

## The Effects of Fruit Maturity and 1-Methylcyclopropene (1-MCP) Treatment on $\alpha$ -farnesene Metabolism in Scald Resistant and Susceptible Cultivars of Apple Fruit

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### Abstract

**Background:** It was found that, climatic conditions and fruit ripeness are important factor for the occurrence and intensity of superficial scald. 1-MCP is an inhibitor of ethylene action in plants and inhibits many ripening related processes along with the production of ethylene and volatiles.

**Objective:** Apples are often held for several months at low temperature either in air (RA) or in controlled atmosphere (CA) storage, therefore they are prone to the occurrence of a large number of physiological disorders. In this work an investigation was made in order to determine the effect of 1-methylcyclopropene ( $1.0 \mu\text{l l}^{-1}$ ) treatment on superficial scald development and  $\alpha$ -farnesene metabolism on scald-resistant '*Golden Delicious*' and scald-susceptible '*Imperial Delicious*' apples.

**Methods:** Fruits harvested on two dates and stored 24 weeks in refrigerated air storage (RA) at  $0.5-1^\circ\text{C}$ . Peel tissue samples were taken at harvest after 6, 12 and 24 weeks. A reversed-phase high-performance liquid chromatographic procedure with UV detection at 232 nm and 269 nm has been used for the determination of  $\alpha$ -farnesene and Ctols contents.

**Results:** Scald symptoms were restricted to untreated control fruit of both harvests of *Imperial* as during storage period increased to 65 and 45%, respectively for the first and second harvest compared with no scald symptoms in those in *Golden Delicious*. At  $1.0 \mu\text{l l}^{-1}$ , 1-MCP completely eliminated superficial scald during storage period in *Imperial* apples. Large increases in peel tissue content of  $\alpha$ -farnesene observed in untreated fruit of both harvest dates in *Golden Delicious* and *Imperial* cultivars. 1-MCP strongly decreased  $\alpha$ -farnesene and conjugated trienols accumulation in treated fruit peel tissues at both maturity stages of two cultivars during storage. The results confirm the proposed role of Ctols in scald induction and indicate that  $\alpha$ -farnesene production is strongly regulated by ethylene.

**Conclusion:** In general, we conclude that only  $\alpha$ -farnesene synthesis and its oxidation products (Ctols) levels in peel tissue could not be involved to the inherent nature of scald susceptibility or resistance in two cultivars tested.

**Keywords:** Apple fruit,  $\alpha$ -farnesene, Conjugated trienols, Superficial scald, Cultivar difference

## Introduction

Apples are often held for several months at low temperature either in air (RA) or in controlled atmosphere (CA) storage, therefore they are prone to the occurrence of a large number of physiological disorders [1] such as Superficial scald which manifested as brown or black patches on the fruit skin and develops during cold storage of susceptible cultivars of apple fruit or may be a chilling injury [2]. There is a genetic component to its occurrence, since some cultivars (e.g., 'Granny Smith' and 'Delicious') are very susceptible, while others (e.g., 'Gala', 'Empire' and 'Golden Delicious') are scald resistant [3]. Apple fruit volatiles are complex mixtures of organic compounds, the synthesis of which occurs primarily during ripening. The majority of apple aroma compounds are volatile esters; however, apples also produce a relatively large amount of  $\alpha$ -farnesene, a sesquiterpene hydrocarbon [4].  $\alpha$ -Farnesene has been linked to the formation of superficial scald via its oxidation products [5].  $\alpha$ -farnesene production peaks with the respiratory climacteric following the ethylene burst in apple. The relationship between ripening-related ethylene synthesis and  $\alpha$ -farnesene accumulation is well documented [6 - 8]. Treatment with ethylene synthesis inhibitors, such as aminoethoxy-vinylglycine [9], or ethylene action inhibitors including diazocyclopentadiene [10] and 1-methylcyclopropene (1-MCP) [11] greatly reduces scald incidence,  $\alpha$ -farnesene and conjugated trienes contents. [8,12]. Production of the sesquiterpene  $\alpha$ -farnesene and its oxidation, forming various conjugated trienes, plays an important role in scald

development; therefore, the prevention of  $\alpha$ -farnesene oxidation in scald resistant cultivars may directly lead to scald control [13, 14]. The primary oxidation products of  $\alpha$ -farnesene were identified as conjugated trienes (CTs), which are readily detected in hexane-dip extracts of apple fruit due to their characteristic UV absorbance maxima at 259, 269, and 281nm. Anet [15] elucidated the structures of several hydroperoxide and epoxide CT products of in vitro autoxidation of  $\alpha$ -farnesene, and proposed that free radicals generated during decomposition of these compounds are the toxic agents that induce scald. It has been shown that over 99% of the CT species accumulation in apple skin in vivo are major (7E, 9E,  $\geq 90\%$ ) and minor (7E, 9Z,  $\leq 10\%$ ) isomers of 2, 6, 10-trimethyl-dodeca-2, 7, 9, 11-tetraen-6-ol (Ctols) rather than the CT hydroperoxides and epoxides identified by Anet [16 - 17].

In this respect, *Golden Delicious*, that is reported as a scald resistant cultivar, as well with scald susceptible 'Imperial' *Delicious* apples, have been chosen in this experiment to evaluate 1-MCP postharvest treatment and maturity at harvest effects on  $\alpha$ -farnesene metabolism in relation to superficial scald development. Therefore, the main objective of the present study was to determine the relation between the amount of  $\alpha$ -farnesene synthesis and its oxidation to conjugated trienols in scald resistant 'Golden Delicious' apple with scald susceptible 'Imperial' *Delicious* apples in response to 1-MCP treatment and maturity stages during cold storage.

## Materials and Methods

### Plant material and handling

In this experiment fruits were harvested from mature trees of 'Golden Delicious' (standard) and 'Imperial' (standard) *Delicious* apples, growing at a commercial orchard in Agajary region of Marageh in East Azerbaijan province on October 1 (H1), and October 11 (H2), 2006. Harvest dates were during the commercial harvest for each cultivar. Starch index was measured at harvest where 1= full starch and 8= no starch, and indices were between 2 to 3 for the first harvest of each cultivar, and 3 to 4 for the second harvest of each cultivar. All apples were allowed to equilibrate at 20 °C overnight upon arrival in Karaj. For each cultivar, fruit were sorted for uniformity and freedom from blemishes and were randomly divided into two lots of 240 fruit and placed in twelve plastic field boxes, each one contained 20 fruit, in each harvest date. One lot was placed into 0.5-1 °C storage (control) with relative humidity (RH) of >85%, the second was used for 1-MCP treatment. Three replicate samples were used for each treatment combination.

### 1-MCP treatment

Twelve plastic field boxes of each harvest date and of each cultivar were divided in two lots and placed in 0.3-m<sup>3</sup> polyethylene bags which could be sealed with wide duct tape and were exposed to 1.0 µl l-1, 1-MCP (Smartfresh, Agrofresh, Inc., Rohm and Haas, Springhouse, PA) for 24h at 20 °C. After 24h, fruit boxes were vented, and placed in cold storage. The 1-MCP concentration was calculated according to the percent active ingredient (0.14% active ingredient by weight)

and release from the Smartfresh™ powder into the free headspace of the sealed bags as described in the Technical Bulletin provided by Rohm and Haas. Treatment bags were equipped with fans to circulate the atmosphere.

### Sampling time and storage period

Two 10-fruit samples from each replicate of each treatment combination, were randomly used for the following experiments in four sampling times as, harvest time (T<sub>0</sub>), 6 (T<sub>1</sub>), 12 (T<sub>2</sub>), and 24 weeks (T<sub>3</sub>) during cold storage period.

### Assessment of superficial scald

Superficial scald development was recorded visually. For each treatment 30 fruit were individually ranked according to the scale: (0) no scald; (1) 0–25% of the fruit surface affected; (2) 25–50% of the surface affected; or (3) >50% of the surface with scald in each sampling time after holding them for 7 days at 20 °C. A scald index was calculated as described by Zanella [18]. Mean scores were calculated for the replicates.

### Analysis of α-farnesene and Ctols

At each sampling time, upon removal from storage, fruit peel samples were taken from 10 fruit and frozen in liquid N<sub>2</sub>. The total samples from each sampling time × cultivar × harvest time × treatment were then sealed in a large plastic bag and stored at -80 °C until used for measurement of α-farnesene and Ctols contents. α-farnesene standard was obtained from Aldrich, Kosher and Ctols269 standard isolated and purified spectrophotometrically from apple peel tissue according to the method

described by Anet [19]. HPLC analysis  $\alpha$ -farnesene and Ctols contents were performed by reversed-phase HPLC (Waters Chromatography, Alliance, USA) at 232 and 269 nm, respectively according to the method described by Whitaker et al [20] with some modifications, as followed.

Ten peel discs were removed from the equatorial region of 5 apple fruit using a cork borer (1.cm diameter) and cortical tissue was removed using a razor blade, and the skin (105±12.mg fresh weight) was extracted for 24 h in 2ml hexane. After hexane evaporation under the gentle stream of nitrogen, 400  $\mu$ l of methanol was added and filtered through 0.45  $\mu$ m PTFE membrane prior to HPLC analysis. Thereafter, the samples were diluted to 1:1 ratio with 50% of methanol, and 50  $\mu$ l of sample were injected into HPLC. Separation of the components was performed at room temperature under the following chromatographic conditions: Spherisorb C<sub>18</sub> column (4.6  $\times$  250 mm) 5  $\mu$ m, using methanol: acetonitrile: water (90: 5: 5) as the mobile phase at a flow rate of 0.8 ml min<sup>-1</sup>. Quantification of each metabolite was performed using a standard curve prepared with the corresponding authentic compound and expressed as  $\mu$ g. g<sup>-1</sup> on a fresh weight basis ( $\mu$ g. g<sup>-1</sup> fw).

### Statistical analysis

The experimental design was a completely randomized design (23  $\times$  4  $\times$  3 factorial), two cultivars, two harvest dates, one 1-MCP concentration, four sampling times, with three replicates. The results were subjected to the analysis of variance (ANOVA) of SAS software (SAS Institute, Inc., Cary, NC, USA),

using Excel and comparisons among means at each time of sampling were made using Duncan's multiple range test at  $p \leq 0.05$ .

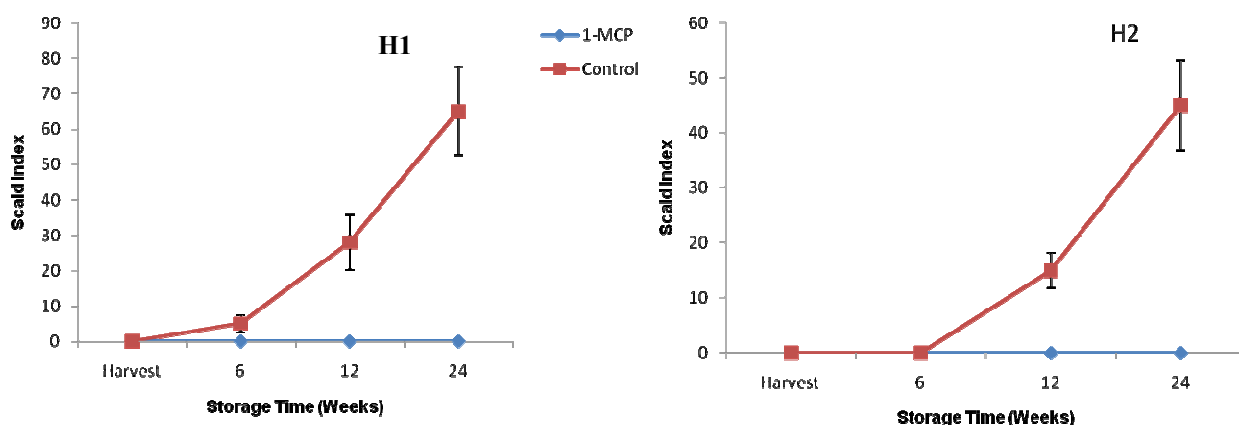
## Results

### Superficial scald development

Scald symptoms did not develop on control and 1-MCP treated fruit of 'Golden Delicious' apples harvested at both maturity stages, during storage period in post storage holding period at 7 days at 20 °C. There was a significant difference between scald indexes at both harvest times of 'Imperial' apples during storage period (Fig. 1). After 6 weeks of storage and subsequent holding period of 7 days at 20 °C, only in control fruit of the first harvest, a low incidence of superficial scald (5%) was observed (Fig. 1). Scald development increased in control fruit of both harvest dates at week 12, as 28 and 15% for the first and second harvest fruit, respectively. In the prolonged storage period, scald symptoms was developed more severe in control fruit of both harvests, as 65 and 45% of the control fruit from the early and optimal harvest, respectively (Fig. 1). At weeks 12 and 24, scald index in fruit from the first harvest, was 54% and 69%, respectively, higher than in the second harvested fruit. As shown in Fig. 1 1-MCP (1 $\mu$ l l<sup>-1</sup>) treatment prevented successfully superficial scald development in the fruit of both harvest stages of 'Imperial' apples during storage for 24 weeks.

### Concentrations of $\alpha$ -farnesene and conjugated trienols during storage

At harvest time,  $\alpha$ -farnesene was detected in small amounts (between 2 and 4  $\mu$ g. g<sup>-1</sup> fw) in the peel tissue of untreated control and

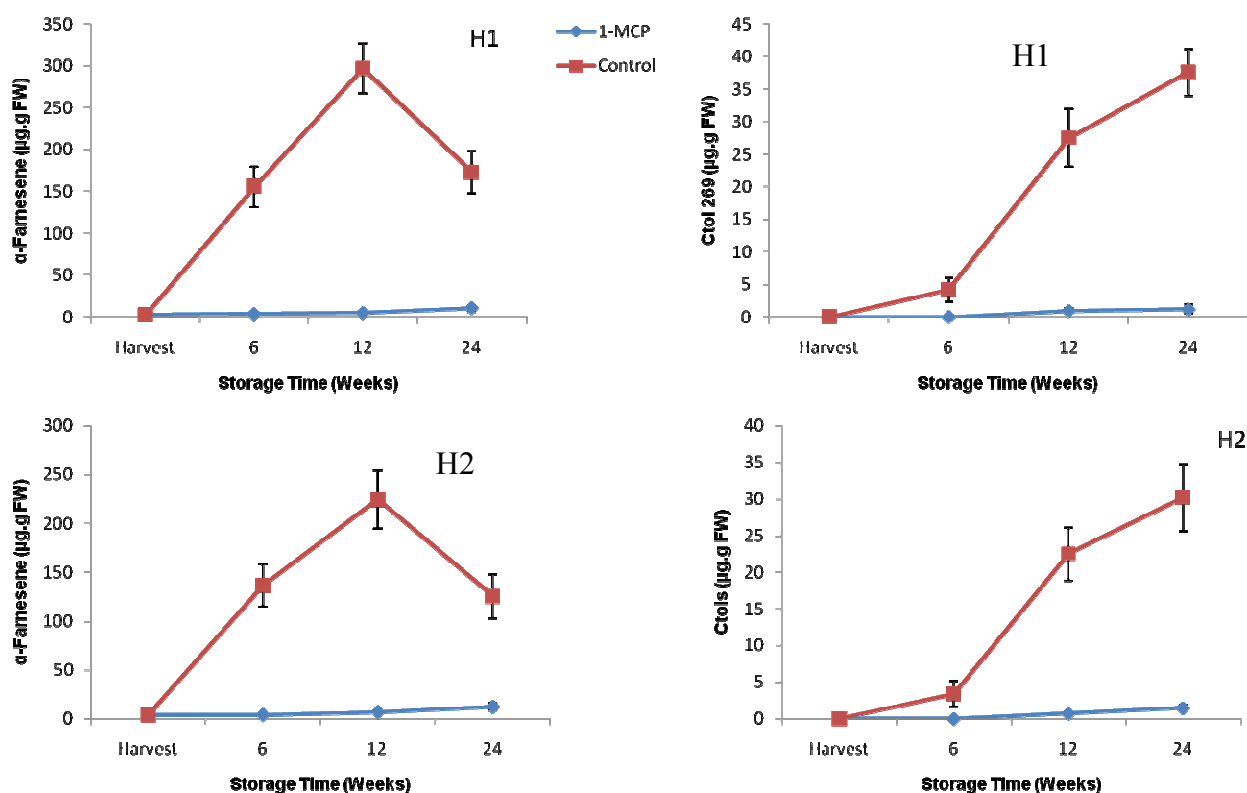


**Fig. 1-** Effect of storage duration on percentage of control and 1-MCP (1 $\mu$ l l<sup>-1</sup>) treated fruit of 'Imperial' Delicious from two harvest dates (H1 and H2), developing superficial scald. Values are means  $\pm$  SE., n= 30 in each treatment at each sampling time

1-MCP treated fruit at both harvest maturity stages of both cultivars (Fig. 2 and 3). The general pattern of  $\alpha$ -farnesene accumulation in both harvests of two cultivars was similar but the amount of  $\alpha$ -farnesene accumulation differed significantly. In untreated control fruit of 'Golden Delicious' apples from the early and second harvests,  $\alpha$ -farnesene increased from 155.7 and 136.4  $\mu$ g. g<sup>-1</sup> fw at 6 weeks after harvest and achieving a peak of 296.6 and 224.4  $\mu$ g g<sup>-1</sup> fw at week 12 of storage period, and declined thereafter (Fig. 2). While, in untreated control fruit of 'Imperial' apples at early and optimal harvests,  $\alpha$ -farnesene content increased from 102.5 and 85.8  $\mu$ g. g<sup>-1</sup> fw at 6 weeks after harvest and achieving a peak of 185.4 and 132.6  $\mu$ g g<sup>-1</sup> fw at week 12 of storage period, and declined thereafter (Fig. 3). The increase in  $\alpha$ -farnesene concentration was greater in control than in treated fruit. 1-MCP (1  $\mu$ l/L) inhibited  $\alpha$ -farnesene accumulation in fruit peel of both cultivars effectively. The peaks of  $\alpha$ -farnesene concentration in control fruit of both harvests were observed only after twelve weeks, whereas

1-MCP (1 $\mu$ l /L) completely prevented the accumulation of  $\alpha$ -farnesene in both cultivars.

Ctol 269 level was not detectable in peel tissue of control and treated fruit of both cultivars, at all harvest dates and The cumulative amount of Ctol 269 produced in storage by two cultivars was different (Fig. 2 and 3). Scald resistant 'Golden Delicious' untreated control fruits had the lowest amount of Ctol 269 in each harvest dates in comparison with 'Imperial' control apples (Fig. 3). The pattern of conjugated trienol accumulation during storage period was similar for each harvest maturity of both cultivars. As, Ctol 269 level in peel tissues of untreated control fruit of two cultivars at both maturity stages increased and reached maximum values by the end of storage (Fig. 2 and 3). In comparison with untreated control of *Golden Delicious* apples, 1-MCP treatment caused a reduction of 97 and 95 % in Ctols levels of the first and second harvest, respectively, accumulated after 24 weeks in storage (Fig. 2). At the same sampling time



**Fig. 2-** Effect of 1-MCP (1 $\mu$ l l<sup>-1</sup>) treatment on  $\alpha$ -farnesene and conjugated trienols (Ctols) during storage of ‘Golden Delicious’ apple fruit harvested at two times (H1, H2) and stored at 0.5-1 °C. Values are means  $\pm$  SE., n= 30 in each treatment at each sampling time

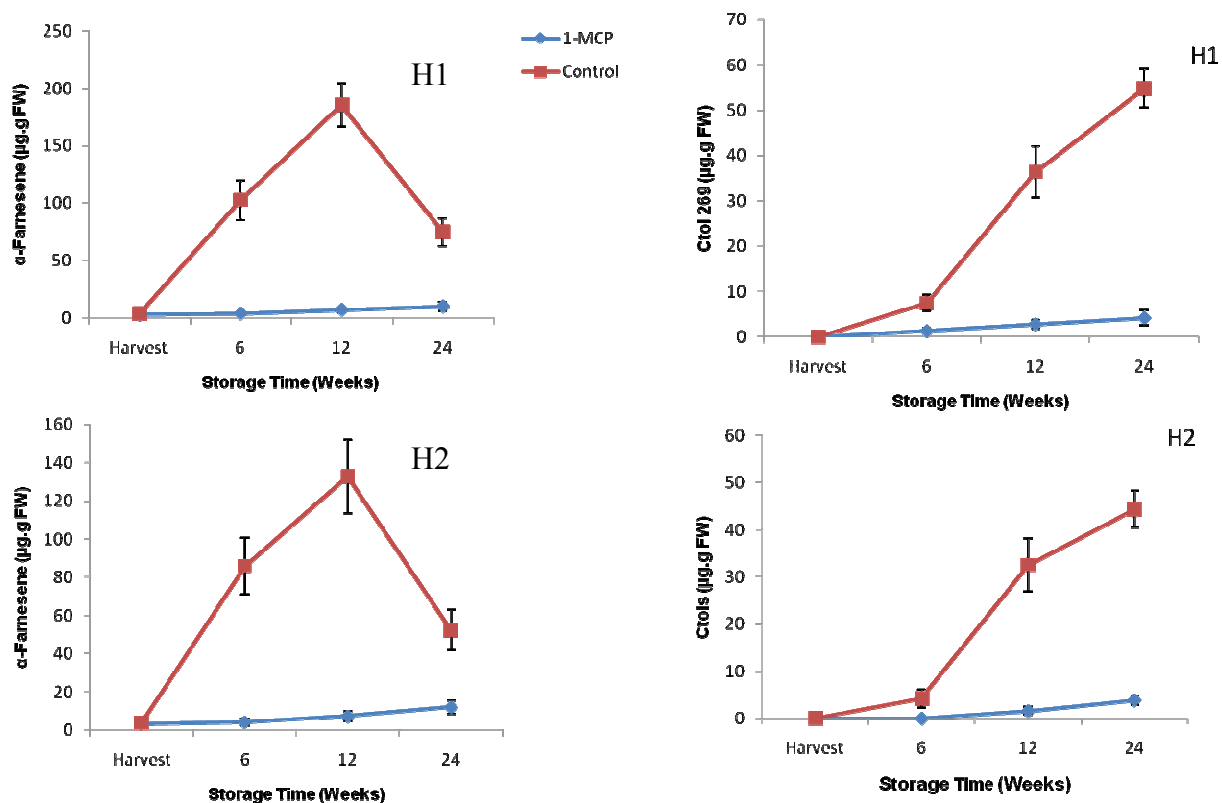
(24 weeks), 1-MCP (1 $\mu$ l /L) had reduced the levels of conjugated trienols by 92 and 90 % in relation to control fruit from the first and second harvests, respectively, in *Imperial* apples (Fig. 3).

## Discussion

It was found that, climatic conditions and fruit ripeness are important factors for the occurrence and intensity of superficial scald [21-22]. In addition, storage conditions and the length of storage result in incidence of scald in many apple cultivars [23-24]. In this study, scald symptoms developed in the first harvest control of ‘*Imperial*’ *Delicious* apples (scald susceptible) within 6 weeks and appeared at week 12 in second harvested fruits, but there were no scald symptoms in two harvests of

‘*Golden Delicious*’ apples, until end of storage period (Fig.1).

1-MCP is an inhibitor of ethylene action in plants and inhibits many ripening related processes along with the production of ethylene and volatiles [25]. Cultivar can affect product responses to 1-MCP [24]. It was shown that the effective concentration of 1-MCP is 1  $\mu$ l l<sup>-1</sup> [23]. In agreement with these findings, 1-MCP treated fruits of both harvests of two cultivars tested in this experiment showed no scald during the total storage time (Fig.1). Our results showed that the effect of 1-MCP at 1  $\mu$ l l<sup>-1</sup> in inhibition of superficial scald development was similar in both harvest dates of ‘*Imperial*’ apples as 1-MCP reduced completely scald even during long-term storage in normal air storage (Fig. 1).



**Fig. 3-** Effect of 1-MCP (1 $\mu\text{l}$ , 1-1) treatment on  $\alpha$ -farnesene and conjugated trienols (Ctols) during storage of 'Imperial' Delicious apple fruit harvested at two times (H1, H2) and stored at 0.5-1  $^{\circ}\text{C}$ . Values are means  $\pm$  SE., n= 30 in each treatment at each sampling time

Fruit maturity was an important factor determining  $\alpha$ -farnesene synthesis and conjugated trienols accumulation in fruit peel tissues of both apples. As, with progressing fruit maturity stage the content of  $\alpha$ -farnesene synthesis and conjugated trienols accumulation in untreated control fruit of both cultivars declined (Fig.2 and 3). Small amounts of this compound were detected at the beginning of storage [14, 26] which is in accordance with our results. The  $\alpha$ -farnesene concentrations decreased from week 12, again in accordance to similar reported literature [24, 27]

In our experiment,  $\alpha$ -farnesene concentration in fruit peel of two cultivars with different scald susceptibility, increased during storage to attain a maximum at twelve

weeks after storage.  $\alpha$ -farnesene starts to accumulate in the first 15 weeks of storage. Sometimes this period is different depending on fruit susceptibility with scald or other factors. Our results, showed that decrease in  $\alpha$ -farnesene concentration after attaining maximal levels in untreated control fruit, during storage was more evident in scald susceptible 'Imperial' Delicious than scald resistant 'Golden Delicious' (Fig. 2 and 3).

$\alpha$ -Farnesene generally showed no correlation with scald occurrence, which is in accordance to the literature. Rupasinghe et al. [28] found that scald-resistant cultivars 'Mutsu' and 'Idared', respectively, produced the highest and lowest levels of  $\alpha$ -farnesene during 22 weeks of storage period. In agreement with this finding, our results

showed that  $\alpha$ -farnesene content in the peel of untreated control fruit of scald-resistant 'Golden Delicious' cultivar, at both maturity stages, in comparison with 'Imperial' apples does not correlate with its inherent scald-free characteristic (Fig. 2 and 3).

$\alpha$ -farnesene and Ctols accumulation in the skin were dramatically inhibited in 1-MCP treated fruit, than for untreated fruit during storage period (Fig 2 and 3). These data illustrate the  $\alpha$ -farnesene accumulation in the skin of apples is closely associated with ethylene production by fruit, with patterns of change of  $\alpha$ -farnesene generally reflecting those for ethylene production. 1-MCP reduced 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) activity by 30% and respiration by 60% in apples, resulting in a 72% reduction in  $\alpha$ -farnesene [29]. While, E,E  $\alpha$ -farnesene synthase activity was no different in 1-MCP-treated apples when compared with the control, 1-MCP treatment greatly reduced  $\alpha$ -farnesene in apple skin [30]. More recently, the use of 1-MCP, which markedly reduces  $\alpha$ -Farnesene synthesis [8,12], proved to be an effective means to prevent scald. In this regard, given the very low  $\alpha$ -farnesene content in 1-MCP treated of 'Golden Delicious' and 'Imperial' apples, there was very low amount of  $\alpha$ -farnesene oxidation (Fig. 2 and 3). As, 1-MCP at  $1\mu\text{l l}^{-1}$  was very effective in preventing  $\alpha$ -farnesene accumulation (Fig. 2 and 3). The levels of  $\alpha$ -farnesene in fruit treated with  $1\mu\text{l l}^{-1}$  of 1-MCP were significantly lower than those observed in untreated controls of both cultivars and presumably this accounts for the reduction in conjugated trienols (Fig. 2 and 3) and superficial scald (Fig. 1).

Conjugated trienols (Ctols) result from the in vivo oxidation of  $\alpha$ -farnesene and are believed to be directly responsible for primary events leading to development of superficial

scald. The susceptibility of apple to scald decreases with advanced ripening stage and seems to depend more from conjugated trienols (CTs) accumulated in the peel and from the chilling effect of low storage temperature than from ethylene or  $\alpha$ -farnesene production. Fruit maturity seemed to be an important factor, since the relationship between Ctols level and scald incidence depended on harvest date.  $\alpha$ -Farnesene peaked and then declined well before the trienols, which remained elevated through the end of storage [20]. In agreement with these findings, our results showed the similar trend for  $\alpha$ -farnesene and conjugated trienol levels in both harvests of 'Imperial' apples, throughout the storage period.

Anet [15] emphasized that detection of CTs early in storage was the best predictor of scald development and proposed that natural antioxidants delay  $\alpha$ -farnesene oxidation and thus are important in scald resistance. In accord with this, scald resistance was correlated with the level of lipophilic cuticular antioxidants present at harvest [30], or maintained during storage [5]. Therefore, higher concentration of  $\alpha$ -farnesene with no scald index in scald resistant cultivar 'Golden Delicious' indicate the ability of fruit to suppress  $\alpha$ -farnesene oxidation. Rao et al. [26] suggested that superficial scald is also related to the cellular efficiency in metabolizing active oxygen species. Ju and Bramlage [9] reported that the total antioxidant activity of 'Golden Delicious' was higher than 'Empire Delicious' apples which was similar to their relative scald resistance. Whitaker [13] suggested that 'Gala' apples, a scald resistant cultivar, but fruit of this cultivar also generally produce little  $\alpha$ -farnesene and conjugated trienol, which alone may be sufficient to prevent scald. Ctol content in 1-MCP treated fruit with different maturity stages remained very low in both cultivars (Fig. 2 and 3) and

no scald developed during the storage period (Fig. 1). 1-MCP appears to inhibit  $\alpha$ -farnesene synthesis and thereby limits the precursor for oxidation to Ctols.

In general, we conclude that the poor relationship between high production of  $\alpha$ -farnesene and low amount of its oxidation products (Ctols) in scald resistant 'Golden Delicious' apples show that there is an alternative mechanism for scald control in this cultivar compared to scald susceptible 'Imperial' apples.

The results confirm the proposed role of Ctols in scald induction and indicate that  $\alpha$ -farnesene production is strongly regulated by ethylene in both cultivars tested in this study. As 1-MCP treatment inhibited  $\alpha$ -farnesene

metabolism in both cultivars, however scald occurrence not only affects by  $\alpha$ -farnesene metabolism but also by other modifying factors with equal importance that their effects are consistent in superficial scald control in scald resistant 'Golden Delicious' compared to scald susceptible Imperial cultivar. This alternative mechanism may relate to the special components of their fruit peel antioxidant system that is more potential in scald resistant 'Golden Delicious' than scald susceptible Imperial apples. Therefore, we conclude that only  $\alpha$ -farnesene synthesis and its oxidation products (Ctol) levels in peel tissue could not be involved to the inherent nature of scald susceptibility or resistance in two cultivars tested.

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