerant of stress, but studies of environmental stress tolerance are scant for non-timber species from oak forests. Moreover, besides broad biogeographical and morpho-physiological patterns, high-temperature tolerance is unknown for orchids in general [18,20].

Air temperature influences all biological processes, from controlling the rates of enzymatic activity, to determining plant phenology over the course of a year, to limiting the distribution of a species [21]. For plants, high air temperature, which is often coupled with high insolation, can range from temporary inhibition of photosynthesis to permanent physiological damage [22,23]. These processes can be monitored by means of chlorophyll fluorescence [24,25]. High quantum yields can be indicative of growth for orchids [26]. In contrast, the quantum yield (Fv/Fm) shrinks by half for epiphytic orchids from the Yucatán Peninsula during the dry season, when air temperatures are the highest [18].

While mean air temperatures are a most useful predictor of plant performance in the field, extreme air temperature events (very high or very low), however rare, can actually kill an entire population [21,27], and plant responses to extreme events can be delayed up to several years [28,29]. Therefore, a useful laboratory method for quickly determining extreme temperature tolerance by plants has been developed that measures cell viability following the exposure of plant tissue to increasingly extreme high or low temperatures [21,27]. By expressing cell viability—scored as electrolyte leakage or as cells taking up a vital stain—as the proportion of cells that remain viable after exposure to extreme temperatures, a parameter can be found, $LT_{50}$, which is a good predictor of tolerance to extreme temperature. The high-temperature $LT_{50}$ can reach values of up to 70 °C for *Cylindropuntia acanthocarpa*, 64 °C for *Agave americana*, 55 °C for the hemiepiphytic cactus *Hylocereus undatus*, and 43 °C for the understory legume *Lupinus elegans* [27,30,31]. When plants are incubated at different mean air temperatures, differences in the $LT_{50}$ indicate a plant's ability to acclimate to changing environments. In particular, the $LT_{50}$ for *C. acanthocarpa* increases by 0.6 °C for each degree that the incubation temperature is raised; acclimation is 0.3, 0.1, and 0 °C per degree that the incubation temperature is raised for *A. americana*, *H. undatus*, and *L. elegans*, respectively [31–33]. Alas, both the $LT_{50}$ and the acclimation capacity of orchids are unknown.

Because it is uncertain whether the Mayflower will be able to survive the substantially warmer environment projected to occur during the present century or increasingly frequent high air temperature episodes, we tested its high-temperature tolerance and acclimation for two-year-old *in vitro* propagated plants and adult individuals of natural origin, to evaluate the utility of *in vitro* propagation for conservation of this endangered orchid.

## Methods

High-temperature tolerance was determined for *in vitro* propagated individuals of Mayflower (*Laelia speciosa* (Kunth) Schltr.) and compared with that of mature individuals that were originally obtained from a wild population. In particular, one-year-old *in vitro* propagated individuals of *L. speciosa* were acclimated to greenhouse conditions (mean air temperature of 25 °C, ranging from 15 to 38 °C) at the Instituto de Investigaciones en Ecosistemas y Sustentabilidad (19°38'55.9"N; 101°13'45"W; 1967m), Universidad Nacional Autónoma de México, for an additional year prior to the start of the experiment [9,34]. The mature wild-grown individuals had been rescued in 2004 from a construction site about 7 km from campus. These plants were kept on their original substrate, *i.e.,*, branches of *Quercus deserticola*, and placed on a mesh bench inside a shadehouse (mean air temperature of 23 °C, ranging from 15 to 35 °C), allowing the plants to remain under semi-natural conditions. The individuals used in this experiment had an average of eight pseudobulbs, and were presumably eight years old, as this species produces one new pseudobulb every year [3].

On 23-26 April 2008, 10 individuals from each age group were placed inside growth chambers (Percival Scientific, Boone, Iowa, USA) according to a randomized block design, where they were exposed to a 12-h photoperiod and allowed to acclimate to three day/night air temperature regimes: 25/15, 30/20, and 35/25 °C, for 30 days under 50% relative humidity. The plants' photosynthetic performance under the experimental temperature regimes was assessed with measurements of chlorophyll fluorescence (quantum yield, $F_v/F_{max}$) conducted with a FluorPen Handheld Fluorometer (Qubit Systems, Kingston, Ontario, Canada).

Plant tolerance to high temperatures was also determined by electrolyte leakage, an indicator of cell viability that increases as the membrane degrades in response to stress [27,31,35]. In particular, several leaf discs (6 mm in diameter) were obtained with a cork borer from each plant and placed in 1.5 ml microcentrifuge tubes that contained cotton damped with distilled water to prevent tissue desiccation. The tubes were placed in a Tropicooler benchtop cooler/heater (Boekel Scientific, Feasterville, Pensilvania, USA) for exposure to high temperatures. A given high temperature was maintained during one hour, following which a leaf disc was removed from the tube, placed in a glass vial containing 15 ml of deionized distilled water and placed for 40 min in an orbital shaker set at 200 rpm. At the same time, a second disc was removed from the vial and boiled for 5 min to fully disrupt cell membranes and obtain the maximum electrolyte leakage, before being placed in the orbital shaker as described above. Electrical conductivity of the incubation solutions was measured following agitation with an Orion 3 Star conductivimeter (Thermo Electron Corporation, Waltham, Massachusetts, USA), and electrolyte leakage was expressed as percentage of the maximum. For the remaining leaf discs, the incubation temperature was progressively raised in 5 °C increments (rate of 0.5 °C $min^{-1}$) for additional successive 1-h incubation periods. This process was repeated until the electrolyte leakage reached 100% of the maximum. The temperature at which half of the maximum electrolyte leakage occurred (lethal temperature-50, $LT_{50}$) was identified for both the propagated and the rescued individuals as an indicator of temperature tolerance in the field [27].

Chlorophyll fluorescence and $LT_{50}$ were analyzed with a two-way ANOVA (factors were plant age × air temperature) followed by pairwise Tukey Tests ($p < 0.05$) [36]. Statistical analyses were performed with SigmaStat 3.5 (SYSTAT Software, Point Richmond, CA, USA). Data are shown as mean ± s.e. (n = sample size).

## Results

The experimental temperature regimes had an effect on both chlorophyll fluorescence and high-temperature tolerance of the Mayflower orchid, which depended on plant age (Table 1). In particular, the photosynthetic quantum yield (Fv/Fm) tended to be higher for the mature plants than for the propagated individuals, especially under the warmest treatment. Indeed, Fv/Fm ranged from 0.64 ± 0.02 for the propagated plants under 35/25 °C, to 0.77 ± 0.01 for the mature plants growing under 25/15 °C. The $LT_{50}$ also changed with incubation temperature for both the propagated and the mature individuals (Table 1). In this case, some acclimation occurred as the $LT_{50}$ was 47.67 ± 0.40 °C for plants incubated under 20/10 °C, increasing by 3.34 °C for plants incubated under 25/15 °C, *i.e.,* 0.67 °C for each degree that the incubation temperature was raised. The highest experimental incubation temperature led to a dramatic decrease of the $LT_{50}$ regardless of plant age.

**Table 1.** Acclimation parameters for micropropagated one-year-old and rescued three-year-old individuals of *Laelia speciosa* kept at the indicated day/night temperature regime during 4 weeks. $LT_{50}$ refers to the temperature at which half of the cell membrane viability, expressed as percent of maximum, is lost. Acclimation refers to the displacement of the $LT_{50}$ relative to that for plants kept at 25/15 °C. Data are shown as mean ± 1 S.E. (n = 10). For a given parameter different letters indicate a statistical difference with ($p < 0.05$).

| Temperature (day/night °C) | Chlorophyll fluorescence (Fv/Fm) | | $LT_{50}$ (°C) | |
|---|---|---|---|---|
| | Young | Mature | Young | Mature |
| 25/15 | 0.74 ± 0.01 *a,b* | 0.77 ± 0.01 *b* | 47.55 ± 0.73 *a* | 47.78 ± 0.06 *a* |
| 30/20 | 0.72 ± 0.01 *a* | 0.74 ± 0.01 *a,b* | 50.87 ± 0.26 *b* | 51.17 ± 0.16 *b* |
| 35/25 | 0.64 ± 0.02 *c* | 0.72 ± 0.01 *a,b* | 39.99 ± 1.32 *c* | 44.48 ± 0.66 *d* |

## Discussion

High-temperature tolerance tended to be greater for the mature Mayflower individuals than for the two-year old plants from *in vitro* propagation. This agrees with a frequently found pattern among various life-forms, in which increasing plant age leads to greater tolerance of environmental stress [37]. For instance, cuttings of the hemiepiphytic cactus *Hylocereus undatus* are not able to withstand air temperatures above 45 °C, but adult individuals are often exposed to this temperature in commercial plantations from the Yucatán Penninsula, Mexico [30,38,39]. Also, the high temperature $LT_{50}$ for young stems of *Opuntia ficus-indica* is 6.5 °C lower than for those that are 10 years old [40].

The quantum yield (Fv/Fm) was relatively stable for the Mayflower, except for the *in-vitro* propagated individuals incubated under the highest temperature treatment, which had a substantial reduction in variable fluorescence. Similarly, the quantum yield is reduced for epiphytic orchids from the Yucatán Peninsula during the time of year when the air temperatures are highest [18]. In contrast, high quantum yields can be indicative of growth for some orchids [26]. If this was the case for the Mayflower orchid, even the highest temperature treatment of 35/25 °C could lead to some growth for the mature individuals considered in the present study, but not for the two-year old plants from *in vitro* propagation. For the latter, the quantum yield was consistent with that of plants subjected to environmental stress [25].

While plant performance is greatly influenced by mean air temperature, extreme events, even if rare, can actually kill individuals that should otherwise be able to survive or even thrive at a given location [27,32]. It is likely that an increase in the frequency and the magnitude of high-temperature events will develop during the current century, including within the distribution range for the Mayflower [14,16,41]. However, the $LT_{50}$ determined here for the Mayflower was the lowest among 31 succulent plant species of different growth forms; the closest species is the hemiepiphytic cactus *Hylocereus undatus*, whose $LT_{50}$ is still 5-16 °C higher than the one determined here for the orchid [30,33]. However, the $LT_{50}$ for the Mayflower orchid as higher than that of the sympatric understory shrub *Lupinus elegans* ($LT_{50}$ = 43°C) [31].

Incubation temperatures, including the prevalent mean air temperature at a given site, can influence plant extreme temperature tolerance. Indeed, for 31 succulents, the $LT_{50}$ increases by 0.42 ± 0.04 °C for each degree that the incubation temperature increases [27]. For the Mayflower orchid, the $LT_{50}$ increased by 0.6-1.0 °C for each degree that the incubation temperature increased, up to 30/20 °C, indicating a higher acclimation capacity for this orchid than for some of the other succulent species [30]. However, the fact that a further increase of the incubation temperature led to a substantially lower $LT_{50}$ suggests that the Mayflower requires a fairly stable air temperature to remain physiologically active. The ability to acclimate to changing environmental conditions will be crucial for the survival of species that will be exposed to the accelerated temperature increase projected to occur during the present century.

## Implications for conservation

*In vitro* propagation can be a useful tool for the conservation of plant species [8,10]. Consequently, specific propagation protocols have been developed for numerous species of orchids, including for the Mayflower orchid [6,42–44]. At least for the orchid considered in our study, high-temperature tolerance of *in vitro* propagated individuals is fairly similar to that of mature individuals, except under the highest experimental treatment, as discussed above. Either an acclimation protocol needs to be developed that induces temperature hardening for *in vitro* propagated individuals, or the actual age when acclimation becomes possible needs to be determined if a reintroduction program is to be implemented. However, because the lethal temperatures determined here are still rare in its area of distribution, including the city of Morelia, where most of the illegal trade in wild Mayflowers occurs, marketing of *in vitro* propagated individuals able to tolerate current environmental conditions could relieve extractive pressure on wild populations.

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