Title Use of 1-methylcyclopropene and hydrogen peroxide to study apple ripening physiology

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Abstract

Unintended consequences of the ethylene action inhibitor 1-methylcyclopropene (1-MCP) are increased susceptibility to rot and decay pathogens, and fruit failure to develop aroma volatiles. In 1-MCP-treated apples, the effects of hydrogen peroxide (H_2O_2) to trigger ethylene biosynthesis and signaling were investigated. Transcription of antioxidative enzyme genes, antioxidant capacity, and catalase activity also were examined. H_2O_2 caused an increase in ethylene emission in 1-MCP-treated 'Golden Delicious' and 'Delicious' apples. In 'Golden Delicious', an increase in transcription of *Md-acs1* in 2005 and *Md-acs3* in 2006 matched the onset of the ethylene burst. In 2005, transcript levels of the putative *ein2, Md-acs1, Md-etr1* , and *Md-ers1* were lower in fruit treated with 3 $\mu l\, l^{-1}$ 1-MCP and dipped for an hour in 30 mM H_2O_2 , compared to treated and untreated fruit dipped in water. In 2006, transcription of *Md-ers1* increased in fruit treated with 1$\mu l\, l^{-1}$ 1-MCP dipped in 30 mM H_2O_2 compared to treated fruit dipped in water. In 2005, transcript levels of *Md-gpx* and putative *sod* and *cat* increased over time in 1-MCP-treated water-dipped fruit compared to the other treatments. In contrast, transcript levels of putative *apx* varied with treatments and year.

Constantly, 1-MCP-treated fruit always had lower catalase activity compared to untreated ones. In 2006, dipping fruit in H_2O_2 significantly increased catalase activity during the first 12 days overall, however exogenous H_2O_2 did not significantly change catalase activity of 1-MCP-treated fruit. H_2O_2 infiltration in 'Delicious' apples after storage significantly decreased catalase activity. In 2005, dipping 1-MCP-treated fruit in H_2O_2 delayed a decrease in total water-soluble antioxidant capacity by a minimum of nine days. In 2006, 1-MCP significantly prevented a change in total water-soluble antioxidant capacity caused by H_2O_2.

In addition, while 1-MCP-treated apples suffered from higher rot incidence compared to untreated ones, those treated with H_2O_2 had higher induction of PR2 and PR5; two pathogenesis-related proteins linked to heightened disease resistance.

With 1-MCP it is difficult to ascertain treatment status or effectiveness. *Md-acs1* and *Md-pg* , two gene candidates as molecular markers of 1-MCP application were tested using RT-PCR. Both genes are

effective molecular markers for 1-MCP application at harvest, after storage, when exposed to exogenous ethylene, and regardless of formulation.