Relationship between Ethylene Response Manipulation and Volatile Production in Jonagold Variety Apples

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The ripening of Jonagored Jonagold apple fruit (*Malus* × *domestica* Borkh.) during development was manipulated with preharvest applications of ReTain or a combination of ReTain plus Ethrel. The fruits, harvested preclimacteric at approximately the same stages of maturity, were stored in refrigerated air (RA) for 45 days or in controlled atmosphere (CA) for 180 days at 0 °C. Volatile evolution, ethylene production, and respiration of stored fruit were studied during poststorage holding at 22 °C. ReTain reduced volatile production by 19%, but application of Ethrel to ReTain-treated fruit increased production to control levels. The inhibition of volatile production by ReTain appears to be independent of respiration but may be related to the ethylene-producing capacity of the fruit. Although ReTain reduced flavor-related volatile esters, it did not affect levels of the compound responsible for the typical spicy flavor in Jonagored Jonagold fruit, 4-methoxy-2-propenylbenzene. The CA-stored fruit had a much reduced production of volatile compounds compared to RA-stored fruit, with more discernible effects in ReTain-treated fruit. Ethrel application to ReTain-treated fruit improved the volatile production intermediate between the ReTain alone and control in CA-stored fruit. The data collectively suggest that ReTain may have some promise for better scheduling of harvest of apples with no appreciable loss in RA-stored fruit quality. Reduction in production of α-farnesene by ReTain may also reduce the potential for scald development in CA-stored fruit.

Keywords: *Malus* × *domestica; ReTain; AVG; Ethrel; harvest management; quality; aroma*

INTRODUCTION

Ethylene plays a central role in initiating and accelerating ripening-related processes that we perceive as changes in dessert quality of most climacteric fruits such as apple and pear (i.e., firmness, soluble solids, acidity, flavor, etc.). For ethylene to exert such effects, it has to be biosynthesized by fruit or, alternatively, it may be supplied by external sources (Yang, 1985).

Most commercially grown cultivars of apple ripen either at once (with a very small harvest window) or very sporadically in time (demanding multiple manual harvests). However, the onset of fruit ripening is associated with a burst in ethylene production in all apple cultivars (Dilley and Dilley, 1985). 2-(Chloroethyl)-phosphonic acid (Ethrel) is used commercially to advance apple color development (Larrigaudiere et al., 1996) through its ability to advance fruit ripening (Murphey and Dilley, 1988).

1-Aminocyclopropane-1-carboxylic acid (ACC) synthase, which converts *S*-adenosylmethionine (SAM) to ACC, is the main site of control of ethylene biosynthesis (Miyazaki and Yang, 1987). This enzyme requires pyridoxal phosphate for maximal activity (Yu et al., 1979) and has been shown to be strongly inhibited both in vivo (Adams and Yang, 1979) and in vitro (Boller et al., 1979) by inhibitors of pyridoxal phosphate-dependent enzymes such as aminooxyacetic acid (AOA) and L-2-amino-4-(2-aminoethoxy)-*trans*-3-butenoic acid (AVG). Ethylene inhibition by AVG, resulting in delayed fruit maturity and ripening, with improved fruit condition and storage life in apple (Autio and Bramlage, 1982; Bangerth, 1978; Bramlage et al., 1980; Williams, 1980) and pear (Wang and Mellenthin, 1977), has been shown. The commercialization of these practices was hampered because the only available options were high cost, pure chemical formulations of AVG. Recently, an organic formulation (ReTain) that contains 15% (w/w) AVG has been registered for use on apples in the United States (Abbott Laboratories, Chicago, IL).

ReTain is used to prevent premature fruit drop in apple and for harvest management by delaying fruit ripening. It is not known whether Retain-treated fruit harvested at the same stage of maturity as untreated fruit behaves similarly in storage. Further, it is not known what modifying effect Ethrel may have on maturity and ripening characteristics of ReTain-treated fruit.

In this investigation, we examine the effects of artificially manipulated fruit maturity by ReTain or a combination of ReTain plus Ethrel on volatile production, ethylene production, and respiration of refrigerated air (RA)-stored and controlled atmosphere (CA)-stored apple fruit. We used a rapid, nondestructive, and sensitive technique of sampling, solid-phase microextraction (Zhang and Pawliszyn, 1993) combined with gas chromatography (GC) time-of-flight mass spectrometry (TOFMS) for volatile analysis.

MATERIALS AND METHODS

Fifty grams of ReTain (Abbott Laboratories) and 20 oz of a wetting agent, Sylgard-309 (Dow Corning Corp., Midland, MI), were dissolved in 100 gal of water. Approximately 0.4 ha of

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10.1021/jf981150k CCC: $18.00
Published on Web 06/23/1999

Jonagored Jonagold apple trees in the Fruit Ridge area in Michigan was sprayed with this solution to the drip point ~4–5 weeks ahead of anticipated harvest (August 28, 1997). This treatment was found to delay maturity by 5–10 days (Mir and Beaudry, unpublished results, 1996). Half of the ReTain-treated trees (0.2 ha) were sprayed with 50 gal of 1 mM Ethrel (Rhone-Poulenc, Research Triangle Park, NC) on September 24, 1997, to accelerate color development. Trees of another 0.2 ha land area in the same orchard as above were sprayed with water to serve as control.

Ten fruits from each treatment were brought to the laboratory beginning ~1 month ahead of commercial harvest and subsequently twice a week at an interval of 3–4 days for assessing fruit maturity using standard protocols (Beaudry et al., 1993). Treated and control fruits were harvested at equivalent maturities, having a preclimacteric condition (internal ethylene < 0.2 $\mu L \cdot L^{-1}$) on October 6 (control and ReTain plus Ethrel-treated) and October 16 (ReTain-treated) in 1997 for storage. The harvested fruits (200 per treatment, selected at random from various trees within a treatment block) were immediately brought to the laboratory and were kept overnight at 22 °C to equilibrate with ambient temperature and remove field heat. The following day, 80 fruits in each treatment that were free from surface defects and uniform in size and shape were sorted. Half of the sorted fruits (40 in each treatment) were stored separately in plastic buckets at 0 °C in humidified [>95% relative humidity (RH)] air for 45 days. The remaining half of the sorted fruits were stored separately in plastic buckets at 0 °C in CA (1.5 kPa O_2, 3 kPa CO_2) in a flow-through system for 180 days.

The fruits were removed from the storage buckets at the end of the storage period (45 days for RA and 180 days for CA storage), warmed for 6 h at 22 °C, and used for analyses of volatiles, respiration, and ethylene production over a holding period of 8 days at 22 °C.

Twelve fruit from each treatment/storage with no visual defects were removed from storage at 45 days (RA) and 180 days (CA) and held at 22 °C for 8 days in 1.95-L glass containers ventilated with humidified air. Each container held three fruits (485–512 g), which were taken as the unit of replication. There were four replications per treatment. The flow rates were 20 mL min^{-1} (for RA fruit) and 10 mL min^{-1} (for CA fruit), which ensured aerobic conditions as well as detectable levels of ethylene, CO_2, and organic volatiles in the exit gas stream. Tubing was 3 mm i.d. flexible Teflon tubing, which is largely impermeable to all vapors. Sampling was performed at glass tees fitted with a Teflon-lined septum at the inlet and outlet of the container. The data shown are an average of three to four replications (9–12 fruits) in each treatment.

Respiration and ethylene production were determined daily by withdrawing 0.5 and 1 mL of gas sample, respectively, from the exit gas stream using an insulin-type plastic syringe. CO_2 measurements were made with an infrared gas analyzer (model 225-MK3; Analytical Development Co., Hoddesdon, England). Ethylene concentrations were determined using a gas chromatograph (Carle Series 100 AGC; Hach Co., Loveland, CO) equipped with a flame ionization detector. Gas concentrations were calculated relative to a certified gas mixture (4.85% CO_2, 1.95% O_2, and 0.979 $\mu L\ L^{-1}$ C_2H_4 balanced with N_2) and converted to partial pressures assuming an atmospheric pressure of 1×10^5 Pa.

Volatile Collection, Separation, and Detection. Volatiles were sampled by means of solid-phase microextraction (SPME) using 1-cm-long fibers coated with a 100-μm-thick layer of poly(dimethylsiloxane) (Supelco Co., Bellefonte, PA). Before use, the fibers were conditioned at 250 °C for 60 min. The absorption time was 5 min. Separation and detection were by GC (HP-6980, Hewlett-Packard Co.) and TOFMS (FCD-650, LECO Corp., St. Joseph, MI) according to the method of Mir et al. (1999). Identification of compounds was confirmed by comparison of collected mass spectra with those of authenticated reference standards and spectra in the National Institute for Standards and Technology (NIST) mass spectra library, Search Version 1.5. The quantification was performed relative to the known standards that had similar or approximately similar molecular weights and retention times. Volatiles were measured at 1–2 day intervals for a period of 8 days after transfer of fruit from RA or CA to 22 °C.

Table 1. Effect of ReTain (R) and ReTain plus Ethrel (RE) Application on Fruit Color (Percent), Fruit Firmness (Pounds), and Starch Rating (1–8) of Jonagold Apples at Harvest[a]

	control ± SD	R ± SD	RE ± SD
fruit color	95.5 ± 9.1	94.0 ± 4.4	91.3 ± 6.1
fruit firmness	18.1 ± 2.6	18.9 ± 2.9	18.3 ± 3.1
starch rating	4.5 ± 2.0	4.9 ± 1.5	4.3 ± 1.7

[a] The values shown are for optimal harvest, and each data point is an average of 10 fruits.

Statistical Analyses. For treatment comparisons, the least significant range test was performed. The data were analyzed by the analysis of variance (ANOVA) procedure using a graphics and statistics software package (PlotIT, Scientific Programming Enterprises, Haslett, MI). Unless otherwise stated, only data significant at $p = 0.05$ are discussed.

RESULTS

Fruit Maturity. The measures of fruit maturity at harvest, fruit color, fruit firmness, and starch rating were similar in fruits of all the treatments (Table 1).

Ethylene. The ethylene production of RA-stored fruit (Figure 1A) at day 1 of holding was highest in control (1.2 nmol kg^{-1} s^{-1}), followed by ReTain plus Ethrel-treated (0.4 nmol kg^{-1} s^{-1}) and ReTain-treated (0.1 nmol kg^{-1} s^{-1}) fruit. The ethylene evolution was relatively stable during holding in control and ReTain plus Ethrel-treated fruit. In contrast, the ethylene evolution in ReTain-treated fruit continued to increase gradually from 0.1 nmol kg^{-1} s^{-1} at day 2 to 0.2 nmol kg^{-1} s^{-1} at day 12 of holding.

CA storage caused a 3-fold reduction in ethylene production for control fruit and 11- and 7-fold reductions in ethylene production for ReTain-treated and ReTain plus Ethrel-treated fruit, respectively (Figure 1B).

Respiration. The rates of CO_2 production of RA-stored fruit (Figure 2A) were similar in control, ReTain-treated, and ReTain plus Ethrel-treated fruits. The CO_2 evolution increased from 0.25 mmol kg^{-1} s^{-1} at day 0 to 0.8 mmol kg^{-1} s^{-1} at day 3. The rate of CO_2 evolution was stable from day 3 through day 8 and declined thereafter in all treatments.

CA storage caused an ~3-fold reduction in respiration for fruit of all treatments (Figure 2B).

Organic Volatiles in RA-Stored Fruit. The volatile compound production of fruit stored in RA at 0 °C in air for 45 days and subsequently ripened at 22 °C for 7 days is presented in Table 2. Total volatile compound productions at day 7 were 1477 pmol kg^{-1} s^{-1} in control fruit and 1196 pmol kg^{-1} s^{-1} in ReTain-treated fruit (Table 2). This volatile production in ReTain-treated fruit was ~81% that of control fruit. Volatile esters were reduced by 21%. The maximal reduction in volatile esters occurred in butyl acetate (57%), propyl 2-methylbutanoate (57%), and propyl hexanoate (50%).

Ethrel application to ReTain-treated fruit increased the volatile production approximately to control levels at day 7 (Table 2). Although Ethrel application to ReTain-treated fruit increased the production of most of the volatile compounds that were suppressed by ReTain treatment, the suppression in production of butyl butanoate remained uninfluenced by Ethrel treatment (Table 2). In contrast, Ethrel application to Re-

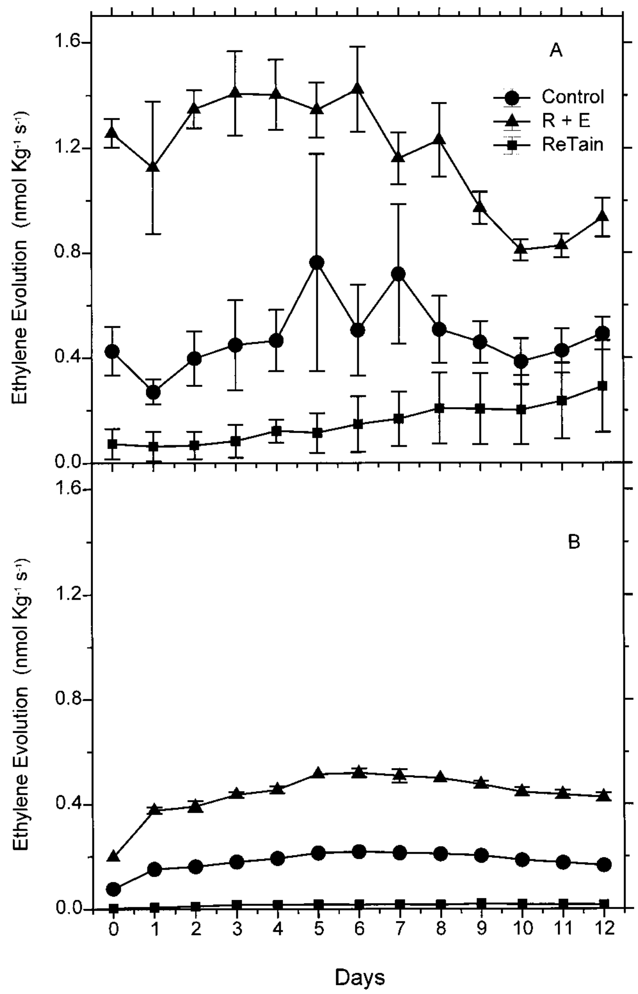

Figure 1. Ethylene evolution at 22 °C of Jonagored Jonagold fruit stored either for 45 days in air at 0 °C (A) or for 180 days in CA (1.5 kPa O_2, 3 kPa CO_2) at 0 °C (B). The data at each sampling point are an average of four replications, each replication comprised of three fruits. The bars represent the standard deviation (SD) of the population.

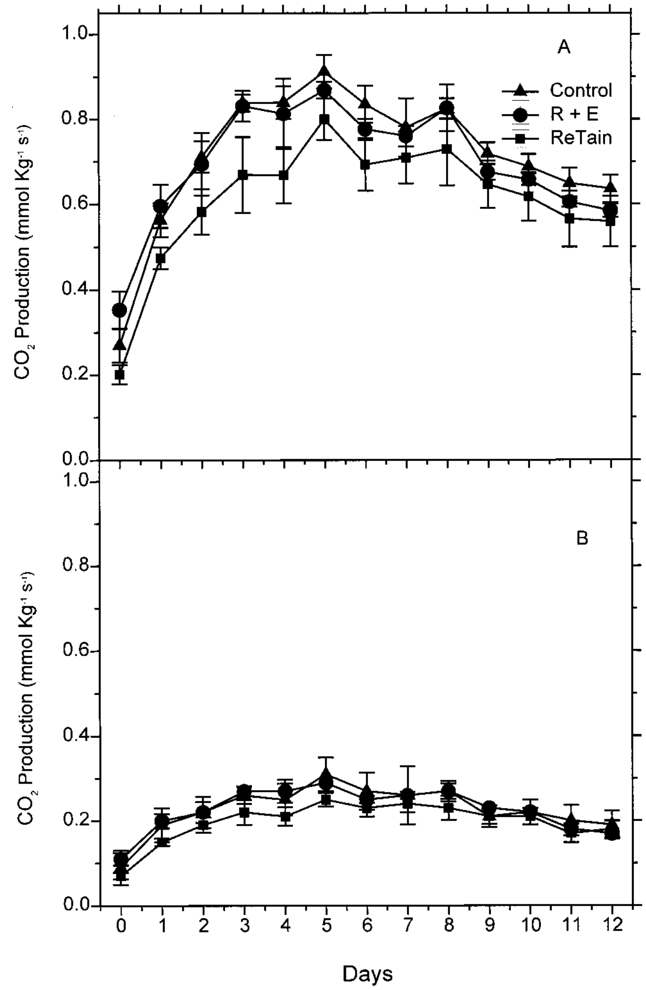

Figure 2. Respiration rate at 22 °C of Jonagored Jonagold fruit stored either for 45 days in air at 0 °C (A) or for 180 days in CA (1.5 kPa O_2, 3 kPa CO_2) at 0 °C (B). The data at each sampling point are an average of four replications, each replication comprised of three fruits. The bars represent the SD of the population.

Tain-treated fruit resulted in increased production of butyl, pentyl, and hexyl hexanoates and α-farnesene (Table 2).

In control fruit, the predominant branched-chain esters were, in decreasing order of abundance, hexyl 2-methylbutanoate, propyl 2-methylhexanoate, propyl 2-methylbutanoate, 2-methylbutyl acetate, and butyl 2-methylbutanoate. The predominant straight-chain esters were, in decreasing order of abundance, butyl acetate, hexyl acetate, pentyl acetate, hexyl hexanoate, butyl hexanoate, butyl propanoate, hexyl propanoate, and hexyl butanoate. Among the various esters, hexyl 2-methylbutanoate was produced in the highest (253 pmol kg^{-1} s^{-1}) and 2-methylpropyl acetate in the lowest (1.89 pmol kg^{-1} s^{-1}) amounts (Table 2). The sesquiterpene, α-farnesene, was evolved at the rate of 223 pmol kg^{-1} s^{-1} (Table 2), which accounted for 15% of the total amount of the 25 volatile compounds measured in RA-stored fruit. The rate of production of 4-methoxy-2-propenylbenzene, a volatile compound responsible for the spicy flavor in apple, was 13 pmol kg^{-1} s^{-1} (Table 2). Only one ketone, acetone, was detected.

Organic Volatiles in CA-Stored Fruit. The volatile compound production of fruit stored in CA (1.5 kPa O_2, 3 kPa CO_2) at 0 °C for 180 days and subsequently ripened at 22 °C for 7 days is presented in Table 3. At day 7 of holding, CA storage caused a 5–6-fold reduction in total volatile synthesis for control fruit and approximately 20- and 8-fold reductions in total volatile synthesis for ReTain- and ReTain plus Ethrel-treated fruit, respectively (compare Tables 3 and 2).

The volatile compound production was reduced from 259 pmol kg^{-1} s^{-1} in control fruit to 58 pmol kg^{-1} s^{-1} by ReTain application (Table 3). This reduction in volatile production in ReTain-treated fruit was ∼78% lower than control. Volatile esters were reduced by 89%, and α-farnesene was reduced by 73%.

Ethrel application to ReTain-treated fruit increased the volatile production intermediate between ReTain alone and control fruit (Table 3).

The sesquiterpene, α-farnesene, evolved at the rate of 184 pmol kg^{-1} s^{-1} (Table 3), which accounted for 71% of the total amount of the 18 volatile compounds measured in CA-stored Jonagored Jonagold fruit. The volatile esters accounted for 28% of the total volatile compound production. Pentyl acetate (25 pmol kg^{-1} s^{-1}), hexyl hexanoate (14 pmol kg^{-1} s^{-1}), butyl hexanoate (9 pmol kg^{-1} s^{-1}), hexyl butanoate (8 pmol kg^{-1} s^{-1}), and hexyl acetate (5 pmol kg^{-1} s^{-1}) were the predominant straight-chain esters. The branched-chain esters were, in decreasing order of abundance, butyl 2-methylbutanoate, 2-methylbutyl acetate, and pentyl 2-methylbu-

Table 2. Effect of ReTain (R) and ReTain plus Ethrel (RE) Application on Volatile Compound Production ($pmol{\cdot}kg^{-1}{\cdot}s^{-1}$) by RA-Stored Jonagold Apples during Holding at 22 °C[a]

compound	control ± SD	R ± SD	relative change (%)	RE ± SD	relative change (%)
acetone	0.03 ± 00	0.04 ± 0.01	+32	0.03 ± 0.00	0
propyl acetate	8.89 ± 0.89	5.43 ± 1.28	−39	7.09 ± 0.33	−20
2-methylpropyl acetate	1.89 ± 0.15	1.92 ± 0.31	+2	1.75 ± 0.08	−7
propyl propanoate	5.57 ± 0.56	5.05 ± 3.78	−9	5.32 ± 3.79	−5
butyl acetate	189.33 ± 22.1	80.68 ± 17.1	−57	180.71 ± 9.30	−5
2-methylbutyl acetate	95.10 ± 8.82	83.40 ± 5.30	−12	84.84 ± 3.19	−11
propyl butanoate	8.75 ± 0.88	4.53 ± 1.27	−48	6.51 ± 1.01	−26
butyl propanoate	57.15 ± 9.33	39.34 ± 7.20	−31	52.90 ± 8.16	−7
pentyl acetate	49.09 ± 4.93	37.93 ± 4.31	−23	43.34 ± 2.17	−12
butyl butanoate	11.90 ± 2.22	6.96 ± 2.57	−42	6.54 ± 0.78	−45
1-butanol 2-methylpropanoate	2.38 ± 0.33	1.76 ± 1.25	−26	1.73 ± 0.03	−27
propyl 2-methyl butanoate	56.49 ± 8.51	24.14 ± 16.8	−57	57.35 ± 4.01	+2
hexyl acetate	98.27 ± 6.88	78.90 ± 5.36	−20	100.66 ± 5.06	+2
butyl 2-methyl butanoate	30.74 ± 1.02	22.95 ± 4.33	−25	26.51 ± 1.74	−14
propyl hexanoate	10.88 ± 0.73	5.47 ± 1.30	−50	9.08 ± 0.54	−17
hexyl propanoate	43.12 ± 5.54	34.41 ± 3.16	−20	44.49 ± 5.25	+3
pentyl 2-methylbutanoate	2.03 ± 0.05	1.74 ± 0.52	−14	1.57 ± 0.15	−23
butyl hexanoate	39.94 ± 2.68	30.34 ± 1.19	−24	38.63 ± 1.38	−3
hexyl butanoate	61.33 ± 8.11	39.77 ± 22.9	−35	72.09 ± 6.34	+18
propyl 2-methylhexanoate	141.52 ± 97.9	150.63 ± 16.4	6	142.87 ± 98.8	+1
4-methoxy-2-propenylbenzene	12.80 ± 2.17	10.91 ± 0.80	−15	13.82 ± 1.34	+8
hexyl 2-methylbutanoate	253.00 ± 148	272.38 ± 27.0	8	238.05 ± 150	−6
pentyl hexanoate	2.88 ± 0.49	2.34 ± 0.47	−19	3.22 ± 0.47	+12
hexyl hexanoate	71.03 ± 11.8	56.72 ± 2.34	−20	79.04 ± 5.08	+11
α-farnesene	222.70 ± 21.6	197.81 ± 72.8	−11	242.35 ± 24.4	+9
total volatiles	1476.81	1195.55	−19	1460.51	−1
total esters	1241.28	986.79	−21	1204.31	−3

[a] Volatile compounds are shown in order of their retention time. On removal of fruit from 45 days of RA storage, volatile compounds were collected at 1−2 day intervals over a period of 8 days of holding at 22 °C. The data were corrected for flow rates, and fruit mass and pooled data in each treatment for day 7 are shown only for treatment comparisons. Data are an average of three jars; each jar contained three fruits.

Table 3. Effect of ReTain (R) and ReTain plus Ethrel (RE) Application on Volatile Compound Production ($pmol{\cdot}kg^{-1}{\cdot}s^{-1}$) by CA-Stored Jonagold Apples during Holding at 22 °C[a]

compound	control ± SD	R ± SD	relative change (%)	RE ± SD	relative change (%)
acetone	0.45 ± 0.28	0.19 ± 0.11	−57	0.38 ± 0.07	−17
2-methylpropyl acetate	0.31 ± 0.01	0.06 ± 0.02	−81	0.11 ± 0.13	−65
butyl acetate	3.42 ± 0.59	0.16 ± 0.17	−95	3.29 ± 0.39	−4
2-methylbutyl acetate	25.39 ± 2.86	0.70 ± 0.95	−97	13.80 ± 9.74	−46
butyl propanoate	0.86 ± 0.18	0.06 ± 0.02	−93	0.42 ± 0.28	−51
pentyl acetate	1.53 ± 0.65	0.09 ± 0.02	−94	1.37 ± 0.93	−10
butyl butanoate	0.03 ± 0.01	0.04 ± 0.03	+33	0.03 ± 0.01	0
hexyl acetate	5.44 ± 0.63	0.38 ± 0.50	−93	3.91 ± 20.6	−28
butyl 2-methylbutanoate	2.52 ± 0.26	0.14 ± 0.09	−94	1.54 ± 1.06	−39
propyl hexanoate	0.42 ± 0.07	0.03 ± 0.03	−92	0.21 ± 0.12	−50
hexyl propanoate	0.73 ± 0.96	0.07 ± 0.06	−90	0.37 ± 0.43	−49
pentyl 2-methylbutanoate	0.32 ± 0.21	0.04 ± 0.01	−87	0.14 ± 0.15	−56
butyl hexanoate	8.43 ± 0.81	1.54 ± 1.10	−82	5.69 ± 3.84	−33
hexyl butanoate	9.38 ± 1.22	1.56 ± 1.10	−83	6.43 ± 4.13	−31
4-methoxy-2-propenylbenzene	0.83 ± 0.19	0.06 ± 0.05	−93	0.38 ± 0.23	−55
pentyl hexanoate	0.83 ± 0.09	0.24 ± 0.13	−71	0.55 ± 0.35	−34
hexyl hexanoate	14.16 ± 1.41	3.17 ± 1.98	−78	9.47 ± 5.95	−33
α-farnesene	184.35 ± 14.57	49.67 ± 33.39	−73	127.68 ± 60.75	−31
total volatiles	259.40	58.20	−78	175.77	−32
total esters	73.77	8.28	−89	47.33	−36

[a] Volatile compounds are shown in order of their retention time. On removal of fruit from 180 days of CA storage, volatile compounds were collected at 1−2 day intervals over a period of 8 days of holding at 22 °C. The data were corrected for flow rates and fruit mass, and pooled data in each treatment for day 7 are shown only for treatment comparisons. Data are an average of three jars; each jar contained three fruits.

tanoate. Among the various esters, pentyl acetate was produced in the highest (25 pmol kg^{-1} s^{-1}) and butyl butanoate in the lowest (0.03 pmol kg^{-1} s^{-1}) amounts (Table 3). The rate of production of 4-methoxy-2-propenylbenzene, a volatile compound responsible for the spicy flavor in apple, was 0.8 pmol kg^{-1} s^{-1} (Table 3). Only one ketone, acetone, was detected.

Effect of Treatments on the Production of Selected Volatiles by RA-Stored Jonagored Jonagold Fruit. The production rates of aroma-related representative volatiles from straight-chain esters (butyl acetate), its corresponding branched-chain ester (butyl 2-methylbutanoate), and a unique compound responsible for the spicy flavor in some cultivars of apple (4-methoxy-2-propenylbenzene) are shown in Figure 3. The production rate of butyl acetate increased from 100 pmol kg^{-1} s^{-1} on day 1 to 170 pmol kg^{-1} s^{-1} on day 3 and was stable thereafter during poststorage holding (Figure 3A). ReTain-treated fruit produced approximately half the amount of butyl acetate (60 pmol kg^{-1} s^{-1}) compared

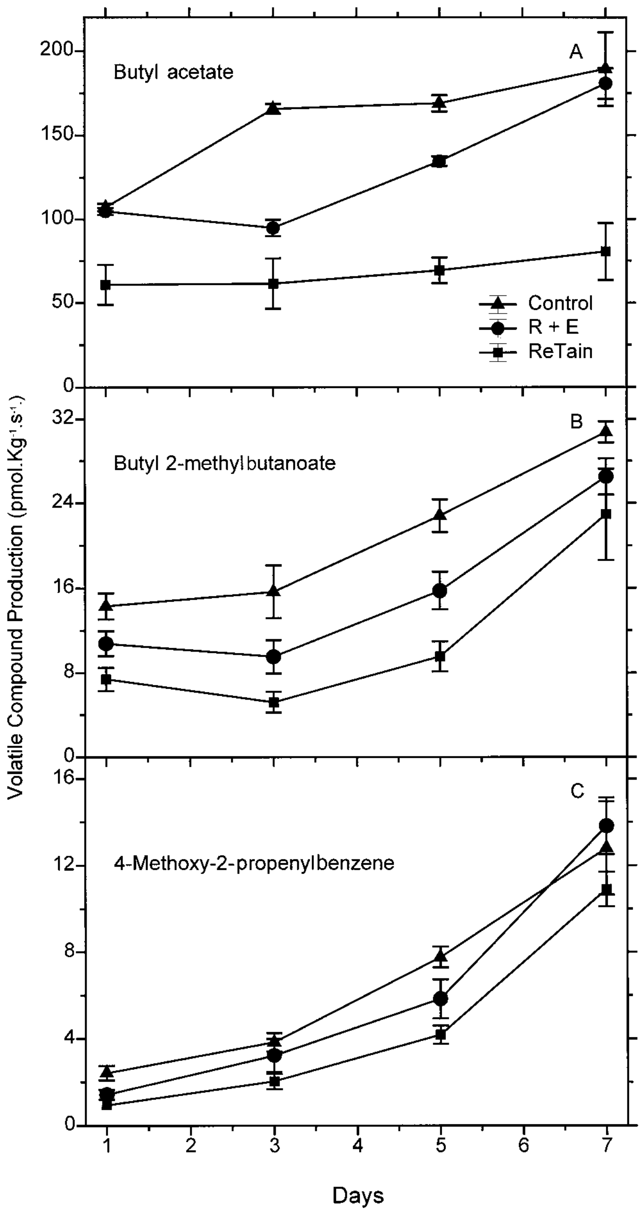

Figure 3. Butyl acetate (A), butyl 2-methylbutanoate (B), and methoxy-2-propenylbenzene (C) evolution at 22 °C of Jonagored Jonagold fruit stored for 45 days in air at 0 °C. The data at each sampling point are an average of three replications, each replication comprised of three fruits. The bars represent the SD of the population.

to control on day 1 of holding. The production rate of butyl acetate in ReTain-treated fruit increased gradually from 60 pmol kg^{-1} s^{-1} on day 1 to 75 pmol kg^{-1} s^{-1} on day 7 of holding. Ethrel application to ReTain-treated fruit increased the butyl acetate production approximately to control levels.

The production of butyl 2-methylbutanoate was stable during the initial 3 days of holding. However, it increased from 15 pmol kg^{-1} s^{-1} on day 3 to 30 pmol kg^{-1} s^{-1} on day 7 of holding (Figure 3B). ReTain application reduced the production of butyl 2-methylbutanoate by 3-fold during the initial 3 days of holding. Ethrel application to ReTain-treated fruit increased the production of butyl 2-methylbutanoate to intermediate levels.

The production of 4-methoxy-2-propenylbenzene increased gradually from 2.5 pmol kg^{-1} s^{-1} on day 1 to 3.4 pmol kg^{-1} s^{-1} on day 3 and sharply thereafter to 12 pmol kg^{-1} s^{-1} on day 7 of poststorage holding (Figure 3C). ReTain and Ethrel applications did not influence the production of 4-methoxy-2-propenylbenzene.

DISCUSSION

The reduction in internal ethylene and delay in maturation achieved by ReTain application are similar to responses previously measured (Autio and Bramlage, 1982; Bangerth, 1978; Bramlage et al., 1980; Wang and Mellenthin, 1977; Williams, 1980) and are consistent with the known mode of action of AVG. The ability of Ethrel to partially reverse these effects suggests that the reduction in internal ethylene brought about by reduced synthesis can be ameliorated in the field by the application of ethylene-releasing compounds.

The apple-like or fruity smell in various cultivars of apple is due to a class of volatile compounds known as esters (Fellman et al., 1993; Flath et al., 1967). Apple cultivars may be either "acetate-ester producing" such as Golden Delicious, Red Delicious, Fuji (Dixon and Hewett, 1997; Fellman et al., 1993) and Jonagored Jonagold (Tables 2 and 3) or "non-acetate-ester producing" such as Cortland and McIntosh (Yahia et al., 1990). Whereas esters in apple impart a generic fruity aroma (Fellman et al., 1993), the spicy flavor of Jonagored Jonagold apples could be related to 4-methoxy-propenylbenzene (Williams et al., 1977). The qualitative composition of aroma volatiles in this study is similar to that found by other investigators in other cultivars of apple (Fallik et al., 1997; Yahia et al., 1990).

Whereas pathways of volatile synthesis in fruit are poorly understood, autocatalytic ethylene production seems to precede and is likely associated with volatile production in apple (Fan et al., 1998). Despite the reduced ethylene production by ReTain-treated RA fruit, the similar levels of total volatile production and respiration rates suggest the accumulated internal ethylene was sufficient to result in similar levels of ethylene response. Although reduced volatile production with AVG application has been previously reported in apple (Halder-Doll and Bangerth, 1987; Child et al., 1984) and pear (Romani et al., 1983), RA storage for 45 days permitted the fruit to largely overcome the effects of ReTain. The fact that the production of 4-methoxy-propenylbenzene, a compound responsible for the spicy flavor in apple (Williams et al., 1977), was not influenced by applied chemicals suggests that fruit treated with ReTain or a combination of Retain plus Ethrel may have a level of consumer preference similar to that of control fruit. This contention is supported by the results of Child et al. (1984) with Cox's Orange Pippin, who have shown no effect of preharvest sprays of AVG on consumer acceptability of RA-stored fruit.

Reduced aroma production by long-term CA storage relative to RA storage is consistent with volatile compound loss in many apple cultivars following CA storage (Brackman et al., 1993; Yahia et al., 1990). Although it is conceivable that evaporation of volatile compounds during long-term storage may contribute to their decline, it is generally believed that loss of substrates and enzymes essential for formation of esters may also take place (Patterson et al., 1974). A study by Williams and Knee (1977) showed that esterifying enzymes operate at similar rates in fruit stored in air or CA. Therefore, the suppression of volatile compounds was suggested to be due to the limited supply and further metabolism of volatile precursors (Knee and Sharples, 1981). The low production of volatile compounds in CA as compared to RA may have been due to the differences in the length of storage or known effects of CA suppression on ethylene perception (Beaudry, 1999) and subsequent volatile production (Williams and Knee, 1977; Yahia et al., 1990).

The further reduction in volatile biosynthesis in ReTain-treated fruit suggests even though fruit are at similar stages of maturity at harvest, the effect of ReTain continues to suppress ethylene production and hence ethylene responses in low-O_2 storage. Thus, where RA storage confers sufficient ethylene responsiveness to overcome reduced ethylene accumulation, the same is not true for CA storage. ReTain treatment effects may therefore be exaggerated somewhat by long-term CA storage. The inability of Ethrel application to completely overcome the effects of ReTain on aroma production is consistent with the ability of the Ethrel application to only partially restore ethylene production.

The suppression in the production of α-farnesene by ReTain application in CA-stored fruit may also suppress the development of storage scald in apple. α-Farnesene, a sesquiterpene hydrocarbon, accumulates in the peel of apple and pear during low-temperature storage and has been associated with the induction of superficial scald in apple fruit (Anet, 1969; Ingle and D'Souza, 1989). Fruit cultivars that produce fewer α-farnesene oxidation products such as 6-methyl-5-hepten-2-one (MHO) and conjugated trienols are less likely to develop superficial scald during storage (Whitaker et al., 1997). It has also been shown that MHO may causally be related to the development of superficial scald in apple (Mir et al., 1999). Treatments that reduce α-farnesene metabolism in fruit (such as low-O_2 storage, use of some antioxidants) or that remove possible toxic volatile products of α-farnesene oxidation, such as MHO, from the storage environment (continuous purging with low-O_2 atmospheres or hypobaric storage) appreciably reduce storage scald in apple (Ingle and D'Souza, 1989). The reduced production of α-farnesene and lack of its oxidation product, MHO, in ReTain-treated fruit may suggest that superficial scald development may be reduced or delayed by preharvest application of ReTain to apple fruit.

This study demonstrates that ReTain may have some promise for harvest management of apple. For RA-stored fruit, compromised fruit quality loss as measured by flavor-related volatile compounds appears not to be serious. However, CA-storage in combination with ReTain may be detrimental to sensory quality. Ethrel application can partially overcome this and other ethylene-related effects.

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Received for review October 19, 1998. Revised manuscript received March 15, 1999. Accepted May 10, 1999.

JF981150K