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# NON-EQUILIBRIUM COOLING OF *Poncirus trifoliata* (L.) EMBRYONIC AXES AT VARIOUS WATER CONTENTS

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**Abstract**

The present study investigated the rate of temperature change within axes of *Poncirus trifoliata* during cooling and warming by various methods. Cooling rates ranged between 0.17 and 1700°C $s^{-1}$, and warming rates of 1.25 and 600°C $s^{-1}$ were measured when axes were warmed at room temperature or in water at 40°C, respectively. Partial drying increased the cooling rate within axes in direct contact with the cryogen, but did not affect the cooling or warming rates within axes enclosed in a double layer of lightweight aluminium foil. The procedures described illustrate the orders of magnitude that separate extremes of the range of cooling or warming rates attained using methods commonly employed in cryopreservation studies. Quantifying these rates allows the relationship between cooling rate, water content and survival of hydrated embryonic axes to be explored.

**Keywords:** embryonic axes, rapid cooling, dehydration, *Poncirus trifoliata*, warming, recalcitrance, cryopreservation

## INTRODUCTION

The rate of cooling to cryogenic temperatures influences the location, size and number of ice crystals formed within biological cells and tissues. Previous studies (e.g. 23, 16, 6, 18, 25) have shown that cooling rates ≥10°C $min^{-1}$ exceed the limit required for equilibrium freezing in plant and yeast cells, resulting in supercooling and subsequent freezing within the cell interior. Increasing the rate of cooling above 100°C $s^{-1}$ can limit the amount of ice formed in cells and solutions at physiological concentrations (12, 13, 14). Even faster cooling is exploited in the preparation of native biological tissues for cryo-electron microscopy (8, 22) using procedures that must meet stringent criteria in order to preclude freezing artefacts. A salient criterion amongst these is that hydrated biological specimens should not exceed 0.1 mm linear dimensions (2) in order to surpass the benchmark rate of $10^4$ °C $s^{-1}$ (e.g. 17). However, given the relatively large size of most cryobiological samples, it seems that complete avoidance of intracellular freezing in hydrated tissues during non-equilibrium cooling is unattainable (9, 11, 21) and that rapid cooling may, at best, only limit the amount of ice formed.

Since water stores the largest reserve of heat within cells (1) and contributes to the overall mass of the specimen, drying pre-treatments reduce the heat to be dissipated during cooling. Hydrated embryonic axes of recalcitrant seeds tolerate moderate degrees of drying, and the corresponding reduction in both heat capacity and mass facilitates faster cooling under forced convection (33). While it is difficult to calculate accurately the amount of heat to be dissipated