Quantifying Reactive Oxygen Species (ROS) in Austrian pine (*Pinus nigra*) Shoot Tissue by Using Fluorimetric Dye – Amplex Red

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Reactive oxygen species (ROS) are a byproduct of fundamental metabolic processes in plants. Biotic and abiotic stresses elevate production of ROS, which in turn participate in defense signaling. Among other ROS in plants, hydrogen peroxide (H_2O_2) has the longest half-life and is critical for downstream defense signaling. H_2O_2 is produced in plant tissues by enzymatic reduction and/or dismutation of superoxide. Despite the importance of ROS, methods for their absolute quantification in plants are not available. Here we describe the development of a fluorimetric technique using the dye Amplex Red to quantify H_2O_2 and other ROS. In this experiment, 20 four-year old Austrian Pine (*Pinus nigra*) trees were used. The trees were placed in a greenhouse at 27 ± 8 °C and ten of the trees received abiotic stress for five weeks as drought. After the five weeks, three shoots were taken from each of the trees and their needles were removed. These samples were immediately flash frozen in liquid N_2 and ground to a fine powder. The samples were then weighed and extracted in phosphate buffer (pH 7.4) for further analysis. Amplex red was prepared in DMSO and diluted to a 5 mL phosphate buffer stock solution containing horseradish peroxidase. Calibration curves were prepared with different concentrations of H_2O_2 standards. As expected we observed higher levels of ROS in the shoots of droughted Austrian pine trees compared to the non-droughted trees, suggesting that this technique is a viable tool to quantify ROS in plant tissue.