Photosynthetic gas exchange and temperature-induced damage in seedlings of the tropical alpine species *Argyroxiphium sandwicense*

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Abstract The capacity of *Argyroxiphium sandwicense* (silversword) seedlings to acclimate photosynthetic processes to different growing temperatures, as well as the tolerance of *A. sandwicense* to temperatures ranging from \(-15\) to \(60\, ^\circ\text{C}\), were analyzed in a combination of field and laboratory studies. Altitudinal changes in temperature were also analyzed in order to explain the observed spatial distribution of *A. sandwicense*. *A. sandwicense* (Asteraceae) is a giant rosette plant that grows at high elevation on two Hawaiian volcanoes, where nocturnal subzero temperatures frequently occur. In addition, the soil temperatures at midday in the open alpine vegetation can exceed \(60\, ^\circ\text{C}\). In marked contrast to this large diurnal temperature variation, the seasonal variation in temperature is very small due to the tropical maritime location of the Hawaiian archipelago. Diurnal changes of soil and air temperature as well as photosynthetic photon flux density were measured on Haleakala volcano during four months. Seedlings were grown in the laboratory, from seeds collected in ten different *A. sandwicense* populations on Haleakala volcano, and maintained in growth chambers at \(15/5\), \(25/15\), and \(30/25\, ^\circ\text{C}\) day/night temperatures. Irreversible tissue damage was determined by measuring electrolyte leakage of leaf samples. For seedlings maintained at each of the three different day/night temperatures, tissue damage occurred at \(-10\, ^\circ\text{C}\) due to freezing and at about \(50\, ^\circ\text{C}\) due to high temperatures. Tissue damage occurred immediately after ice nucleation suggesting that *A. sandwicense* seedlings tend to avoid ice formation by permanent supercooling. Seedlings maintained at different day/night temperatures had similar maximum photosynthetic rates (\(5\, \mu\text{mol m}^{-2}\text{s}^{-1}\)) and similar optimum temperatures for photosynthesis (about \(16\, ^\circ\text{C}\)). Leaf dark respiration rates compared at identical temperatures, however, were substantially higher for seedlings maintained at low temperatures, but almost perfect homeostasis is observed when compared at their respective growing conditions. The lack of acclimation in terms of frost resistance and tolerance to high temperatures, as well as in terms of the optimum temperature for photosynthesis, may contribute to the restricted altitudinal range of *A. sandwicense*. The small seasonal temperature variations in the tropical environment where this species grows may have prevented the development of mechanisms for acclimation to long-term temperature changes.

Key words *Argyroxiphium sandwicense* • Photosynthesis • Seedlings • Supercooling • Temperature acclimation

Introduction

A notable feature of tropical alpine vegetation in many parts of the world is the presence of giant rosette plants with monocarpic life history traits. These long-lived perennials flower only once in their lives producing a giant inflorescence with a large number of fruits before the parent plant senesces. Flowering only occurs during certain years and more or less synchronously in plant that have reached a relatively large size. *Argyroxiphium sandwicense* (Asteraceae) is a well-known example of the evolution of a monocarpic giant rosette plant in a tropical alpine environment. *Argyroxiphium* is one of three genera of Hawaiian tarweeds that have evolved from a single North American ancestor (Carr 1985; Baldwin et al. 1991). The genus consists of six species, all of which share some of the same features. Most are giant rosettes growing at relatively high elevations on bogs, open wet forests, or cinder deserts. *A. sandwicense* is one of the
few Hawaiian plants, and the only member of its genus, that can grow between 2000 and 3000 m above sea level (a.s.l.) where subzero nighttime temperatures are common (Lipp et al. 1994). *A. sandwicense* is an erect, single-stemmed rosette plant, approximately 0.5–1.0 m in diameter, 0.5–1.0 m tall when vegetative, and with the flowering stems up to nearly 2 m tall.

In most plant populations with overlapping generations, mortality rates tend to vary with age and size of individuals. Theoretical survivorship patterns established long ago by Pearl and Miner (1935) indicate that mortality can be highest among juveniles, highest among mature adults, or constant throughout the lifespan. Other more complex functions have also been used to describe mortality with age in long-lived tree species (e.g., Hett and Loucks 1976). Death rates in polycarpic giant rosette species of the genus *Espeletia* are greatest during the first few years and at old ages (Goldstein et al. 1985). The high risk of mortality in young plants of this species is a consequence of their small volume of internal water storage tissues, and the lack of ability to osmotically adjust to periods of low soil water availability. Mortality rates of *A. sandwicense* are also higher during the seedling stage than at juvenile or adult stages (Loope and Crivellone 1986; Rundel and Witter 1994). The end of the life cycle of this species is triggered by the development of a large inflorescence followed by the death of the plant probably due to massive nutrient, carbohydrate and water transport from the vegetative to the reproductive part of the plant. Most seeds of *A. sandwicense* remain viable in the seed bank for less than 1 year (Powell 1992) and therefore establishment of new seedlings during a given year fluctuates according to the number of plants that enter the reproductive stage that year.

Tropical alpine environments are characterized by high-amplitude diurnal, rather than seasonal, temperature fluctuations, and by frequent freezing nighttime temperatures (Goldstein and Meinder 1983; Goldstein et al. 1984; Robichaux et al. 1990). Because of the lack of continuous vegetation cover and the resulting high radiant energy input during the day and large longwave radiant energy loss at night (Korner and Larcher 1988), the environment close to the soil surface is subjected to the most extreme temperature fluctuation. Soil temperatures at Haleakala volcano, where most *A. sandwicense* populations grow, can drop below −5 °C during the night and reach 60 °C at midday on clear days, particularly on dark cinder deposits. The seedling stage is a critical stage in the life cycle of any plant, but particularly for species such as *A. sandwicense* that are close to the barren soil where temperature conditions are extreme. Here we investigated the effect of temperature on photosynthetic gas exchange of *A. sandwicense* seedlings. In addition, tolerance to extreme high and low temperatures as well as ice nucleation temperatures of seedlings maintained at different growing temperature conditions were determined. This information may help to identify thermal requirements and potential altitudinal ranges of *A. sandwicense* on mountain slopes of the Hawaiian archipela-go. One of the main goals of the laboratory experiments was to determine whether or not the species has the potential for temperature acclimation.

### Materials and methods

**Plant material and field measurements**

*Argyroserpium sandwicense* ssp. *macrocephalum* seeds (Haleakala silversword) were collected from 15 plants from 10 different populations in Haleakala National Park on the island of Maui (20°45′N, 156°12′W). The seeds were germinated in petri dishes, then immediately transplanted into pots containing a mixture of three parts fine basalt ash to one part peat moss. Seedlings were grown for 5 months in a growth cabinet at 20 °C and 340 μmol m⁻² s⁻¹ white fluorescent lights, and regularly watered and fertilized with NPK fertilizer plus micronutrients. Five month-old seedlings (leaf length was 5–7 cm) were transferred to growth chambers and subjected to 15/5, 25/15, and 30/25 °C day/night temperatures with a photoperiod of 12 h for 40 days. Irradiance was kept at around 600 μmol m⁻² s⁻¹. Growing conditions of the seedlings were kept as close as possible to the growing conditions in the field. The substrate and the nutritional status of the laboratory grown plants were comparable to the field grown plants. Light levels in the growth chambers were lower than field irradiance at midday during clear days but kept at or above light saturation for photosynthesis for the species (unpublished results).

A network of five microclimatological stations, known as HaleNet, was installed in June 1988 (at elevations of 950, 1650, and 2130 m) and in April 1990 (at elevations of 2600 and 3000 m) on the western slope of Haleakala volcano (Giambellucci and Nullet 1991). At each station, several climatological variables are monitored, including air temperature (measured with the thermistor of the temperature-humidity sensor model HMD30UYB, Vaisala, Helsinki, Finland) reported herein. The temperature sensors are connected to dataloggers LI-1000 (LiCor, Lincoln, Nebraska) which measure every 5 s and record mean values hourly.

Field air, leaf and soil temperatures on Haleakala were monitored intensively during May to July 1992 at 2740 m elevation and during March 1995 at 2940 m elevation. Temperatures were measured with copper-constantan thermocouples (36 gauge) which were shielded from direct solar radiation, and solar radiation with Eppley pyranometers (model 8-48 or PSP, Eppley Laboratory, Newport, R.I.). Data were sampled every 5 s and average values were recorded at 15-min intervals with CR-10 and 21X dataloggers (Campbell Scientific, Logan, Utah).

### Freezing and heat injury

Irreversible tissue damage was determined for *A. sandwicense* seedlings by subjecting leaf samples to different temperatures ranging from −15 °C to 60 °C. Damage was determined by measuring electrolyte leakage of leaf samples exposed to the different temperatures (Flint et al. 1967). Eight plants were randomly selected from each acclimation temperature. Four or five recently expanded leaves were removed from each plant and cut into 1-cm segments. The 35–50 segments from each plant were pooled, mixed, and divided among 14 scintillation vials, and weighed. The vials were tightly sealed to avoid tissue dehydration. One vial from each plant was then subjected to one of 14 different temperature treatments ranging from −70 °C to 100 °C (8 plants x 3 acclimation temperatures x 14 temperature treatments=336 vials).

Twelve of the vials were immersed in a refrigerated water bath inside plastic bags and subjected to 20, 40, 45, 50, 55 and 60 °C in the warm treatment or 0, −5, −7.5, −10, −12.5 and −15 °C in the cold treatment. The temperature of each water bath started at 20 °C and was changed at a rate of 7 °C h⁻¹ and held constant for 30 min at each desired temperature, after which one bag of samples was removed. After removal, vials were warmed or cooled slowly