Plant Ecophysiology
https://www.sciltp.com/journals/PlantEcophys

Review

Supplementary Information: Cold Air Pools (CAPs) as Natural Freezers for the Study of Plant Responses to Low Temperatures

Enara Alday [1,*], Usue Pérez-López [1], Beatriz Fernández-Marín [1,2], Jaime Puértolas [2], Águeda M. González-Rodríguez [2], José Luis Martin Esquivel [3] and José Ignacio García-Plazaola [1]

[1] Department of Plant Biology and Ecology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa 48940, Spain
[2] Department of Botany, Ecology and Plant Physiology, Facultad de Farmacia, University of La Laguna, La Laguna 38200, Spain
[3] Teide National Park, La Orotava, Tenerife, Islas Canarias 38300, Spain
* Correspondence: enara.alday@ehu.eus

How To Cite: Alday E, Pérez-López U, Fernández-Marín B, Puértolas J, González-Rodríguez AM, Esquivel JLM, & García-Plazaola JI. (2025). Cold air pools (CAPs) as natural freezers for the study of plant responses to low temperatures. *Plant Ecophysiology, 1*(1), 6. https://doi.org/10.53941/plantecophys.2025.100006.

Materials and methods (Section 8, Figure 7)

Study site and plant material. *Descurainia bourgeauana* was sampled at Cañada del Portillo, located in the large CAP of Seven Cañadas (28°17′35″ N, 16°33′45″ W). Plant material from inside or outside the CAP, was collected at noon, during the growing season (May 2024). Healthy, adult leaves were collected and acclimated for 24h in saturated atmosphere. This plant material was used for measuring freezing tolerance and osmotic potential.

Photosynthesis and fluorescence measurements. Gas exchange analysis was performed with an infrared gas analyzer with a Multiphase FlashTM Fluorometer (LI-6800F, LI-COR Inc., Lincoln, NB, USA). Four replicates per species and per area (inside and outside CAP) were measured in situ ±2 h around noon. Light-saturated net assimilation (An), stomatal conductance to CO_2 (g_{sc}), substomatal CO_2 concentration (Ci) and photochemical yield of photosystem II (ΦPSII) at chamber CO_2 (420 µmol mol^{-1}), saturating light (1500 µmol m^{-2} s^{-1}), ambient humidity (50–70%) and 25 °C (block temperature), were recorded as instantaneous photosynthesis measurements each day between 10:00 and 16:00 h, after reaching steady-state conditions (15–30 min). The maximum photochemical efficiency of PSII in samples acclimated in the dark (30′) (Fv/Fm) was assessed with a photosynthesis yield analyser Mini-PAM (Walz, Effeltrich, Germany) which also enabled to measure ΦPSII of illuminated leaves.

Water and osmotic potential Midday xylem water potential was measured in four individuals per sampling site by suppressing leaf transpiration in twigs (Begg and Turner, 1970). Between 11:30 and 12:00, twigs were wrapped in aluminium foil, 2–3 h before detaching them from the plant with a razor blade. They were unwrapped, immediately covered in vaseline, rewrapped and introduced in a sealed plastic bag with a damp piece of tissue, which was stored in a cooled box for transport to the laboratory. This procedure ensures that water loss in the sample is minimal for at least several hours (Perera-Castro et al., 2024). Once on the laboratory twig water potential was measured with a Scholander-type pressure chamber (Model 1505D, PMS Instrument Company, OR, USA)

Leaf osmotic potential (Ψo) was measured by analyzing the freezing point of sap of leaf segments using an OSMOMAT 030 cryoscopic osmometer (Gonotec GMBH, Berlin, Germany) and calculated as $\Psi o = M \times T \times 0.00832$, where M denote the concentration (osmol) and T the temperature of the sample (298 °K).

Freezing tolerance. The evaluation of freezing tolerance was performed following Arzac et al. (2024). Fresh sample discs (n = 4 per site) were introduced in thermoelectric device (International Patent WO2024028532). Before freezing (t_0) and after recovery (t_f), the Fv/Fm was monitored with the fluorometer Junior-PAM (Walz, Germany) to test for freezing induced variations in PSII photochemical efficiency.