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Superficial scald susceptibility and α-farnesene metabolism in 'Bartlett' pears grown in California and Washington

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ABSTRACT

'Bartlett' pears grown in northern California (CA) consistently show development of the physiological storage disorder superficial scald, particularly after prolonged storage of 4–5 months in air. In contrast, fruit of this cultivar grown in central Washington (WA) are typically less susceptible to scald, exhibiting mild or no symptoms. Conjugated triene (CT) oxidation products of the sesquiterpene α-farnesene are thought to play a key role in scald induction in apples and pears. This study compared accumulation of α-farnesene and its CT products in peel tissue of CA- and WA-grown 'Bartlett' pears during air storage at −1 °C in relation to scald development after transfer to 20 °C. Pears were harvested from commercial orchards in 2006 and 2007 and stored under nearly identical conditions for up to 24 weeks. Peel tissue samples taken at harvest and at 2–4-week interval during storage were analyzed by HPLC to determine concentrations of α-farnesene and CTs. Measurements of flesh firmness, respiration, and ethylene production were also made at harvest and/or from 1 to 8 d after removal from storage to 20 °C. WA fruit from the second harvest in 2006 developed light superficial scald after 20 weeks of cold storage plus 5 d at 20 °C; all others were scald-free. By contrast, all CA fruit from both seasons showed light scald after 12–14 weeks, and moderate scald after 20–24 weeks, plus shelf life. Correspondingly, α-farnesene and CTs accumulated more rapidly and on average reached about twofold higher concentrations in CA compared with WA fruit over the first 8–12 weeks of storage. CA fruit also had an earlier rise in ethylene production, higher respiratory rates, and lower flesh firmness at harvest than WA fruit. These suggest advanced maturity, which may have contributed to the increased rates of α-farnesene synthesis and oxidation, and higher incidence of scald.
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1. Introduction

Superficial scald is a commercially important physiological storage disorder that occurs in apples and pears. Symptoms manifest as brown or black patches on the skin and typically worsen after removal from storage, rendering the fruit unmarketable. Oxidative stress is the generally accepted cause of superficial scald (Whitaker, 2004), and conjugated triene (CT) oxidation products of the sesquiterpene α-farnesene are thought to play a key role in induction of the disorder (Huelin and Coggiola, 1970; Anet, 1972; Chen et al., 1990b; Rowan et al., 2001), although other factors are clearly involved (Rao et al., 1998; Whitaker et al., 2000). There is wide variation in superficial scald susceptibility among fruits of commercial apple and pear cultivars, those most prone to the disorder often, but not always, exhibiting high level accumulation of CTs in peel tissue after a few months of cold storage (Huelin and Coggiola, 1970; Anet, 1972; Chen et al., 1990b; Whitaker et al., 1997; Gapper et al., 2006; Tsantili et al., 2007).

The major CT oxidation products of α-farnesene that accumulate in apple and pear peel tissue during cold storage have been identified as conjugated trienols (CTols), 3*E* and 3*Z* isomers of 3,7,11-trimethyldodeca-1,3,5(*E*),10-tetraen-7-ol (Rowan et al., 1995; Whitaker et al., 1997; Whitaker, 2007). Application of these CTols and their corresponding hydroperoxides to apple fruit prior to storage induced symptoms indistinguishable from naturally occurring superficial scald (Rowan et al., 2001). Pre-storage treatment of apples and pears with the antioxidants diphenylamine and ethoxyquin inhibits oxidation of α-farnesene and largely controls superficial scald development (Huelin and Coggiola, 1970; Chen et al., 1990a,b). In addition, exposure of apple and pear fruit to the blocker of ethylene action 1-methylcyclopropene (1-MCP) greatly curtails α-farnesene production and markedly reduces superficial scald incidence and severity (Fan et al., 1999; Watkins et al., 2000; Argenta et al., 2003; Chen and Spotts, 2005; Lurie et al., 2005;

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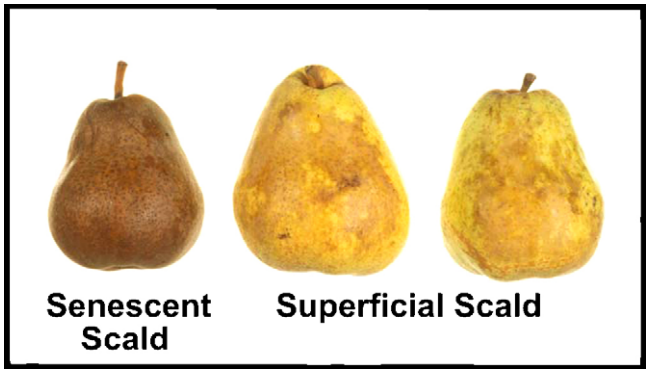

Fig. 1. 'Bartlett' pears grown in northern California exhibiting severe symptoms of senescent scald (left) and superficial scald (center and right) after 5 months of storage in air at −1 °C plus 5 d at 20 °C.

Gapper et al., 2006). Results from the studies with 1-MCP, as well as several prior reports (Du and Bramlage, 1994; Watkins et al., 1995; Whitaker and Solomos, 1997; Ju and Curry, 2000a), have shown that ethylene production and perception, and tissue responsiveness to ethylene, are involved in regulation of α-farnesene synthesis and induction of superficial scald in apples and pears.

Other factors that influence the occurrence and severity of superficial scald include fruit maturity at harvest, storage conditions (e.g., ventilation, and O_2 and CO_2 concentrations), mineral nutrition, pre-harvest temperatures, and orchard locality (Ingle and D'Souza, 1989; Ingle, 2001; Chen, 2004). The last two of these factors as well as maturity are most pertinent to our observation that 'Bartlett' pears grown in northern California (CA) consistently show development of superficial scald, particularly after long-term storage of 4–5 months in air (Bower et al., 2003; Ekman et al., 2004), whereas fruit of this cultivar grown in central Washington (WA) are typically less susceptible to the disorder, developing mild or no symptoms. It must be noted that senescent scald is generally regarded as a more common and serious problem than superficial scald in 'Bartlett' pears (Meheriuk et al., 1994; Zoffoli et al., 1998; Chen, 2004). However, symptoms of the two storage disorders, shown in Fig. 1, are readily distinguished. Symptoms of senescent scald typically start as uniform dark blotches around the calyx end of the fruit, spreading upward with longer storage duration to often cover the entire fruit surface (Zoffoli et al., 1998). By contrast, superficial scald symptoms first arise as small blotches on the neck of the fruit, spread non-uniformly on mainly one side, often appear only after removal from storage, and on 'Bartlett' fruit are generally much paler than senescent scald symptoms (Fig. 1).

The present study was conducted over two seasons (2006–2007 and 2007–2008) to evaluate metabolic differences between CA- and WA-grown 'Bartlett' pears that could produce the disparity in superficial scald susceptibility exhibited in fruit from these two regions. Our main focus was on accumulation of α-farnesene and its CTol oxidation products in peel tissue during cold storage in relation to scald incidence and severity. Limited data on other physiological and metabolic parameters, including fruit flesh firmness, skin color, and rates of ethylene production and respiration, were also acquired.

2. Materials and methods

2.1. Fruit, storage conditions, and tissue sampling

'Bartlett' pear (*Pyrus communis* L.) fruit were obtained from commercial orchards in Ukiah, CA and Finley, CA in the 2006 and 2007 seasons, respectively, and in Monitor, WA in both seasons. California fruit were picked on 14 and 21 August in 2006 (Harvest 1 and Harvest 2; average flesh firmness 73.8 and 69.0 N, respectively), and on 21 August in 2007 (Harvest 1; average flesh firmness 63.6 N). Washington fruit were picked on 22 and 25 August in 2006 (Harvest 1 and Harvest 2; average flesh firmness 80.9 and 78.4 N, respectively), and on 7 and 14 August in 2007 (Harvest 1 and Harvest 2; average flesh firmness 83.4 and 80.1 N, respectively). Defect-free fruit from each harvest were placed on fiber trays, packed in boxes with perforated plastic liners (60 fruit per box), and stored in air at −1 °C and >90% relative humidity.

Fruit from both locations were sampled at harvest and immediately after removal from cold storage at 2, 4, 8, 12, 16, and 20 weeks. Peel tissue, including the epidermis and 1–2 mm of hypodermal cortex, was excised with a stainless steel fruit peeler from the equatorial region of 8–10 randomized fruit from each box and immediately frozen in liquid N_2. Pooled samples of about 25–30 g were stored at −80 °C in zip-lock bags until shipped packed in dry ice to the USDA, ARS Food Quality Laboratory by overnight courier. Upon arrival, the peel tissue samples were stored at −80 °C until used for extraction and analysis of α-farnesene and CTols.

2.2. Firmness, respiration, and ethylene measurements, and evaluation of scald and color

Parameters of fruit quality and physiology, including flesh firmness, rates of respiration and ethylene evolution, and scald incidence, were measured at harvest and after 2, 4, 8, 12, 14, 16, 20, and/or 24 weeks of −1 °C storage. Measurements were made at 0–8 d after transfer to 20 °C.

Flesh firmness was determined at the University of California-Davis with a model GS-14 Fruit Texture Analyzer (Güss Manufacturing Ltd., Strand, South Africa) and at the USDA-ARS TFRL in Wenatchee, WA with a Mohr Digi-Test computerized penetrometer (Mohr and Associates, Richland, WA), both using an 8-mm diameter probe. Two measurements were obtained per fruit (18–24 fruit total) on opposite sides of the equator after removal of 20-mm diameter peel discs.

Respiration (CO_2 evolution) and ethylene production at 20 °C were measured by placing 4–6 fruit (total mass about 1 kg) into a 3.7-L jar, which was sealed it for 10–60 min prior to withdrawal of headspace gas samples for analysis. At the University of California-Davis, CO_2 and ethylene concentrations in headspace gas samples were determined, respectively, using a rapid gas analyzer, model VIA510 (Horiba, Japan), and a gas chromatograph (GC), model AGC Series 400 (Hach-Carle Co., USA), with a flame ionization detector (FID) and fitted with an alumina column. At the USDA-ARS TFRL, an HP 5890 GC-FID (Hewlett Packard, Avondale, PA) equipped with a methanizer (John T. Booker, Austin, TX), and an HP 5880A GC-FID, both instruments fitted with Poropak Q columns, were used to measure CO_2 and ethylene concentrations, respectively, as described by Fan et al. (1998).

Superficial scald was assessed visually in 18–24 fruit from each storage duration 5 d after transfer from cold storage to 20 °C. Scald incidence was defined as the percentage of pears exhibiting slight to severe scald symptoms on ≥10% of the fruit surface. Scald severity was evaluated subjectively using the scale: 0, none; 1, slight; 2, moderate; 3, severe, according to the criteria established by Chen et al. (1990a). For the CA fruit, color was also evaluated visually using the California Department of Agriculture color chart (1, green; 2, light green; 3, light yellow; 4, yellow).

2.3. Extraction and quantification of α-farnesene and conjugated trienols (CTols)

Hexane extraction of α-farnesene and CTols from samples of each set of pooled frozen pear peel tissue was conducted as described in Whitaker et al. (1997) with the exception that 3 g of

tissue were immersed in 12 mL of hexane. Four replicate tissue samples were extracted for each set. A 1.5-mL aliquot of the 12-mL extract was transferred to a 2-mL glass vial, the hexane was evaporated with a gentle stream of N_2, and the residue was dissolved in 400 μL of methanol. After centrifugation for 2 min at 2000 × *g* to pellet insoluble wax, the supernatant was transferred to a clean 2-mL vial with a fine-tipped Pasteur pipet. The methanolic samples were analyzed by high-performance liquid chromatography (HPLC) using a Hewlett-Packard Series 1100 HPLC system (Agilent Technologies) with a quaternary pump, autosampler, and diode array detector (DAD). Sample vials were placed in the autosampler and 25-μL aliquots were injected onto a Luna 5 μm particle size C18(2) column (250 mm long, 4.6 mm i.d.) from Phenomenex (Torrance, CA) and eluted with isocratic methanol:water:acetonitrile, 90:5:5 (v/v/v), at a flow rate of 13.3 μL s^{-1}. DAD monitoring at 232 and 269 nm was used to determine levels of α-farnesene and CTols, which eluted at 13.8 and 5.8 min, respectively. HPLC-purified apple α-farnesene and CTol samples were used as external standards for quantification (Whitaker et al., 1997). Concentrations of these standards were calculated using the molar extinction coefficients $\varepsilon_{232\,nm}$ = 27,740 for α-farnesene (Huelin and Coggiola, 1968) and $\varepsilon_{269\,nm}$ = 42,500 for CTols (Anet, 1969).

3. Results

3.1. Accumulation of α-farnesene and CTols in pear peel tissue during storage

Concentrations of α-farnesene in peel tissue of CA and WA 'Bartlett' pears harvested on two dates (H1 and H2) in mid to late August in 2006 and stored up to 20 weeks in air at −1 °C are compared in Fig. 2. Regardless of fruit origin or maturity, only trace levels of α-farnesene were detectable at harvest and after 2 weeks of storage. Thereafter, patterns of change were closely similar for H1 and H2 CA fruit, with a sharp increase from 4 to 8 weeks, a modest decline from 8 to 12 weeks, and a more rapid decline through 20 weeks. The concentration also rose sharply from 4 to 8 weeks in peel of WA pears, but the pattern after 8 weeks differed in H1 and H2 fruit, decreasing linearly in H1 and increasing to a maximum at 12 weeks in H2 before falling off. Regardless of harvest date, α-farnesene accumulated more rapidly and to higher levels in CA than in WA fruit. Concentrations were about three- to fivefold greater at 4 weeks, and nearly twofold greater at 8 weeks, in CA compared with WA pears. Maximum levels in CA and WA fruit were, respectively, about 113 and 65 mg kg^{-1} peel fresh weight for H1, and 99 and 73 mg kg^{-1} for H2.

Fig. 3 shows the corresponding comparison of CTol concentrations in peel tissue of CA and WA 'Bartlett' pears from the two harvests in 2006. In all fruit, CTols were undetectable at harvest and after 2 weeks of storage. As observed for α-farnesene, the levels CTols and their patterns of change during storage were remarkably similar in CA fruit from H1 and H2. Only trace levels were detected at 4 weeks, followed by a steady, rapid increase through 12 weeks, and a linear decline thereafter. Generally, with the exception of the final 4 weeks of storage, these same trends were observed in WA fruit from both H1 and H2. However, from 8 to 16 weeks the level of CTols was on average about 2.6-fold lower in WA than in CA fruit. Late in storage, from 16 to 20 weeks, CTols in WA fruit increased slightly (H1) or leveled off (H2). Maximum CTol concentrations in CA and WA fruit were, respectively, 7.3 and 3.1 mg kg^{-1} peel fresh weight for H1, and 7.0 and 3.3 mg kg^{-1} for H2.

Only a single harvest of late maturity CA 'Bartlett' pears, H1, was available in 2007; hence, accumulation of α-farnesene and CTols in these fruit was compared with that of WA pears from the second 2007 harvest, H2 (Fig. 4). The pattern of accumulation and levels of α-farnesene in the CA fruit (Fig. 4, upper panel) were much the same as those observed for both H1 and H2 CA fruit in the previous season (Fig. 3), with a maximum at 8 weeks of 109 mg kg^{-1}. The same was generally true of CTol accumulation and levels in 2007 CA fruit (Fig. 4, lower panel), although the maximum concentration at 12 weeks (7.9 mg kg^{-1}) was slightly higher than that in 2006 H1 and H2 CA fruit (Fig. 3), and the levels declined more slowly after 12 weeks. The pattern of α-farnesene accumulation in 2007 H2 WA fruit (Fig. 4, upper panel) most closely resembled that in 2006 H2 WA fruit (Fig. 2, lower panel), but was more clearly biphasic, reaching the highest concentration at 20 weeks (90 mg kg^{-1}) after a small decline from 12 to 16 weeks. As was noted for CA and WA pears from the 2006 harvests (Fig. 2), higher levels of α-farnesene accumulated over the initial 12 weeks of storage in CA compared with WA H2 fruit harvested in 2007 (Fig. 4, upper panel). Despite the fact that α-farnesene levels in 2007 WA H2 fruit were about the same as those in 2006 WA H2 fruit through 12 weeks, and were substantially higher at 16 and 20 weeks, accumulation of CTols was more gradual, reaching a maximum of 3.2 mg kg^{-1} at 20 weeks (Fig. 4, lower panel).

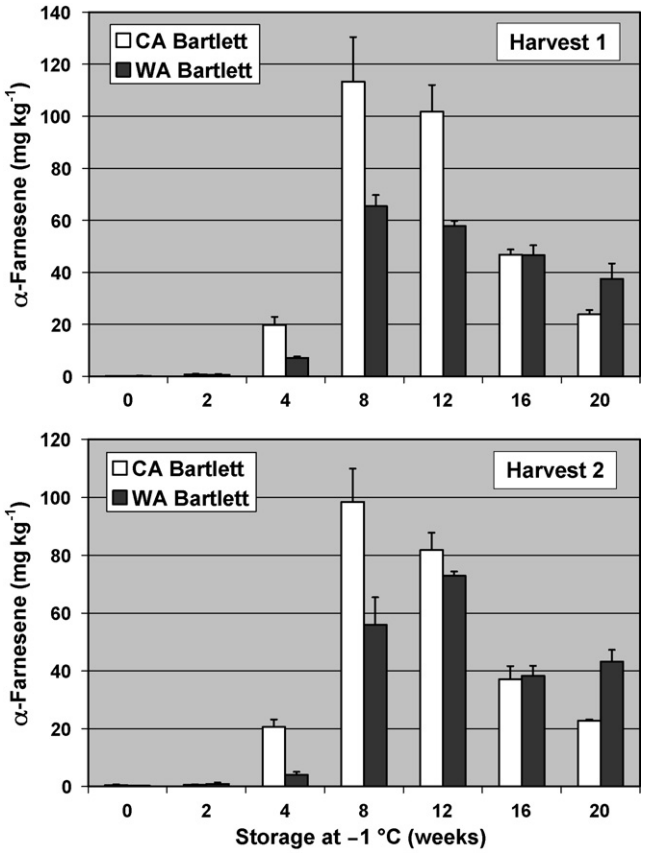

Fig. 2. Concentrations of α-farnesene in peel tissue of 'Bartlett' pears grown in California (CA) and Washington (WA) in 2006, and stored from 0 to 20 weeks in air at −1 °C and >90% relative humidity. Harvest 1 fruit (upper panel) were picked on 14 August (CA) and 22 August (WA), and Harvest 2 fruit (lower panel) were picked on 21 August (CA) and 25 August (WA). Vertical bars represent the mean concentration from four replicate tissue samples and error bars indicate 1 S.D.

3.2. Scald incidence and severity

In the 2006–2007 season (harvests in August, 2006), H1 and H2 CA fruit were evaluated for scald incidence and severity immediately after removal from −1 °C air storage and/or after 5 additional days at 20 °C, whereas CA fruit in the 2007–2008 season, and WA fruit in both seasons, were evaluated only after 5 d post-storage at 20 °C (Table 1). Regardless of maturity, CA pears harvested in 2006

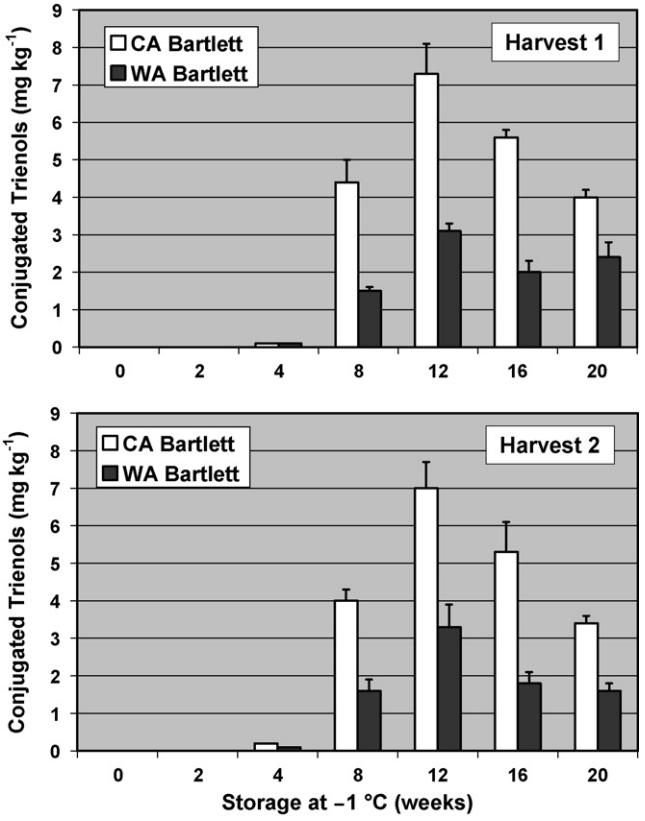

Fig. 3. Concentrations of conjugated trienol oxidation products of α-farnesene in peel tissue of 'Bartlett' pears grown in California (CA) and Washington (WA) in 2006, and stored from 0 to 20 weeks in air at −1 °C and >90% relative humidity. Harvest 1 fruit (upper panel) were picked on 14 August (CA) and 22 August (WA), and Harvest 2 fruit (lower panel) were picked on 21 August (CA) and 25 August (WA). Vertical bars represent the mean concentration from four replicate tissue samples and error bars indicate 1 S.D.

showed no scald directly after 12 weeks of storage; however, after 14 weeks plus 5 d at 20 °C, there was light scald on over 50% of H1 and over 80% of H2 fruit. Accordingly, there was also light scald on 25% of the CA fruit from the one harvest in 2007 after 12 weeks plus 5 d shelf life. H2 2006 CA pears had a low incidence of light scald when removed after 16 weeks, and two-third of the 2007 CA pears exhibited moderate scald after 16 weeks plus 5 d at 20 °C. Scald incidence and severity in 2006 H1 and H2 CA fruit directly out of storage at 20 weeks were about the same as after 14 weeks plus 5 d shelf life. Directly after 24 weeks of storage, scald severity (but not incidence) was increased, particularly in H2 fruit. A high incidence of moderately severe scald developed on all CA fruit after 24 weeks (2006) or 20 weeks plus 5 d at 20 °C (2007). By contrast, no scald was observed on the WA pears with the exception of H2 fruit from the 2006 harvest, one-third of which developed light scald after 20 weeks plus 5 d at 20 °C.

3.3. Fruit flesh firmness and skin color

Over the two seasons, fruit flesh firmness at harvest ranged from an average of 63.6–73.8 N for CA pears and 78.3–83.4 N for WA pears. Fruit from the second harvest (H2) were invariably less firm than those from the first harvest (H1). CA fruit from all three harvests in 2006 and 2007 softened completely after 8 d at 20 °C (average firmness 6.9 N), whereas WA pears from all four harvests had softened only slightly (average decrease about 4 N) after 5 d at 20 °C (Table 2; data not shown for WA 2007 H1). Because WA fruit were substantially firmer at harvest, they were generally firmer

Table 1
Incidence (SI) and severity (SS) of superficial scald on California and Washington 'Bartlett' pears at harvest and after 12–24 weeks of storage in air at −1 °C plus 0 or 5 d shelf life at 20 °C.

Week	Day	2006 harvest 1[a]		2006 harvest 2[b]		2007 harvest 1[c]	
		SI[d]	SS[e]	SI	SS	SI	SS
California							
12	0	0	0.0	0	0.0	–	–
	5	–	–	–	–	25	1.0
14	5	55	1.2	81	1.3	–	–
16	0	0	0.0	8	1.0	–	–
	5	–	–	–	–	67	2.0
20	0	50	1.2	75	1.1	–	–
	5	–	–	–	–	92	2.0
24	0	58	1.4	58	1.9	–	–
	5	81	2.2	94	2.1	–	–

Week	Day	2006 harvest 1[a]		2006 harvest 2[b]		2007 harvest 2[c]	
		SI	SS	SI	SS	SI	SS
Washington							
12	5	0	0.0	0	0.0	0	0.0
16	5	0	0.0	0	0.0	0	0.0
20	5	0	0.0	33	1.0	0	0.0

[a] California and Washington fruit were picked on 14 and 22 August, respectively.
[b] California and Washington fruit were picked on 21 and 25 August, respectively.
[c] California and harvest 2 Washington fruit were picked on 22 and 14 August, respectively.
[d] Scald incidence was evaluated visually as the percent of fruit exhibiting slight to severe symptoms (n = 18–24).
[e] Scald severity was evaluated visually using the scale 0, no scald; 1, slight; 2, moderate; 3, severe (n = 18–24).

Table 2
Flesh firmness in California and Washington 'Bartlett' pears at harvest and after up to 24 weeks of storage in air at −1 °C plus 0–8 d shelf life at 20 °C.

Week	Day	2006 harvest 1[a] Firmness (N)[d]	2006 harvest 2[b] Firmness (N)	2007 harvest 1[c] Firmness (N)
California				
0	1	73.8 (0.8)	69.0 (3.0)	63.6 (0.7)
	8	6.7 (0.3)	6.5 (0.2)	7.6 (0.1)
12	0	–	–	50.8 (4.9)
	6	–	–	8.0 (0.7)
14	0	62.3 (1.2)	54.3 (2.2)	–
	5	12.1 (0.2)	15.7 (0.7)	–
20	0	–	–	45.8 (2.0)
	4	–	–	15.6 (1.3)
24	0	52.5 (1.2)	45.8 (0.8)	–
	5	17.3 (0.8)	12.5 (1.1)	–

Week	Day	2006 harvest 1[a] Firmness (N)	2006 harvest 2[b] Firmness (N)	2007 harvest 2[c] Firmness (N)
Washington				
0	1	80.9 (1.5)	78.3 (1.3)	80.1 (1.2)
	5	77.9 (1.8)	74.5 (1.9)	74.7 (1.1)
12	1	70.7 (1.8)	68.9 (1.1)	64.3 (1.6)
	5	9.1 (0.6)	12.8 (0.5)	12.3 (0.8)
16	1	55.7 (2.3)	54.3 (2.2)	60.5 (2.2)
	5	10.8 (0.4)	15.7 (0.7)	21.8 (1.8)
20	1	53.3 (1.2)	55.6 (1.3)	50.1 (1.9)
	5	14.1 (1.0)	16.6 (1.4)	26.8 (1.9)

[a] California and Washington fruit were picked on 14 and 22 August, respectively.
[b] California and Washington fruit were harvested on 21 and 25 August, respectively.
[c] California and harvest 2 Washington fruit were picked on 22 and 14 August, respectively.
[d] Mean fruit flesh firmness with standard error of the mean shown in parentheses (n = 18–24).

after 12 weeks plus 1 d at 20 °C (average 70.1 N) than CA fruit directly out of storage after 12–14 weeks (average 55.8 N). Nevertheless, CA and WA pears from all harvests were clearly competent to ripen after 12 weeks in air at −1 °C, and overall showed a similar degree of softening after 12–16 weeks of storage plus 5 or 6 d at 20 °C. A trend noted in WA fruit, particularly those harvested in 2007, was a decrease in the loss of firmness during 5 d shelf life with increasing duration of cold storage, suggesting a decline in ripening capacity. This trend was also evident in CA fruit, although generally less pronounced.

Fruit skin color at harvest and immediately after up to 24 weeks of cold storage was evaluated for 2006 H1 and H2 CA fruit. The color change from green to yellow progressed at about the same rate in pears from the two harvests. Fruit remained fully green (color score 1.0) for the first 4 weeks, but at 8 weeks there was a hint of degreening, which was more evident in H2 than in H1 (color scores 1.4 and 1.1, respectively). By 12 weeks there was slight yellowing (H1, 2,4; H2, 2.2) and at 16 weeks fruit were light to medium yellow (H1, 3.0; H2, 3.4). After 20 and 24 weeks of storage, most or all pears from both harvests were fully yellow (color scores 3.8–4.0). Skin color of CA pears from the single harvest in 2007 was evaluated after 0–20 weeks of −1 °C storage plus 5 d at 20 °C. Regardless of storage duration and even with fruit just after harvest, the 5 d ripening period resulted in full yellow color development (all color scores 4.0).

3.4. Ethylene production and respiration rate

Rates of respiration (CO_2 evolution) and ethylene production by ~1 kg fruit samples were determined after harvest and up to 24 weeks of storage plus 1–6 d at 20 °C (Table 3). Regardless of harvest date or season, freshly harvested CA fruit produced low levels of ethylene and had moderate rates of respiration after only 2 d at 20 °C. Moreover, after an additional 4 d at 20 °C, ethylene production had increased many fold, to an average of about 1.0 nmol kg^{-1} s^{-1}, and CO_2 evolution had increased about two- to fourfold, to an average of about 490 nmol kg^{-1} s^{-1}. By contrast, freshly harvested WA fruit from H1 and H2 in both seasons did not produce detectable levels ethylene and had low respiratory rates (CO_2 production < 100 nmol kg^{-1} s^{-1}) when held at 20 °C for 1 or 5 d. WA fruit began to produce low levels of ethylene after 4 weeks of storage plus 1 d at 20 °C, and 5 d after removal the rate increased to an average of 0.71 nmol kg^{-1} s^{-1}. Accordingly, the rate of respiration at 4 weeks plus 1 d was about twice that at harvest plus 5 d, and CO_2 production by WA pears stored 4 weeks had doubled again after 5 d at 20 °C, to about 290 nmol kg^{-1} s^{-1}. From 12 weeks to the end of storage, maximum rates of ethylene production were nearly the same (close to 2.0 nmol kg^{-1} s^{-1}) in CA and WA fruit, although on average the rate was approximately 20% higher in the CA pears. Respiratory rates, however, were overall substantially higher in CA than in WA fruit, with maximum CO_2 production of about 600–700 nmol kg^{-1} s^{-1} in CA and 270–330 nmol kg^{-1} s^{-1} in WA pears.

4. Discussion

In accord with our observations over the past decade or more, 'Bartlett' pears harvested from commercial orchards in northern CA were much more prone to development of superficial scald in

Table 3
Carbon dioxide (CO_2) and ethylene (C_2H_4) production by California and Washington 'Bartlett' pears at harvest and after up to 24 weeks of storage in air at −1 °C plus 1–6 d shelf life at 20 °C.

Week	Day	2006 harvest 1[a]		2006 harvest 2[b]		2007 harvest 1[c]	
		CO_2[d]	C_2H_4[e]	CO_2	C_2H_4	CO_2	C_2H_4
California							
0	2	265 (11)	0.27 (0.01)	133 (2)	0.15 (0.05)	252 (12)	0.05 (0.01)
	6	486 (30)	1.16 (0.05)	530 (5)	1.02 (0.08)	452 (13)	0.97 (0.04)
12	1	–	–	–	–	491 (31)	2.07 (0.07)
	5	–	–	–	–	578 (31)	1.33 (0.04)
14	2	581 (9)	2.05 (0.08)	726 (44)	1.75 (0.10)	–	–
	5	518 (11)	1.54 (0.08)	568 (93)	0.90 (0.15)	–	–
20	1	–	–	–	–	686 (28)	2.04 (0.10)
	4	–	–	–	–	479 (22)	1.03 (0.07)
24	2	345 (91)	1.11 (0.20)	473 (25)	1.56 (0.12)	–	–
	5	385 (13)	0.89 (0.13)	404 (15)	0.72 (0.14)	–	–
Week	Day	2006 harvest 1[a]		2006 harvest 2[b]		2007 harvest 2[c]	
		CO_2	C_2H_4	CO_2	C_2H_4	CO_2	C_2H_4
Washington							
0	1	72 (3)	0.00 (0.00)	29 (2)	0.00 (0.00)	84 (3)	0.00 (0.00)
	5	64 (4)	0.00 (0.00)	97 (4)	0.00 (0.00)	71 (6)	0.00 (0.00)
4	1	144 (8)	0.02 (0.01)	149 (18)	0.11 (0.01)	135 (11)	0.28 (0.08)
	5	306 (7)	0.54 (0.03)	297 (3)	0.61 (0.13)	273 (46)	0.85 (0.27)
12	1	215 (29)	1.05 (0.21)	170 (1)	0.87 (0.10)	221 (19)	1.45 (0.06)
	5	333 (20)	1.11 (0.29)	226 (16)	0.89 (0.09)	191 (14)	0.91 (0.13)
16	1	278 (3)	1.59 (0.06)	244 (27)	1.16 (0.18)	248 (9)	1.96 (0.08)
	5	271 (9)	1.03 (0.09)	252 (47)	1.07 (0.21)	273 (8)	1.11 (0.05)
20	1	307 (7)	1.77 (0.45)	179 (3)	0.88 (0.24)	248 (13)	1.46 (0.14)
	5	287 (13)	0.83 (0.19)	315 (7)	0.91 (0.06)	–	–

[a] California and Washington fruit were picked on 14 and 22 August, respectively.
[b] California and Washington fruit were picked on 21 and 25 August, respectively.
[c] California and harvest 2 Washington fruit were harvested on 22 and 14 August, respectively.
[d] Mean rate of CO_2 production (nmol kg^{-1} s^{-1}) by ~1 kg fruit samples with standard error of the mean shown in parentheses (n = 4–6).
[e] Mean rate of ethylene production (nmol kg^{-1} s^{-1}) by ~1 kg fruit samples with standard error of the mean shown in parentheses (n = 4–6).

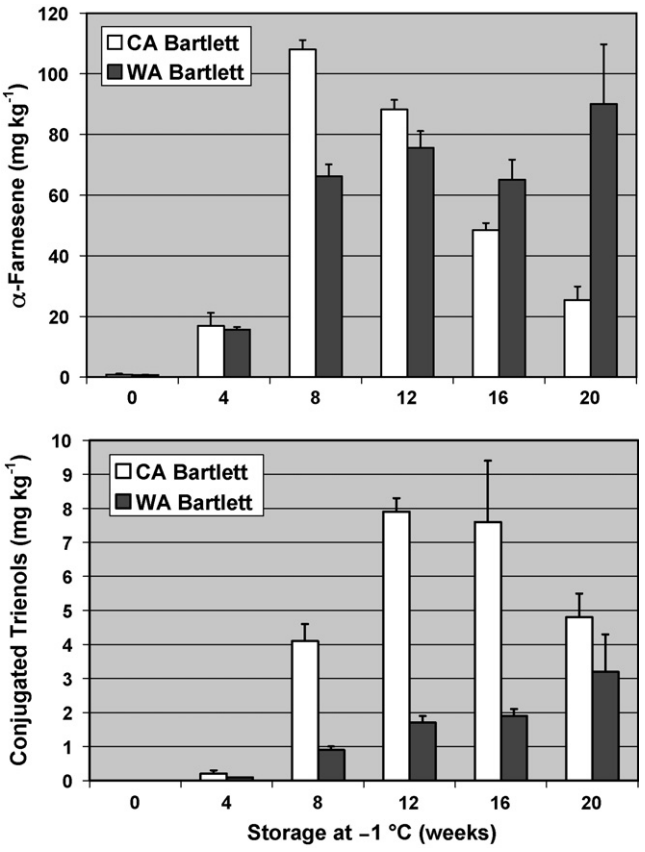

Fig. 4. Concentrations of α-farnesene (upper panel) and conjugated trienols (lower panel) in peel tissue of 'Bartlett' pears grown in California (CA) and Washington (WA) in 2007, and stored from 0 to 20 weeks in air at −1 °C and >90% relative humidity. CA fruit were harvested on 21 August and WA fruit were harvested on 14 August. Vertical bars represent the mean concentration from four replicate tissue samples and error bars indicate 1 S.D.

the 2006–2007 and 2007–2008 seasons than fruit of this cultivar from central WA. Specifically, with long-term air storage of 20–24 weeks plus a ripening period of 5 d after removal, 81–94% of CA pears from all three harvests developed moderate scald, whereas 33% of the WA pears from only one of four harvests exhibited light scald symptoms (Table 1). Ekman et al. (2004) reported a similar high incidence of scald on CA 'Bartlett' pears harvested in the Sacramento area, and Bower et al. (2003) observed slight scald on CA fruit harvested in Mendocino County after only 3 months of storage at −1 °C plus 4 d at 20 °C. It must be noted, however, that in each case, and particularly in the present study, the CA fruit were of late maturity based on criteria such as firmness at harvest and early onset of ethylene production. The WA pears in this study, on the other hand, were harvested at more or less optimal commercial maturity. This is an important distinction when comparing fruit grown in the two regions.

From a practical, commercial standpoint, air storage of long duration is not relevant because of the short chilling requirement for ripening of 'Bartlett' pears and their tendency to soften, yellow, and develop other storage disorders including core breakdown, internal browning, and senescent scald (Blanpied, 1975; Meheriuk et al., 1994; Zoffoli et al., 1998). However, even when the CA fruit in this study were evaluated after 14–16 weeks cold storage plus 5 d shelf life, over 50% developed light to moderate scald, which potentially could result in significant loss during marketing.

The clear difference in scald susceptibility of CA and WA fruit evaluated in this study provides a basis for assessment of factors contributing to development of the disorder in a single, commercially important cultivar. As stated above, by several criteria the most obvious difference in fruit from the two locations is the relatively advanced maturity of the CA pears, despite the more or less comparable harvest dates within the 18-d span from 7 to 25 August over two seasons. In contrast with WA fruit, CA fruit were near the lower limit of firmness considered acceptable for commercial harvest (Agar et al., 1999; Bower et al., 2003; Chen, 2004), and were largely capable of ripening and softening without any chilling period. Moreover, CA pears invariably produced detectable levels of ethylene earlier than WA pears, and on the whole had substantially higher rates of respiration (Table 3). It is well documented that immature, early-harvest apples are generally more prone to scald than mature fruit from subsequent harvests (Ingle and D'Souza, 1989; Ingle, 2001), but the relationship between maturity and superficial scald is not as clear with pears. For the pear cultivars 'd'Anjou' and 'Packham's Triumph', Zoffoli et al. (1998) found that fruit of commercial maturity exhibited the highest incidence and severity of superficial scald. Raese and Drake (2000), on the other hand, reported a higher incidence of scald in late- than in mid-harvest 'd'Anjou' pears, and in a study by Gapper et al. (2006), 'd'Anjou' fruit harvested 2 weeks after commercial maturity had a 100% incidence of scald after 3 months at −1 °C plus 7 d at 20 °C. More pertinent to the present study, Bower et al. (2003) found that late-harvest CA 'Bartlett' pears were more susceptible to superficial scald than fruit from harvests occurring 2–4 weeks earlier. Interestingly, when stored at 2 °C even the early-harvest fruit were prone to the disorder. 'Bartlett' pears of advanced maturity are also particularly susceptible to CO_2 injury (internal browning), core breakdown, and senescent scald (Claypool, 1973; Zoffoli et al., 1998; Ju et al., 2001).

Considering differences in the two growing regions that could influence relative scald susceptibility, climate, and in particular day/night temperatures and rainfall during the weeks preceding harvest, are factors known to play a part in the incidence and severity of scald after storage (Ingle and D'Souza, 1989; Weis et al., 1998; Kupferman, 2001; Villalobos-Acuña and Mitcham, 2008). Comparison of the average day/night temperatures and precipitation in Ukiah, CA and Monitor, WA during the months of July and August (http://www.weather.com) revealed that the two locations are climatically quite similar. Over the 2 months, daytime temperatures average 32.5 and 30.8 °C, and nighttime temperatures 12.8 and 15.8 °C, in Ukiah and Monitor, respectively. Although the differences are fairly small, the cooler nighttime temperatures experienced by the CA-grown fruit could conceivably promote early ethylene production and increase scald susceptibility (Agar et al., 1999). As well, rainfall is slight in both locations over the two summer months, but nearly fourfold lower in CA than in WA. This could be another preharvest stress factor for CA pears, notwithstanding irrigation in the orchards.

The differences in α-farnesene metabolism in CA and WA 'Bartlett' pears found in this investigation are most likely attributable to differences in the physiological status of the fruit. More rapid and abundant accumulation of α-farnesene in CA compared with WA fruit during the initial 8–12 weeks of storage (Figs. 1 and 3) may result from both the relatively early onset of ethylene production and the higher respiratory rates in CA fruit. The sharp increase in α-farnesene production after apples and pears are placed in cold storage is closely associated with ethylene-induced up-regulation of the α-farnesene synthase gene, *AFS1* (Lurie et al., 2005; Gapper et al., 2006; Tsantili et al., 2007). In CA 'Bartlett' pears stored in air at −1 °C, ethylene production increased about 100-fold from 3 to 5 weeks to roughly 50 pmol kg^{-1} s^{-1}, and treatment with 1-MCP at 0.5 or 1.0 μL L^{-1}, respectively, delayed or prevented scald development (Ekman et al., 2004). The high rates of respiration in CA pears could also result in a relatively large pool of acetyl-CoA, the initial substrate required for synthesis of α-farnesene via the

mevalonic acid pathway (Ju and Curry, 2000b; Rupasinghe et al., 2001). Important unanswered questions from this investigation are whether CA 'Bartlett' fruit harvested at commercial maturity would still produce more α-farnesene than WA fruit of equal maturity, and conversely whether α-farnesene synthesis would increase in late-harvest WA fruit. Although not conclusive, from the available data it can be inferred that the answer to these questions is no, inasmuch as for both CA and WA fruit there was a modest decrease in α-farnesene levels with advancing maturity.

As observed in numerous prior studies, the precursor–product relationship of α-farnesene and its CTol oxidation products is clearly evident in Figs. 2–4. The accumulation of twofold or more higher levels of CTols in CA compared with WA fruit (Figs. 3 and 4) is indicative of poorer antioxidative defenses and greater oxidative stress, resulting in more rapid autoxidation of α-farnesene (Anet, 1974; Meir and Bramlage, 1988; Shaham et al., 2003; Whitaker, 2004). It is noteworthy that in 2007 WA H2 compared with 2006 WA H1 and H2 fruit, despite the somewhat higher levels of α-farnesene (Figs. 2 and 4), the levels of CTols remain relatively low until the end of storage (Figs. 3 and 4). This supports the conclusion from many earlier studies (e.g., Huelin and Coggiola, 1968; Anet, 1972) that factors other than α-farnesene accumulation per se determine the levels of CTols produced.

The overall much higher concentrations of CTols in CA than in WA pears at 8 weeks of storage and thereafter (Figs. 3 and 4) is in accord with their hypothesized role in the induction of scald. However, it must be noted that the maximum concentration of CTols in the CA fruit is much less than that determined for a number of other apple and pear cultivars, including a few such as 'Empire' apple (Whitaker, 2000) that are resistant to scald. In a recent study of highly scald-susceptible 'd'Anjou' pears from the Hood River area in Oregon (Gapper et al., 2006), the maximum CTol concentration was more than 10-fold higher than that of the CA 'Bartlett' fruit in this study. The comparison of 'd'Anjou' and 'Bartlett' pears appears to be analogous to that of selected 'Rome Beauty' X 'White Angel' hybrid apple lines investigated by Whitaker et al. (2000). Among the latter, three lines that developed severe scald symptoms accumulated the highest levels of CTols, whereas two lines that exhibited mild to moderate scald had much lower levels. One conclusion is that in certain instances the fruit tissues are so oxidatively compromised that the free radicals generated by even small amounts of CTols are sufficient to induce some injury. Alternatively, processes related to oxidative stress and senescence other than oxidation of α-farnesene may play a role of at least equal importance in scald induction (Rao et al., 1998; Whitaker et al., 2000; Whitaker, 2004). Only when transgenic fruit of a highly scald susceptible apple or pear cultivar silenced for α-farnesene production are available will it be possible to answer the question of whether CTols are the sole or major causal agents of scald.

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Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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