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Superficial Scald and Bitter Pit Development in Cold-Stored Transgenic Apples Suppressed for Ethylene Biosynthesis

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The plant hormone ethylene regulates climacteric fruit ripening and plays a major role in the development of superficial scald in apple fruits during cold storage. The effect of cold storage at 0 °C on development of superficial scald and bitter pit (BP) in transgenic Greensleeves (GS) apples suppressed for ethylene biosynthesis was investigated. Four apple lines were used: untransformed GS; line 68G, suppressed for 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase (ACO); and lines 103Yand 130Y, suppressed for ACC synthase (ACS). Fruits from the transformed lines 68G, 103Y, and 130Y produced very little ethylene during 3 months of cold storage at 0 °C and after subsequent transfer to 20 °C, whereas untransformed fruits produced significant ethylene during cold storage, which increased dramatically at 20 °C. Respiration, expressed as CO_2 production, was similar in all four apple lines. After 2 months at 0 °C, all apple lines showed some BP symptoms, but lines 68G and 103Y were more affected than untransformed GS or line 130Y. Both transformed and untransformed apples produced α-farnesene, but concentrations were lower in yellow fruit than in green fruit in all lines but 68G. Line 68G produced the most α-farnesene after 2 months at 0 °C, including both (*E*,*E*) α-farnesene and (*Z*,*E*) α-farnesene. Concentrations of (*E*,*E*) α-farnesene were 100 times greater than those of (*Z*,*E*) α-farnesene in all lines. After 4 months at 0 °C plus 1 week at 20 °C, untransformed GS apples exhibited the most superficial scald, whereas fruits from lines 68G and 103Y were less affected and line 130Y had no scald. Superficial scald severity was higher in green fruit than in yellow fruit in all affected lines. These lines also exhibited significant production of 6-methyl-5-hepten-2-one (MHO), a major oxidation product of (*E*,*E*) α-farnesene. Line 130Y neither exhibited superficial scald nor produced MHO. It is shown here that even transgenic apples suppressed for ethylene biosynthesis genes can produce α-farnesene, which in turn can oxidize to free radicals and MHO, leading to scald development.

KEYWORDS: Apple cv. 'Greensleeves'; *Malus domestica* Borkh; ACC oxidase; ACC synthase; GC-MS; α-farnesene; 6-methyl-5-hepten-2-one (MHO); ripening; shelf life

INTRODUCTION

During storage at 0 °C, apples (*Malus domestica* Borkh) can develop many physiological disorders, the two most prominent being superficial scald and bitter pit (BP), which cause significant losses to apple growers worldwide. Superficial scald develops during cold storage as hypodermal cells die, causing skin browning. This disorder is attributed to autoxidation products of α-farnesene (*1*, *2*), including conjugated triene hydroperoxides, intermediary free radicals (*3*, *4*), and 6-methyl-5-hepten-2-one (MHO) (*1*). As early as 1970, it was documented that apples produce minor amounts of the (*Z*,*E*)-α-farnesene isomer (*5*) but that autoxidation leading to scald results from the major isomer, (*E*,*E*)-α-farnesene (*3*, *2*).

It is well recognized that α-farnesene synthesis is mediated by ethylene (*6*, *7*). It has recently been demonstrated that the ethylene action inhibitor 1-methylcyclopropene (1-MCP) is very effective in controlling scald in apples and pears, confirming the role of ethylene in scald development and α-farnesene synthesis (*8*−*10*).

Calcium deficiency is believed to contribute to BP development during cold storage. Preharvest foliar applications to increase fruit calcium content are a standard practice worldwide,

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although this does not completely control BP (*11*, *12*). Unlike superficial scald, ethylene has not been implicated in inducing BP symptoms and may actually reduce the incidence of BP.

Slow-ripening apple varieties with better storability are associated with an allele of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (*MdACS1*) that synthesizes less ethylene (*13*). Ripening can also be delayed by transgenic suppression of ACC oxidase (ACO) and/or ACC synthase (ACS) mRNA concentrations (*14*). 'Greensleeves' apples (GS) with reduced ACO activity through antisense suppression show reduced autocatalytic ethylene production, slower ripening, less fruit softening, and less production of some aroma components (*14*). Recent studies reveal that aroma production in apple is controlled by ethylene predominantly at the final step in each biosynthetic pathway (*15*, *16*).

We explored the effect of cold storage at 0 °C on the development of two physiological disorders, superficial scald and BP, in transgenic GS apples suppressed for ethylene biosynthesis. Line 68G produces ACC, but ACC oxidase (ACO) is suppressed, whereas lines 103Y and 130Y have suppressed ACC synthase (ACS) and thus produce very little ACC. Suppression of either ACO or ACS inhibits ethylene production in these lines during storage at 20 °C (*14*, *15*).

MATERIALS AND METHODS

Plant Material. Control and transgenic GS apple fruits suppressed for ethylene biosynthesis genes were harvested at the preclimateric stage, 120 days after full bloom (DAFB), from an experimental orchard in northern California. Three transformed lines were used: the ACO-suppressed line 68G (*14*) and the ACS-suppressed lines 103Y and 130Y (*14*, *15*), with untransformed GS as a control. Apple fruits were harvested at the end of July, when the untransformed fruits started to produce ethylene. Harvest indices were checked on 16 randomly selected fruits from each line, and then fruits from all lines were segregated on the basis of peel color (green or yellow) and stored in boxes (60 fruits/box) at 0 °C in air. The cold room was well ventilated, and ethylene was monitored weekly. We did not detect measurable ambient ethylene in the cold room during storage.

Fruit Quality. Fruits were stored at 0 °C in air for up to 4 months. Sixteen randomly selected fruits were evaluated at harvest and after 2, 3, and 4 months of storage at 0 °C. Quality was also evaluated after 4 months plus 7 days at 20 °C. Peel color indices were recorded at two diametrically opposite sides of each apple fruit using a Chroma Meter CR-310 (Minolta). Results were expressed as hue angle ($h°$, where 90° represents a full yellow color and 180°, full green), lightness (L^*, where 0 = black and 100 = white), and chroma (C^*, color saturation). Firmness was measured as resistance to penetration with an 11-mm probe using a Fruit Texture Analyzer (GUSS, Strand, South Africa) on opposite sides of the fruit after removal of a small area of peel. Total soluble solids (TSS) and titratable acidity (TA) were assayed in fruit juice extracted from a composite sample of four wedges cut from stem to blossom end from four separate fruits. Percent TSS was determined with a digital refractometer (Abbe model 10450; American Optical, Buffalo, NY), and TA (in malic acid equivalents) was determined with an automatic titration system (TIM 850; Radiometer, Copenhagen, Denmark). For starch index determination apples were cut in half at the equator, immersed for 2 min in 3% iodine−potassium iodide, and rinsed with fresh water. Iodine−potassium iodide staining patterns were rated on a 6 point rating scale (1 = full starch, 6 = no starch) (*17*).

Ethylene and Respiration Measurements. During cold storage at 0 °C, four individual fruits from each line, with similar weight and color, were selected and transferred to 1-L jars at 0 °C. The jars were sealed for 2 h to monitor respiration and ethylene production during cold storage. After 4 months of storage, fruits were transferred to 20 °C to determine shelf life. Respiration and ethylene were monitored daily at 20 °C during 1 week of shelf life using the same jars sealed for 1 h. Headspace samples were analyzed for CO_2 using an infrared gas analyzer (Horiba, Irvine, CA) and for ethylene by a gas chromatograph with a flame ionization detector (GC-FID) (Hach Carle, Loveland, CO).

Table 1. Harvest Indices of Apple Fruits from Untransformed (GS) and Transgenic Greensleeves Lines 68G, 103Y, and 130Y[a]

	wt (g)	TSS (%)	acidity (%)	firmness (N)	starch (1−6)	color (hue°)
GS	134.03 b	15.08 a	0.67 a	66.94 c	2.96 a	103.74 b
68G	151.59 a	13.48 b	0.63 ab	77.57 ab	2.98 a	109.92 a
103Y	161.38 a	14.88 ab	0.66 a	72.68 b	2.89 a	103.56 b
130Y	126.83 c	13.55 b	0.56 b	80.91 a	2.37 b	110.77 a

[a] Values followed by the same letter do not differ significantly according to Tukey's test ($p < 0.05$, $n = 16$). Fruit weights are means of around 240 fruits/line; all other indices are means of 16 randomly selected fruits.

Analysis of Volatiles. Volatiles production in situ was checked several times during cold storage and after removal to 20 °C. One-gram pieces of apple peel plus 0.4 g of NaCl were placed in 20-mL amber vials with 2 mL of 20% NaCl. High salt prevents enzymatic activity associated with volatile biosynthesis (*18*) and increases volatile partitioning (*19*). An internal standard of 500 nL/L 1-octanol was added to each vial (*15*). The vials were sealed with screw caps and incubated for 2 h at 30 °C. After incubation, volatiles were absorbed from the headspace for 20 min by solid phase microextraction (SPME) onto a SPME fiber (1 cm long, 100 μm thick) coated with polydimethylsiloxane (PDMS) (Sigma-Aldrich/Supelco). The fiber was desorbed for 10 min at 260 °C in the splitless injection port of a GC-MS (Agilent 6890, Palo Alto, CA) with He as a carrier. Volatiles were separated on a DB-Wax (Agilent 123-7032E, 31 m, 0.26 mm i.d., 0.26-μm film thickness) capillary column. Chromatographic conditions were as follows: initial temperature, 40 °C; maximum temperature, 260 °C; initial time, 1.0 min, then ramps of 10 °C/min to 150 and 15 °C/min to 260 °C. α-Farnesene isomers and MHO were identified by comparison of their mass with those of commercial standards and with spectra published in the National Institute for Standards and Technology (NIST) mass spectral library. Farnesene isomers were confirmed using a mixed farnesene standard (TCI America, Portland, OR; catalog no. F0287) and comparison of retention times and spectra with published values. MHO was confirmed using an external standard (Sigma-Aldrich, catalog no. M48805) and was quantified by comparison with the peak of the internal standard 1-octanol (*15*).

Scald, Bitter Pit, and Decay Evaluation. The superficial scald index was assessed using a 10-point visual peel damage scale (0 = no injury, 1 = slight injury, 5 = moderate injury, and 10 = severe injury) (*20*) and calculated according to the following formula:

$$\text{scald index} = \sum_{0}^{10} \frac{(\text{index level}) \times (\text{fruits at this level})}{\text{total fruits}}$$

BP index was also assessed using a 10-point visual peel damage scale and calculated using the same formula as for scald.

In addition, decay and BP were recorded as the percentage of total fruits that decayed or developed BP in each carton (~60 fruits/carton).

Statistics. Harvest indices and color measurements are means of 16 apples, whereas fruit quality indices are means of three boxes ± SE. Respiration and ethylene production are means of four individual fruits with LSD at the 5% level. Volatiles data are means of three samples. Data were analyzed with the JMP 5.0 software (SAS, Cary, NC) and separated according to Tukey's HSD test at $p < 0.05$.

RESULTS AND DISCUSSION

Fruit Quality at Harvest. The harvest indices of control GS and transformed lines, not segregated by color, are shown (**Table 1**). Fruits from the transformed lines 68G and 103Y were significantly larger than control GS, whereas 130Y fruits were smallest. Control GS fruit had the highest TSS and the lowest firmness, indicating they were riper than fruits from the three transgenic lines. This result is similar to previous findings that

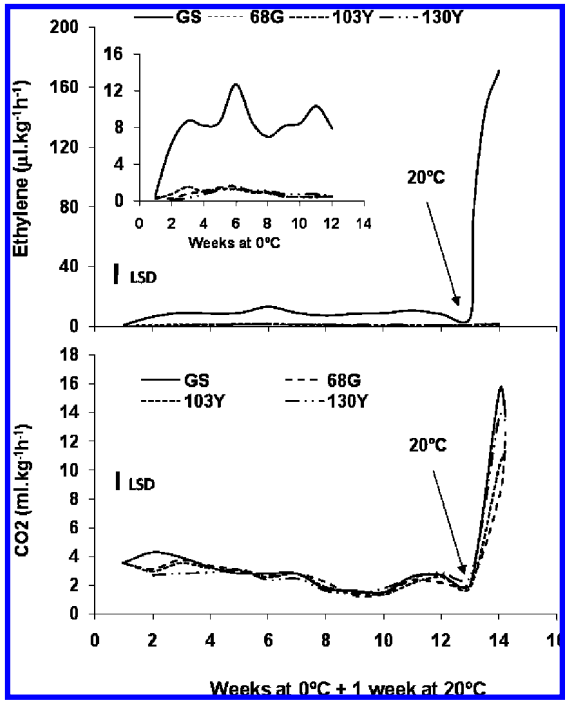

Figure 1. Ethylene and CO_2 production in 'Greensleeves' apples from untransformed (GS) and transgenic lines 68G, 103Y, and 130Y during 13 weeks of cold storage at 0 °C and after transfer to 20 °C. (Inset) Ethylene production at 0 °C; results are means of four individual fruits. The LSD at a 5% confidence level for CO_2 = 0.919; C_2H_4 = 5.450.

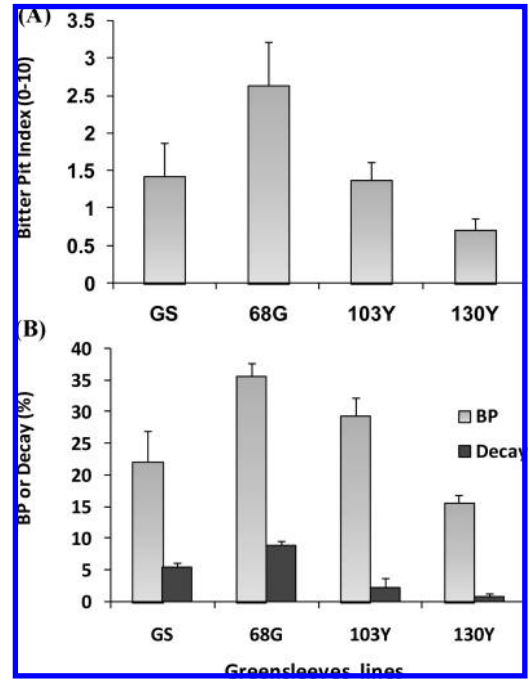

Figure 2. Intensity of bitter pit (BP) symptoms (index, 0−10) (**A**) and percentages of fruits developing BP and decay (**B**) in apples from control (GS) and transgenic lines 68G, 103Y, and 130Y after 2 and 3 months of storage at 0 °C. Results are combined data from 2 and 3 months of storage and are averages of three boxes with 60 fruits each ± SE.

control GS had higher TSS and lower firmness than the transgenic lines 68G and 130Y (*14*). Although fruits from line 130Y were firmer and had more starch, they also had less acidity than control GS or line 103Y. The peel colors (hue°) of control GS and line 103Y fruits were lower than those of lines 68G and 130Y. Greener peel color at harvest in the ethylene-suppressed transgenic lines 68G and 130Y was also found previously (*14*).

Ethylene Production. The transformed lines 68G, 103Y, and 130Y produced very little ethylene (0.3−0.7 μL kg^{-1} h^{-1} during 3 months of cold storage at 0 °C and ∼1.2 μL kg^{-1} h^{-1} after subsequent transfer to 20 °C), whereas control GS produced over 10 times more ethylene (∼8 μL kg^{-1} h^{-1}) during cold storage. This increased about 20-fold after transfer to 20 °C to 170 μL kg^{-1} h^{-1}, which is 140 times higher than transformed lines (**Figure 1**). Respiration expressed as CO_2 production was the same for all four apple lines. Suppression of ethylene production in lines 68G, 103Y, and 130Y became apparent earlier in fruits that were stored at 20 °C after harvest (*14*, *15*). Moreover, exposure of lines 68G (ACO-suppressed) and 103Y (ACS-suppressed) to ethylene did not activate autocatalytic ethylene production during 14 days at 20 °C (*15*). In this study, removal from cold storage after 3 months did not cause a burst of ethylene production in transformed lines suppressed for ethylene biosynthesis genes, unlike in control GS. The very low ethylene production in the transformed lines after cold storage is consistent with results obtained with transformed 'Royal Gala' apples from line AO3, which contain an antisense ACC oxidase gene (*16*). However, CO_2 production by our transformed lines was not statistically different from that of the GS control, whereas in AO3, CO_2 production was 3.5 times lower than in untransformed 'Royal Gala' (*16*). The inhibition of ethylene production in transformed apples after removal from the cold storage is contrary to the well-known phenomena that removal from cold leads to a high burst of ethylene production in apples and that the longer an apple fruit is stored at 0 °C, the greater the ethylene production after chilling ceases (*17*). In addition, the ACO-suppressed line 68G produced tremendous amounts of ACC compared to all other GS lines (*14*), which may influence fruit resistance to superficial scald and BP later during storage.

Bitter Pit and Decay Symptoms. After 2 months at 0 °C, GS apples from transformed and untransformed lines already exhibited symptoms of BP but not scald. After 3 months at 0 °C, there was still no appearance of scald and the severity of BP symptoms remained similar. The most severe BP symptoms occurred after 2 and 3 months in line 68G (**Figure 2A**), which also showed the highest BP incidence (**Figure 2B**). Severity of BP in line 103Y was equal to that in control GS, but BP incidence was higher and decay incidence lower. Line 130Y exhibited the least BP severity and incidence, which was associated with the least decay. BP in apples results from disruption of cell membranes, leading to cell death (*21*, *22*). Calcium deficiency is believed to be the main cause of BP (*11*, *12*), and the transformed lines 68G and 103Y may have a deficiency in intracellular calcium, making them more susceptible to BP. It was also reported that larger fruits are generally more affected by BP than smaller fruits (*23*), which is in accord with our results showing that the larger fruits of lines 68G and 103Y suffered more BP symptoms.

Isolation of the fungi from decayed fruit revealed that mainly *Penicillium* spp. (blue mold) and *Botrytis cinerea* (gray mold) were present. These are the main fungi associated with decay in various apple cultivars (*24*). The most decay was found in line 68G, which correlated with the highest BP symptoms. A study with 1-MCP-treated pears showed a statistically significant increase in susceptibility to *B. cinerea* over control fruit (*25*). It was also shown that application of 1-MCP to inhibit apple ripening can predispose fruit to decay (*26*).

Color Development. The color of control and transformed GS apples at harvest was a light green hue between 103 and

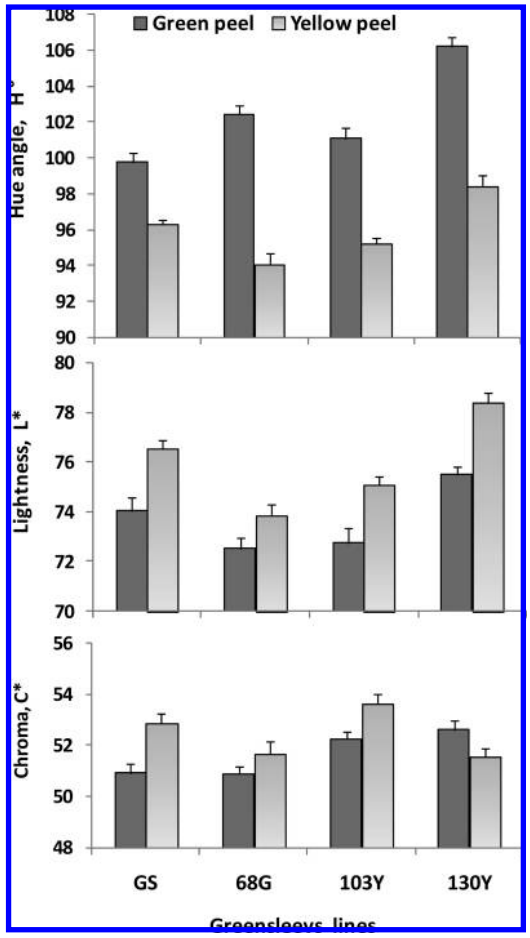

Figure 3. Peel color indices expressed as hue angle, lightness (*L**), and chroma (*C**) in green and yellow apples from control (GS) and transgenic lines 68G, 103Y, and 130Y after 3 months at 0 °C. Results are averages of 16 fruits (32 measurements) ± SE.

110° (**Table 1**). During 3 months of storage at 0 °C, all apples lost much of their green color and became more yellow. However, after 3 months at 0 °C, line 130Y (ACS-suppressed) remained more green and line 68G (ACO-suppressed) was most yellow as indicated by the hue angle (**Figure 3**). The control lost the most chlorophyll during 3 months at 0 °C. The reduction in hue angle of control and transformed lines during 3 months at 0 °C was very similar to that found in the same lines after 12 days at 20 °C (*14*). In addition, *L** values were highest in both green and yellow fruits from line 130Y, indicating more shininess in this line. Color saturation *C** was almost the same for all lines. The significantly greater shine and partial inhibition of green color degradation in line 130Y are similar to the effects of 1-MCP on 'Granny Smith' apples after 6 months at 0 °C (*20*), indicating that ethylene is involved in chlorophyll degradation as in many other fruits (*27*).

α-Farnesene. SPME and GC-MS revealed that after 2 months at 0 °C, green and yellow fruits from all four apple lines produced two stereoisomers of α-farnesene, (*E*,*E*) and (*Z*,*E*). The ACO-suppressed line 68G, which produced ACC but no ethylene, also had the highest concentrations of both α-farnesene isomers after 2 months at 0 °C (**Figure 4**). Headspace concentrations of the (*E*,*E*) isomer were 100-fold higher than those of the (*Z*,*E*) isomer in control and all transformed lines. It is well established that oxidation of the (*E*,*E*) isomer causes scald symptoms (*1*−*4*). Interestingly, in transgenic 'Royal Gala' AO3 apple fruits with antisense ACO, the (*Z*,*E*) isomer was already present at harvest time and increased after 96 and 192 h

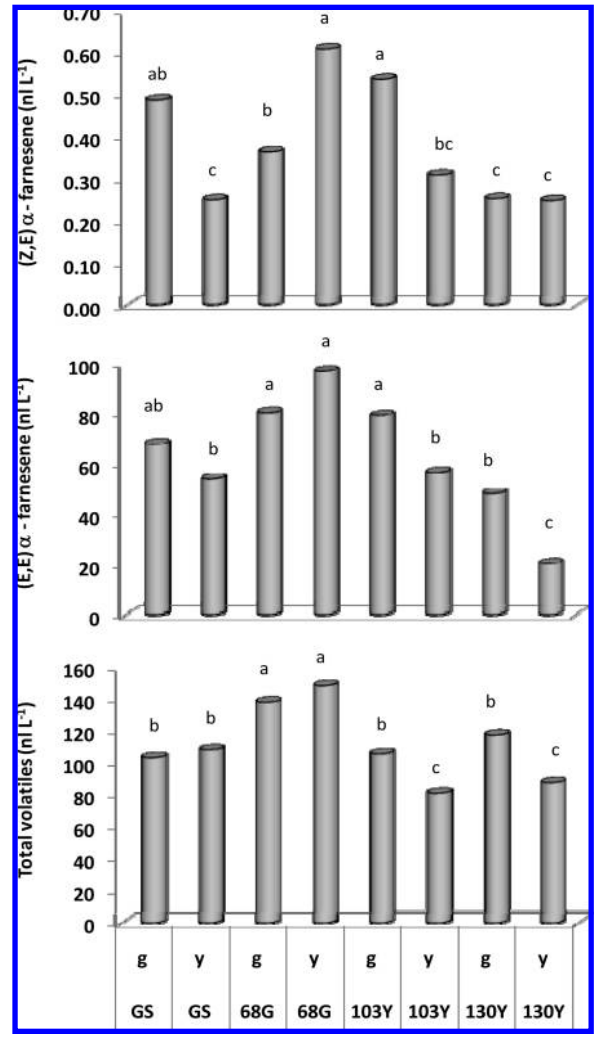

Figure 4. Headspace concentrations of (*E*,*E*)- and (*Z*,*E*)-α-farnesene in control GS and transgenic lines 68G, 103Y, and 130Y after 2 months at 0 °C in green (g) and yellow (y) fruits. All measurements were taken at the apple blossom end. Values marked by the same letter do not differ significantly according to Tukey's test ($p < 0.05$, $n = 3$).

Table 2. Two α-Farnesene Stereoisomers as a Percentage of Total Volatiles Produced after 2 Months at 0 °C in Greensleeves Apples, Not Segregated by Color, from Untransformed (GS) and Transgenic Greensleeves Lines 68G, 103Y, and 130Y[a]

	α-farnesene % of total volatiles	
line	*E*,*E*	*Z*,*E*
GS	57.9 ± 7.86	0.35 ± 0.122
68G	61.7 ± 3.53	0.34 ± 0.074
103Y	72.7 ± 2.55	0.45 ± 0.063
130Y	31.9 ± 9.09	0.25 ± 0.034

[a] All measurements were taken at the apple blossom end and are means of six fruits ± SE.

at 22 °C, although it accounted for <1% of the total α-farnesene present (*16*). (*E*,*E*)-α-Farnesene comprised >58% of the total volatiles produced by untransformed and transgenic apples from lines 68G and 103Y, but only 32% of the volatiles in the transgenic line 130Y (**Table 2**). (*Z*,*E*)-α-Farnesene comprised only 0.25−0.45% of the total volatiles in all GS lines, but the lowest percentage of both isomers was found in line 130Y. The role of the (*Z*,*E*) isomer is not known yet, but in 1-MCP-treated 'Granny Smith' apples that did not exhibit any scald, more (*Z*,*E*)-α-farnesene accumulated during 8 months at 0 °C than did in

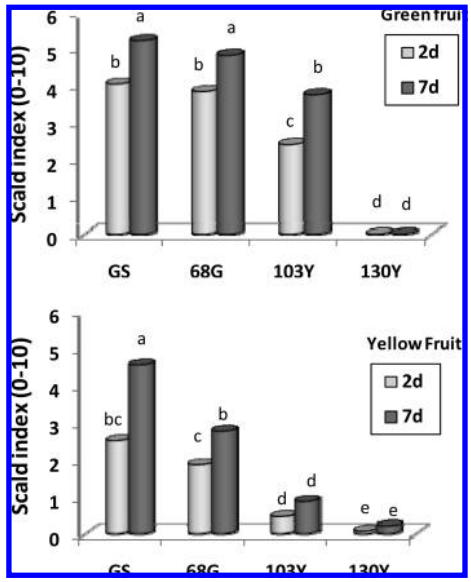

Figure 5. Superficial scald development in green and yellow fruits from control GS and transgenic lines 68G, 103Y, and 130Y after 4 months at 0 °C plus 2 or 7 days at 20 °C. Values marked by the same letter do not differ significantly according to Tukey's test ($p < 0.05$, $n = 3$ boxes).

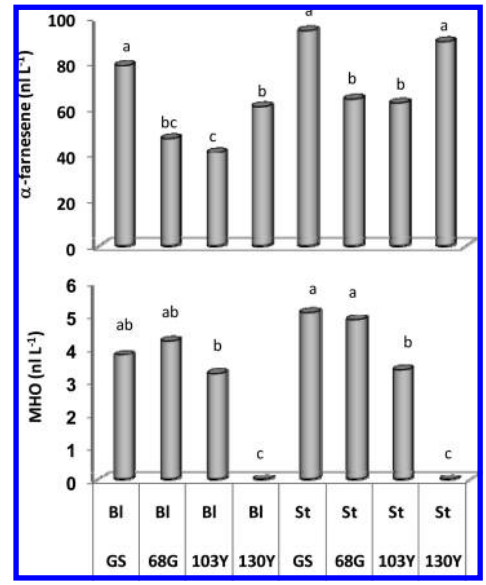

Figure 6. Headspace concentrations of (*E,E*) α-farnesene and 6-methyl-5-hepten-2-one (MHO) in the blossom (Bl) scalded side or the clean stem (St) side of scalded fruit from control GS and transgenic lines 68G, 103Y, and 130Y after 4 months at 0 °C and 2 days at 20 °C. Values marked by the same letter do not differ significantly according to Tukey's test ($p < 0.05$, $n = 3$).

control fruits (unpublished data, E.P.). Moreover, in transgenic 'Royal Gala' apple fruits with ACO antisense there is evidence indicating that there may be a second, ethylene-independent route to (*Z,E*)-α-farnesene production (*16*).

In apple peel tissue, (*E,E*)-α-farnesene is synthesized almost exclusively via the mevalonic acid pathway (*7, 28, 29*). The final, rate-limiting enzyme in the pathway is α-farnesene synthase, which converts farnesyl diphosphate to α-farnesene. This enzyme is induced by ethylene (*9, 30*−*32*). In ACO-suppressed line 68G transgenic apples, α-farnesene concentrations, measured at the blossom end, were higher than in controls after 2 months at 0 °C (**Figure 4**). Moreover, there was significant production of (*E,E*)-α-farnesene in lines 68G and 103Y, accounting for 60−70% of total peel volatiles (**Table 2**). Only in the ACS-suppressed line 130Y was the percentage of (*E,E*) α-farnesene lower after 2 months, which correlated with the absence of scald in this line. Untransformed GS stored only at 20 °C produced double the α-farnesene found in lines 68G and 130Y (*14*). It is not clear how transgenic ACO- and ACS-suppressed apples that produce very little ethylene (**Figure 1**) still produce high concentrations of (*E,E*)-α-farnesene after 2 months in cold storage (**Figure 4**). It is probable that the very low concentrations (0.3−1.2 μL kg^{-1} h^{-1}) of ethylene produced by the transformed lines are still sufficient to enable synthesis of (*E,E*)-α-farnesene. In transgenic 'Royal Gala' apple fruits with ACO antisense, (*E,E*)-α-farnesene and (in much lower quantity) (*Z,E*)-α-farnesene exist at harvest, whereas application of exogenous ethylene induced both (*E,E*)- and (*Z,E*)-α-farnesene production during 192 h at 22 °C (*16*).

Superficial Scald Development. After 4 months at 0 °C plus 2 or 7 days at 20 °C, both green and yellow control apples had the most scald (**Figure 5**). Scald started to appear on the blossom end and spread toward the stem end, whereas its intensity was greater in green fruits than in yellow fruits. The control GS and line 68G exhibited a statistically similar amount of scald development, which intensified after 7 days at 20 °C in both green and yellow fruits. Line 103Y had moderate to severe scald only in green fruits, whereas yellow fruits had very minor scald symptoms associated with lower (*E,E*)-α-farnesene after 2 months at 0 °C (**Figure 4**). Line 130Y had no scald development in either green or yellow fruits (**Figure 5**).

After 4 months at 0 °C, all four lines produced (*E,E*)-α-farnesene, but the control and line 130Y had higher concentrations than lines 68G and 103Y (**Figure 6**). The same trend among lines was found in scalded blossom and in unaffected stem ends, but (*E,E*)-α-farnesene concentrations at the stem ends were a little higher in all lines. In scalded fruits from lines 68G and 103Y and the control, there was significant production of 6-methyl-5-hepten-2-one (MHO), the (*E,E*)-α-farnesene oxidation product, in both blossom and stem ends. Moreover, although the stem ends were not yet scalded, MHO concentrations there were abnormally high, which raises the question of whether this molecule is the cause of scald. In contrast, line 130Y exhibited no scald and produced no MHO at either end. After 4.5 months at 0 °C plus 3 days at 20 °C, the ACO-suppressed line 68G exhibited the most scald symptoms, whereas the ACS-suppressed line 130Y displayed no scald (**Figure 7**).

α-Farnesene concentrations increase during cold storage and then diminish (*33*). In the symptom-free, ACS-suppressed line 130Y, (*E,E*)-α-farnesene concentrations (average of all samples) doubled between 2 and 4 months of cold storage. Delay in (*E,E*)-α-farnesene synthesis is primarily responsible for the delay in scald appearance in 1-MCP-treated apples (*8, 33*). Superficial scald began to appear on the blossom end, as in 'Granny Smith' apples (*20*). Generally, yellow apples had less scald than green apples (**Figure 5**), consistent with lower α-farnesene concentrations in yellow fruits (**Figure 4**). Our results are consistent with the ability of heat pretreatment before cold storage to reduce α-farnesene and its oxidation products, thus reducing scald symptoms (*10*). Our results are also in agreement with a recent report that UV light applied after harvest to previously bagged Granny Smith apples reduced α-farnesene content and scald incidence (*34*). The yellow fruits of GS and transformed lines were probably exposed to higher temperatures in the orchard than green fruits from under the leaf shadow. This higher temperature could have lowered α-farnesene production (except in line 68G). The high concentrations of MHO, the oxidation

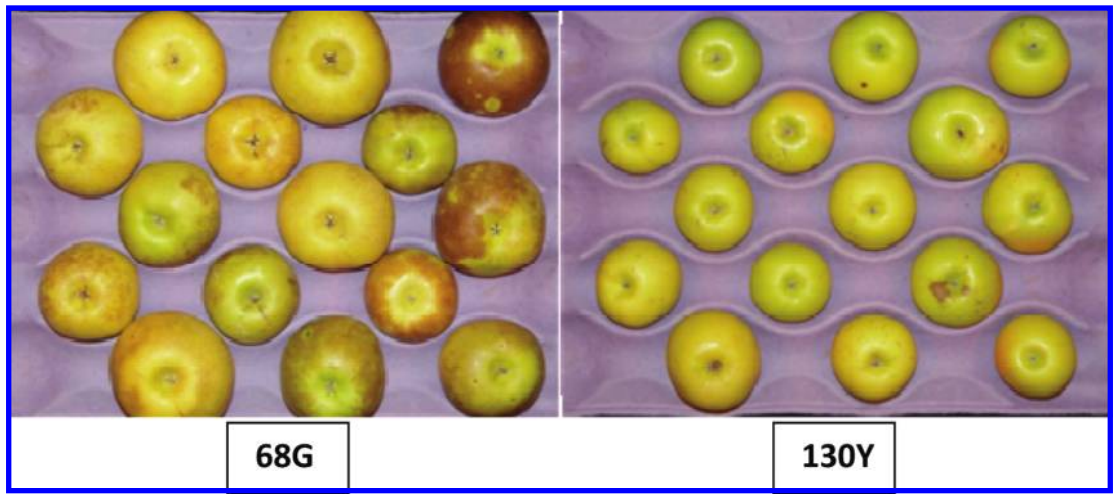

Figure 7. 'Greensleeves' apples from transgenic lines 68G and 130Y after 4.5 months at 0 °C and 3 days at 20 °C.

product of α-farnesene, found in lines 68G and 103Y and the control agree with findings linking MHO to scald symptoms in other apple cultivars (*1*, *2*). Although the free radicals produced from oxidation of α-farnesene are believed to cause the actual scald damage, these products are highly unstable and not measured by the GC-MS technique used here. However, the volatile MHO is produced by decomposition of a farnesyl oxyradical, which in turn is derived from the hydro- and endoperoxide oxidation products of α-farnesene (*2*, *3*). Thus, MHO should be a good marker of free radical degradation of α-farnesene.

We found that MHO was not only emitted from the scalded blossom end but also from the clean peel tissue on the stem side of the same fruit (**Figure 6**). This may indicate that, with time, more free radicals will be produced and superficial scald symptoms would continue to expand toward the stem side, as previously observed in 'Granny Smith' apples (*20*).

The ACO-suppressed line 68G had the most BP and decay development after 2 months at 0 °C. This line produced 32-fold more of the ethylene precursor ACC than the control (*14*). In plants, enhanced ethylene production is an active response to perception of pathogens and is associated with induction of defense reactions (*35*). In avocado fruits, ethylene induces antifungal material and without it, a climacteric fruit becomes more susceptible to developing decay (*36*). It is possible that line 68G became more susceptible to decay, as did 1-MCP-treated 'Golden Delicious' apples (*26*). In addition, line 68G produced bigger apples (**Figure 7**), which could lead to the fruit being more susceptible to damage and decay. Smaller apple fruits generally suffer from fewer injuries during ripening and storage. For example, less BP is found in smaller fruit (*23*), pest-resistant apple cultivars used in organic farming are smaller than common commercial cultivars (*37*), and in Gala apples, smaller fruit are less susceptible to bruising (*38*).

The ACS-suppressed line 130Y kept the best quality by exhibiting higher green color, no scald development, and no MHO production after 4.5 months at 0 °C (**Figure 7**). Although line 130Y produced smaller fruits, which could be more resistant to damage, these fruits also produced no MHO, which is the last product in α-farnesene oxidation (*3*). This result is similar to the ability of 1-MCP to prevent ethylene responses and superficial scald symptoms in various apple cultivars (*8*, *10*, *31*, *39*−*41*). Line 130Y, which had no scald after 4.5 months, produced the least (*E*,*E*)-α-farnesne after 2 months. There was significantly more of this compound after 4 months, consistent with normal trends for α-farnesene accumulation during cold storage in various apples (*33*). However, because line 130Y produces the same small amount of ethylene as lines 68G and 103Y, it seems that α-farnesene production (**Figure 4**) and scald (**Figure 5**) are not necessarily related to the concentration of ethylene alone (**Figure 1**).

α-Farnesene synthesis in GS lines suppressed for ethylene biosynthesis genes raises the question of whether there are other mechanisms to induce it. Alternatively, the very small amount of ethylene found in the transformed apples could be enough to induce α-farnesene synthesis. Another possibility is that in line 130Y, oxidation of α-farnesene is inhibited to a greater extent than in the other two transgenic lines, as shown by no MHO production. MHO, an end product of α-farnesene oxidation, can be an indicator for superficial scald development (*1*), although its possible role in scald induction has been largely ruled out (*2*−*4*).

ABBREVIATIONS USED

BP, bitter pit; GS, Greensleeves; ACC, 1-aminocyclopropane-1-carboxylic acid; ACO, ACC oxidase; ACS, ACC synthase; MHO, 6-methyl-5-hepten-2-one; SPME, solid phase microextraction; PDMS, polydimethylsiloxane; GC-MS, gas chromatography−mass spectrometry.

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