

## Final Report

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**Guest University:** Royal Gardens of Kew – Wakehurst (West Sussex, Ardingly, UK)

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**Title of the Research Project:** The influence of molecular mobility on the effect of oxygen during seed ageing

**Report about visit and future plans** (max. 200 words, English):

The applicant received training in biophysical techniques, including differential scanning calorimetry (DSC) and dynamic thermal analysis (DMA), to detect phase transitions in seeds of *Pinus densiflora*. Supervision was constantly provided by Dr. Charlotte Seal and Dr. Daniel Ballesteros, who have long-standing expertise in the field of seed structural biology. The aim of the visit was to determine the glass-transition temperatures ( $T_g$ s) of seeds equilibrated at different values of relative humidity (RH). This information is essential to select RH conditions associated to different values of seed molecular mobility and will be established in experiments of seed controlled deterioration (CD) under normoxia and hypoxia. The overall aim of the project is to elucidate the influence of oxygen on seed metabolism and longevity, by diagnosing precocious redox and metabolic events occurring during CD. Embryonic axes were dissected from *P. densiflora* seeds equilibrated at different RHs (i.e., 30, 40, 60, 80, and 100%), and DSC analyses conducted on individual endosperms (including seed coats;  $n = 3$ ) and pools of 10 embryonic axes. The identification of putative  $T_g$ s was based on melting scans, which showed only subtle changes at different RHs. This is due to the high seed oil content, which was quantified by nuclear magnetic resonance and was about 29.7%, on a fresh weight basis. However, some conserved patterns across replicates and treatments were revealed: sharp melting peaks at  $-0.87 \pm 0.4$  °C in the endosperms of seeds at 100% RH were indicative of water freezing. Furthermore, in all samples at tested RHs, two peaks related to lipid melting were always evident at  $-96.1 \pm 1.0$  °C and  $-40.0 \pm 1.1$  °C, in both endosperms and embryonic axes. To gain further insights into

changes in molecular mobility, whole seeds at 30% and 60% RH were also compared by DMA. Output scans supported the presence of the two lipid melting peaks detected also by DSC, and showed  $\alpha$ -relaxation peaks, indicative of  $T_g$ s, shifted from about 40 to 15 °C in seeds equilibrated at 60% RH, compared to those at 30% RH. These data confirmed that the transition from glassy to rubbery state occurred at lower temperatures in seeds at high moisture content. In summary, subtle  $T_g$ s could be extrapolated from DSC melting scans of endosperms. Nonetheless, the real  $T_g$ s could be masked by high background lipid signal and require further investigations. **Future plans:** it is intended to analyse further DSC scans ( $n = 2$ ) of the endosperms equilibrated at 30, 40, and 60% RH with a DSC programme, allowing lipid crystals to anneal and potentially stabilise, thus enabling a clearer detection of the  $T_g$ s. Additionally, endosperms equilibrated at around 11% RH for two weeks will be analysed by DSC. Finally, further pools of embryonic axes ( $n = 2$ ) were excised from seeds equilibrated at 30, 40, and 60% RH and will be compared by DSC. Finally, it was agreed to consolidate the DSC scans to elucidate the lipid crystallisation and melting events in seed embryos and endosperm, and use DMA to detect  $T_g$ s under the conditions used for CD. All information will be combined in a phase diagram, summarising the biophysical status of seed tissues.

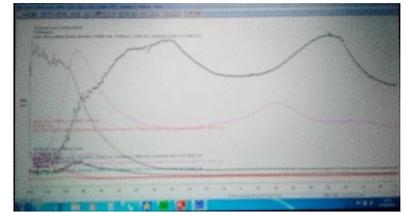
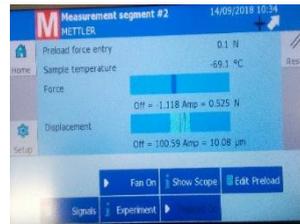
**Picture Credits:** Royal Botanic Gardens of Kew, Wakehurst Place (Davide Gerna)



The Millenium Seed Bank of the Royal Botanic Gardens of Kew - Wakehurst



Differential scanning calorimetry (DSC) analyses of *Pinus densiflora* seeds. From left to right: sample preparation, measurement, and data outputs as thermal scans.



Dynamic mechanical analysis (DMA) of *Pinus densiflora* seeds. From left to right: sample preparation, measurement, and data output.



Snapshots of the Royal Botanic Garden of Kew – Wakehurst.