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Lovastatin Inhibits α-Farnesene Biosynthesis and Scald Development in 'Delicious' and 'Granny Smith' Apples and 'd'Anjou' Pears

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Abstract. Effects of Lovastatin treatment on ethylene production, α-farnesene biosynthesis, and scald development were studied using 'Delicious' and 'Granny Smith' apples [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] and 'd'Anjou' pears (*Pyrus communis* L.) stored in air at 0 °C. During 6 months storage, Lovastatin did not affect internal ethylene concentration but reduced α-farnesene production in a concentration dependent manner in both apples and pears. Lovastatin reduced scald at 0.63 mmol·L^{-1} and inhibited scald completely at 1.25 or 2.50 mmol·L^{-1} in 'Delicious' and 'Granny Smith' apples. In 'd'Anjou' pears, Lovastatin at concentrations from 0.25 to 1.25 mmol·L^{-1} inhibited scald completely. After 8 months storage, inhibition of scald in both apples and pears by Lovastatin was concentration-dependent but none of the concentrations totally eliminated scald. Compared with 11.8 mmol·L^{-1} diphenylamine, Lovastatin treatment reduced scald to the same level at 1.25 mmol·L^{-1} in 'd'Anjou' pear and 2.50 mmol·L^{-1} in 'Delicious' and 'Granny Smith' apples. Lovastatin did not affect apple or pear fruit color, firmness, soluble solids content, or titratable acidity during storage in either apple or pear compared with the controls. Chemical name used: [1S-[1a (R°), 3α, 7β, 8β (2S°, 4S°), 8αβ]]-1,2,3,7,8,8α-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1-naphthaienyl 2-methylbutanoate (Lovastatin).

Since detection of α-farnesene in apple (*Malus sylvestris* var. *domestica*) fruit peel (Murray et al., 1964), the correlation between α-farnesene accumulation and scald development has been well documented (Ingle and D'Souza, 1989; Mir et al., 1999; Whitaker et al., 1997). Correlation analysis alone, however, is inadequate to reveal a cause–effect relationship, and direct evidence in support of this hypothesis is lacking. Applying α-farnesene or its oxidation products to fruit may provide direct evidence but results are inconsistent. In one report, applying α-farnesene directly to fruit was ineffective or caused injury (Huelin and Coggiola, 1970) whereas in another, application to the skin surface reduced scald (Curry, 2000). Furthermore, although applying oxidized α-farnesene (Huelin and Coggiola, 1970) or 6-methyl-5-hepton-2-one (Song and Beaudry, 1996), an α-farnesene oxidation product, induces scald-like symptoms on apple, the symptoms observed could be the result of chemical injury rather than true scald, since acetone or ethanol also induce similar symptoms (Ju and Curry, unpublished data). Preharvest treatment with aminoethoxyvinylglycine (AVG), an ethylene biosynthesis inhibitor, plus low-ethylene storage (Ju and Bramlage, 2000), or treatment with 1-methylcyclopropene (MCP) (Fan et al., 1999; Curry, unpublished data) or diazocyclopentadiene (Gong and Tian, 1998) at harvest, inhibit or reduce α-farnesene production and scald development in apples and pears (*Pyrus communis*). However, it is not clear whether scald inhibition is due to the inhibition of α-farnesene biosynthesis or other changes caused by the inhibition of ethylene biosynthesis or action. Therefore, a different approach is needed to clarify the relationship between α-farnesene and scald development in apples.

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Our previous study (Ju and Curry, 2000a) showed that Lovastatin, a competitive inhibitor of hydroxymethylglutaryl Coenzyme-A reductase (HMGR, EC 1.1.1.34) and a potent cholesterol-lowering agent in humans (Alberts et al., 1980), effectively inhibits α-farnesene production without affecting ethylene biosynthesis during fruit ripening at room temperature. Thus, Lovastatin may be a useful tool to study the relationship between α-farnesene biosynthesis and scald development in fruit. The objective of this research was to investigate the effects of Lovastatin on ethylene production, α-farnesene biosynthesis, and scald development in 'Delicious' and 'Granny Smith' apples and 'd'Anjou' pears after prolonged storage in air at 0 °C.

Materials and Methods

Plant materials. 'd'Anjou' pears were harvested 8 Sept. and 'Delicious' and 'Granny Smith' apples, 23 Sept. and 6 Oct. 1998, respectively, from a commercial orchard in Wenatchee, Washington. At harvest, 10 fruit from each of three replications were used for maturity evaluation, and for ethylene and α-farnesene measurement. Lovastatin emulsion was formulated as described previously by Ju and Curry (2000a) and concentrations used were 0, 0.25, 0.63, and 1.25 mmol·L^{-1} (0, 100, 250, and 500 mg·L^{-1}) for pears and 0, 0.63, 1.25, and 2.50 mmol·L^{-1} (0, 250, 500, and 1000 mg·L^{-1}) for apples. For comparison, diphenylamine (DPA) at 11.8 mmol·L^{-1} (2000 mg·L^{-1}) was also included as a treatment. Two hundred fruit per replication in each treatment were dipped in a solution for 3 min and stored in cardboard boxes in air at 0 °C. Ethylene and α-farnesene production were measured every month for 6 months on 10 fruit per replication. The rest of the fruit were used for scald evaluation after 6 or 8 months of storage following 7 d at 20 °C.

Measurement of fruit quality. Fruit color, firmness, soluble solids content (SSC), and titratable acidity (TA) were assessed both at harvest and after cold storage following 7 d at 20 °C. Fruit color was measured with a Minolta Chromo Meter (DP-301, Minolta, Osaka, Japan). Firmness was measured with an Electronic Pressure Tester (EPT-1, Lake City Tech. Products, Inc., Kelowna B.C.,

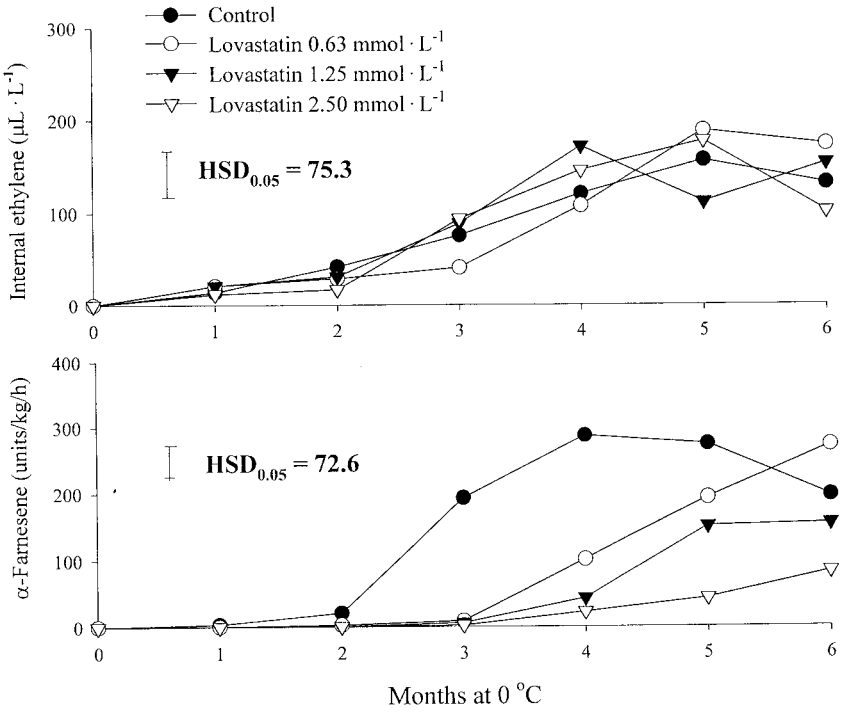


Fig. 1. Effect of Lovastatin treatment on internal ethylene concentration and α-farnesene production in 'Delicious' apples. Fruit were harvested 23 Sept. 1998, treated with Lovastatin at harvest, and stored in air at 0 °C for 6 months. Each symbol/point equals three observations.

Canada) equipped with an 11-mm tip for apple and 8-mm tip for pear. Readings were made on two paired sides of each fruit. SSC was assessed with a Digital Refractometer (PR-1, Atago Co. Ltd., Tokyo, Japan) on a combined sample of juice extracted from 10 fruit in each replicate. TA was measured by titrating 5 mL of juice extracted from 10 fruit in each replicate using a standard pH meter (PHM 82, Radiometer America, Cleveland, Ohio) in conjunction with a Titrator (TTT 80, Radiometer America) and expressed as a percentage of malic acid equivalents. Three replicates of 10 fruit were used.

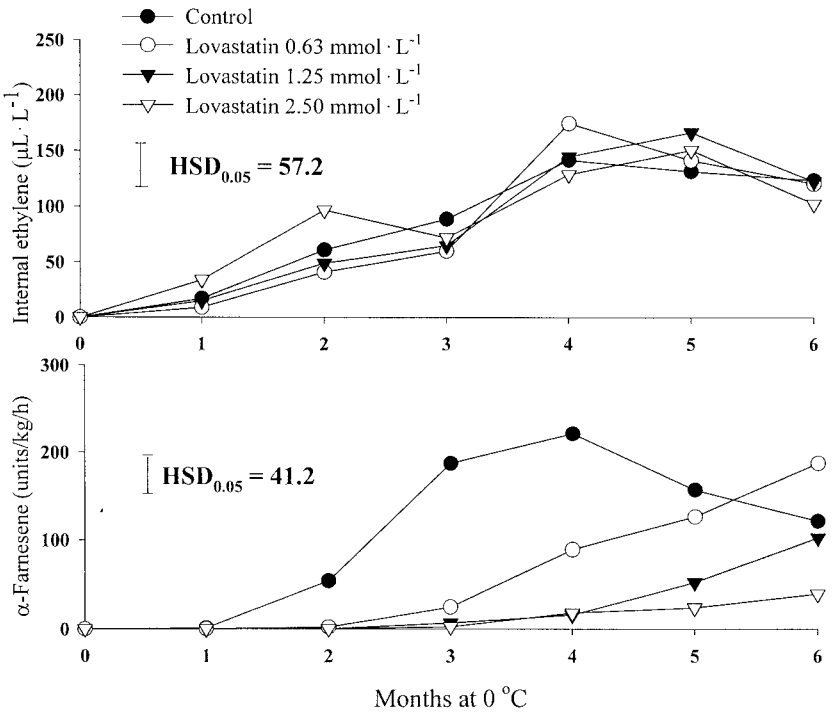
α-Farnesene and ethylene measurement. α-Farnesene in apple fruit was measured by gas chromatography–mass spectrometry (GC–MS) with a solid-phase microextraction (SPME) method (Ju and Curry, 2000a). Twelve fruit from each of the three replications were removed from cold storage, warmed at 20 °C for 4 h, and placed in a 4-L glass jar at 20 °C. The jar was connected to a flowthrough system using scrubbed air with a flow rate of 50 mL·min^{-1}. After 2 h equilibrium, a 100 mm polydimethylsiloxane (PDMS) probe (Supelco, Bellefonte, Pa.) was introduced into each jar and allowed to adsorb volatiles for 10 min. The probe was inserted immediately into the injection port of a gas chromatograph (GC) (HP 5890, Hewlett Packard, San Fernando, Calif.). Adsorbed volatiles were allowed to desorb for 3 min in the injector with a constant temperature of 250 °C. The oven temperature was increased from 35 °C to 250 °C at a rate of 40 °C·min^{-1} and then held for 2 min. α-Farnesene was expressed as units per kg fresh weight (FW) per h. One unit was defined as 1000 in abundance.

Air samples were taken from the cores of apples by inserting a needle in the calyx end and internal ethylene was measured with 10 individual fruit from each replication using a GC. A glass column (610 × 3.2 mm i.d.) packed with Porapak Q (90 to 100 mesh) was used. Oven, injector, and flame ionization detector (FID) temperatures were 50, 50, and 200 °C, respectively. Gas flows for N_2 carrier, H_2, and air were 30, 30, and 300 mL·min^{-1}, respectively.

In pear fruit, discs were taken from fruit peel using a No. 9 brass cork borer (1.2 cm diameter) and were used to measure α-farnesene and ethylene production. Discs were first washed with a 1% (w/v) ascorbic acid solution and then put into a 20 mL test tube. After sealing the test tube with a rubber septum, 2 mL of air was removed from the test tube to accelerate α-farnesene diffusion from the discs. Then, a 100 mm polydimethylsiloxane (PDMS) probe was introduced into the test tube and allowed to adsorb volatiles for 20 min. α-Farnesene was measured as described above and presented as units per g FW per min. Ethylene production rate was measured using a GC by removing a 0.5 mL air sample from the test tube after sampling for α-farnesene.

Scald evaluation. Scald was recorded both as percent incidence and as intensity (scald score) (Ju and Bramlage 2000). Fruit were first graded according to the scalded area of fruit surface using the scale: 0 = none, 1 = 1% to 10%, 2 = 11% to 33%, 3 = 34% to 66%, and 4 = 67% to 100%. Scald incidence was calculated as the percent

Fig. 2. Effect of Lovastatin treatment on internal ethylene concentration and α-farnesene production in 'Granny Smith' apples. Fruit were harvested 6 Oct. 1998, treated with Lovastatin at harvest, and stored in air at 0 °C for 6 months. Each symbol/point equals three observations.



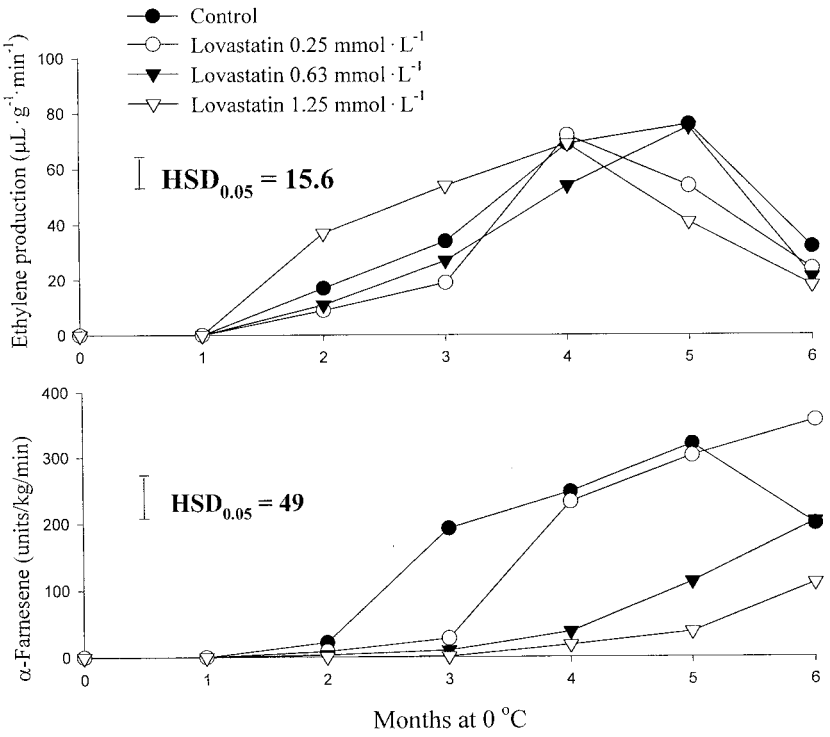


Fig. 3. Effect of Lovastatin treatment on internal ethylene concentration and α-farnesene production in 'd'Anjou' pears. Fruit were harvested 8 Sept. 1998, treated with Lovastatin at harvest, and stored in air at 0 °C for 6 months. Each symbol/point equals three observations.

scalded fruit of the total number of fruit evaluated. Scald score was calculated as the mean of the scale ratings for each individual scald affected fruit. Higher scald scores represent fruit with larger surface areas affected by scald.

Statistical analysis. Data were subjected to analysis of variance (ANOVA) procedures or regression analysis using SAS Statistical Software (SAS Institute Inc., Cary, N.C.). Means were separated by honestly significant difference (HSD) using Tukey's studentized range test. Only results significant at $P \leq 0.05$ are discussed.

Results

Effects of Lovastatin on ethylene and α-farnesene production during storage. Similar trends were found in 'Delicious' and 'Granny Smith' apples (Fig. 1 and 2). In control fruit, internal ethylene concentrations were below detectable levels at harvest, increased during storage, and reached a peak between 4 and 5 months, and remained constant thereafter. α-Farnesene production was undetectable at harvest, started to increase after 1 month storage, reached a peak in production around 4 or 5 months, and declined thereafter. Lovastatin treatment did not affect ethylene production, but delayed and reduced α-farnesene production in a concentration-dependent manner. However, none of the concentrations completely inhibited α-farnesene biosynthesis.

In 'd'Anjou' pears, ethylene and α-farnesene were not detected at harvest or after 1 month storage (Fig. 3), but were detectable after 2 months cold storage. Both ethylene and α-farnesene production showed similar trends, increasing in early storage and then decreasing, after reaching the maximum at ≈4 months. As in apples, Lovastatin inhibited α-farnesene biosynthesis without affecting ethylene production. As the concentration of Lovastatin increased, so did inhibition of α-farnesene, but none of the treatments inhibited α-farnesene production completely.

Effects of Lovastatin treatment on scald development. After 6 months storage, Lovastatin at 0.63 mmol·L^{-1} reduced scald in both 'Granny Smith' and 'Delicious' apples (Table 1). At 1.25 or 2.50 mmol·L^{-1}, Lovastatin eliminated scald. After 8 months storage, inhibition of scald by Lovastatin was concentration dependent and, although none of the concentrations inhibited scald completely in either cultivar, Lovastatin at 2.50 mmol·L^{-1} was as effective in controlling scald as 18.2 mmol·L^{-1} DPA.

In 'd'Anjou' pear, Lovastatin at 0.25 mmol·L^{-1} or higher was effective in controlling scald after 6 months storage. After 8 months, all concentrations were effective in reducing scald but only the 1.25 mmol·L^{-1} treatment was as effective as 18.2 mmol·L^{-1} DPA (Table 1).

Effects of Lovastatin treatment on other fruit quality attributes. Compared with control fruit, Lovastatin treatment did not affect apple or pear fruit color, firmness, SSC, or TA after 8 months storage (data not presented).

Discussion

Results demonstrated that scald development in apples and pears is reduced or eliminated when α-farnesene production is reduced by the specific HMGR inhibitor, Lovastatin. Lovastatin treatment did not affect ethylene biosynthesis (Figs. 1, 2, and 3). Changes in fruit color, firmness, SSC, and TA were similar in both control and Lovastatin-treated fruit (data not presented), supporting previous indications that Lovastatin does not affect other responses to ethylene (Ju and Curry, 2000a). Thus, we provide evidence that α-farnesene biosynthesis in fruit peel is directly related to scald development in apples and pears.

α-Farnesene is completely inhibited by 1.25 or 2.50 mmol·L^{-1} Lovastatin if apples are stored at 20 °C for 30 d (Ju and Curry, 2000a), but, in this study, storage of fruit at 0 °C was not as effective. Possibly, low temperature might affect the absorption of Lovastatin by fruit or the stability of the compound during prolonged air exposure.

The above findings may have the following potential applications. First, due to the effectiveness of Lovastatin in inhibiting α-farnesene biosynthesis and scald development, its structure and mode of action in HMGR inhibition could conceivably provide a useful template with which to find or screen safe, natural products as new scald inhibitors. Second, since α-farnesene plays such an important role in affecting scald, and α-farnesene biosynthesis is regulated by ethylene (Ju and Curry, 2000b), any treatment that reduces ethylene biosynthesis or action during storage may improve scald control. Third, because Lovastatin is a specific HMGR inhibitor (Alberts et al., 1980) and α-farnesene biosynthesis involves HMGR gene expression during fruit ripening (Ju and Curry, unpublished data), further study on this gene and its regulation may provide information for down regulating HMGR genes through bioengineering leading, therefore, to genetic transformations of apple or pear trees to produce fruit highly resistant to scald.

Table 1. Effect of Lovastatin treatment on scald development in 'Delicious' and 'Granny Smith' apples and 'd'Anjou' pears[z].

Concn	6 months		8 months	
(mmol·L^{-1})	Scald (%)[y]	Scald score[x]	Scald (%)	Scald score
		Delicious		
DPA (11.8)	0		7	2.1
Lovastatin				
0	38	2.9	75	3.2
0.63	21	2.5	68	3.0
1.25	0		34	2.3
2.50	0		15	2.7
Regression				
Linear	****		****	***
Quadratic	NS		NS	*
DPA vs. 2.50 mmol·L^{-1} Lovastatin	NS		**	**
		Granny Smith		
DPA (11.8)	0		16	2.1
Lovastatin				
0	54	2.3	86	3.7
0.63	31	1.4	67	3.1
1.25	0		31	2.5
2.50	0		11	1.7
Regression				
Linear	****		****	****
Quadratic	NS		NS	NS
DPA vs. 2.50 mmol·L^{-1} Lovastatin	NS		NS	NS
		d'Anjou		
DPA (11.8)	0		11	1.8
Lovastatin				
0	19	1.2	94	3.5
0.25	0		79	3.2
0.63	0		33	2.6
1.25	0		15	2.1
Regression				
Linear	****		****	****
Quadratic	NS		NS	NS
DPA vs. 1.25 mmol·L^{-1} Lovastatin	NS		NS	NS

[z]'d'Anjou' pears were harvested 8 Sept. and 'Delicious' and 'Granny Smith' apples were harvested 23 Sept. and 6 Oct. 1998, treated with Lovastatin at harvest, and stored in air at 0 °C for 8 months. Scald was evaluated after 6 and 8 months storage plus 7 d at 20 °C.
[y]Scald (%) = percent scalded fruit of the total fruit evaluated.
[x]Scald score = mean of surface area of fruit affected by scald using the scale of 1 = 1% to 10%, 2 = 11% to 33%, 3 = 34% to 66%, and 4 = 67% to 100% of the surface area affected.
[NS,*,**,****]Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, or 0.0001, respectively.

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