Title	Low oxygen and 1-MCP pretreatments delay superficial scald development by reducing
	reactive oxygen species (ROS) accumulation in stored 'Granny Smith' apples
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## Abstract

'Granny Smith' apples are highly susceptible to superficial scald, a symptom of chilling injury. For many crops, low temperature storage results in oxidative stress and chilling injury, caused by increased production of superoxide anions which in turn leads to the generation of other dangerous reactive oxygen species (ROS). Application, prior to cold storage, of low oxygen (LO2, <0.5%) atmospheres, ethanol (<2%) vapour) or 1-methylcyclopropene (1-MCP, 0.5  $\mu$ L L<sup>-1</sup>) at 20 °C, was effective in reducing superficial scald in fruit following 24 weeks of cold storage. ROS levels were measured by confocal laser-scanning microscopy of apple peel treated with the fluorescent probe 2'.7'-dichlorodihydrofluorescein diacetate. In control fruit, ROS levels increased during cold storage and shelf-life and were very high after only 8 weeks, whereas in 1-MCP-, ethanol- and LO2-treated fruit, ROS levels remained low throughout storage. Geneexpression levels of ROS-scavenging enzymes were induced by the various pretreatments: catalase (*MdCAT*) was induced by LO2 treatment, whereas Mn superoxide dismutase (*MdMnSOD*) was induced by 1-MCP treatment. Polyphenol oxidase (MdPPO) gene expression levels were associated with scald symptom development and were highest in control fruit. Ethylene levels and expression of ethylene biosynthesis genes were correlated with  $\alpha$ -farnesene levels and <alpha>-farnesene synthase (MdAFS) gene expression in the variously treated fruit. Accumulation of the  $\alpha$ -farnesene oxidation product, 6-methyl-5hepten-2-one (MHO), was highest in control fruit after 8 weeks, in accordance with ROS accumulation. The LO2 pretreatment mechanism might involve production of anaerobic metabolites, causing a delay in ethylene and  $\alpha$ -farnesene biosynthesis and oxidation; this is different from the mechanism of action of 1-MCP, even though both consequently reduce ROS accumulation and scald symptoms.