The precipitation of black oxide coatings on flooded conifer roots of low internal porosity.

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Summary The blackening of flooded roots of several conifer species was due to precipitation of mixed iron/manganese oxides on the root epidermis and throughout the cortex. These roots were not flood-adapted, and did not have obvious air-filled pore space for oxygen diffusion. Despite this, the amount of oxide precipitate, as measured for flooded roots of two species, red pine and black spruce, was 2240 and 2492 µg/g (dw) Fe and 684 and 1075 µg/g Mn. This compares favorably with oxide precipitation on the very porous roots of wetland species. Oxide precipitation was limited to the flooded roots and did not extend into the surrounding soil. As measured by the concentration of reduced Mn in the xylem sap, the cortical 'oxide sheath' did not prevent uptake of reduced ions into the stele.

Introduction
The roots of wetland species are typically very porous. This internal porosity is essential to support respiration in growing flooded roots. It has also been proposed that internal gas diffusion is essential for the oxidation of reduced solutes such as the ferrous and manganous ions and organic acids which can accumulate under flooded conditions, and which are potentially phytotoxic.

Reddish-brown coatings, comprised primarily of iron oxides, have been frequently described on the flooded roots of wetland species. These oxides precipitate on the root epidermis and the outer root cortex, and sometimes extend into the surrounding rhizosphere soil. This oxidized sheath can interfere with normal plant nutrition by limiting uptake of such nutrients as P, K, Ca and Mg. Besides this detrimental effect, it has been proposed that the oxide coatings have the beneficial effect of interfering with the uptake of reduced toxic solutes.

While investigating the response of conifer species to flooding, the formation of a black precipitate on flooded roots was observed.
This blackening occurred consistently in experiments with many species, including red and white pine, white and black spruce, and European larch. In contrast to the wetland species discussed above, these roots were low in porosity and were not flood-adapted. Roots ceased growth immediately upon flooding, and in many cases root apices did not survive the flooding treatment. The following experiments were conducted to investigate the composition of this precipitate and the conditions under which it formed.

Materials and methods

General plant handling

Plant material used in all experiments was 2 to 4 year old conifer nursery stock. After removal from cold storage, roots were pruned to a length of 15 cm, leaving a residual woody root system from which new root growth emerged. Plants were potted into a 1:1:1 peat:vermiculite:soil (Mardin series, a coarse-loamy, mixed, mesic typic Fragiochrept) mix (pH 5.0) in 3.75 cm diameter by 45 cm long Plexiglass cylinders. The soil was moistened and cold-stored before use to avoid the rapid chemical changes which are incident upon wetting. The cylinders were foil-wrapped or suspended into dark boxes to shield the root systems from light throughout the experiments.

All experiments were conducted in a growth chamber with a 14 h daylength (fluorescent and incandescent) and a 23°C constant temperature. Plants were ready for use after leaf elongation was complete and the newly produced roots were approaching the cylinder bottom. Flooding was imposed by suspending the 45 cm long cylinders into an external water reservoir which maintained a water table at the cylinder midpoint.

Morphology and anatomy of root blackening

Six plants each of European larch (Larch decidua Mill.), red pine (Pinus resinosa Ait), and white spruce (Picea glauca (Moench) Voss.) were flooded to the cylinder midpoint for 11 days. For two plants of each species, the progress of blackening was recorded daily by stoppering the cylinder bottom before removing from the external water reservoir and photographing.

After allowing 2 weeks for root recovery after drainage, roots were removed from the cylinders and washed free of soil. The former water table position was very obvious on the cleaned roots, as only the parts of the root system which had been flooded were black in color.

On roots with replacement laterals, the position of lateral emergence was recorded in cm behind the dead root apex. Anatomical observations were made on at least 20 flooded and 10 control roots of each species. Flooded roots were cut in cross section from the original root apex throughout the blackened zone and above the water table. Comparable sections of control roots were prepared. Both freehand fresh sections and fixed sections were prepared and examined.

Precipitate identification and analysis

Six plants each of black spruce (Picea mariana Mill.) and red pine (Pinus resinosa Ait.) were drained after flooding to the cylinder midpoint for 22 days. Soil samples were collected from the unflooded soil above the water table, and soil and root samples from below the water table. Soil clinging to the roots collected below the water table was shaken free and considered as rhizosphere soil. Samples were quickly transferred to Petri plates and saturated with a solution of 0.5% tetramethyl benzidine in 1% acetic acid. This reagent, like benzidine, is used to detect manganese oxides, as blue color development indicates a reduction reaction which is specific to these species.