Effect of Methyl Jasmonate on Ethylene and Volatile Production by Summerred Apples Depends on Fruit Developmental Stage

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Pre- and postclimacteric Summerred apples (*Malus domestica* Borkh.) were exposed for 12 h to a low concentration of atmospheric methyl jasmonate. Ethylene and volatile production were measured at harvest and through 15 days at 20 °C after treatment. Responses to methyl jasmonate treatment depended on the stage of fruit development. Methyl jasmonate stimulated ethylene, ester, alcohol, and acetic acid production in preclimacteric fruits, while little or no response was elicited from postclimacteric fruits. Ketone and aldehyde emissions were not affected by methyl jasmonate regardless of developmental stage.

Keywords: *Apple fruit; ripening; volatiles; methyl jasmonate*

INTRODUCTION

Jasmonic acid (JA) and its methyl ester, methyl jasmonate (MJ), are regarded as naturally occurring plant growth regulators (Sembdner and Parthier, 1993). JA and MJ are present in low concentrations in a variety of plant organs including flowers, fruits, seeds, buds, germs, shoots, and leaves (Meyer *et al*., 1984). Relatively large amounts (JA 80 ng g^{-1} fresh weight; MJ 390 ng g^{-1} fresh weight) have been reported in Golden Delicious apple fruits (Meyer *et al*., 1984). The biological activities ascribed to MJ and JA include promotion of senescence, petiole abscission, root formation, and synthesis of ethylene, anthocyanin, and carotene. MJ and JA have also been shown to inhibit seed germination, callus growth, root growth, chlorophyll production, lycopene accumulation, and pollen germination (Koda, 1992; Staswick, 1992, and references cited therein).

Non-ethylene volatile compounds are an important component of apple flavor, and production of aroma volatiles increases as apples ripen (Brown *et al*., 1966). Recently, MJ has been shown to inhibit volatile ester production by Golden Delicious apples after controlled atmosphere storage (Olias *et al*., 1992). MJ applied to the fruit surface at a concentration of 0.5% in lanolin paste stimulated ethylene production in both mature green and red ripe tomato fruits (Saniewski and Czapski, 1985). When applied to apples at the same or higher concentrations, MJ promoted ethylene production by preclimacteric apples but inhibited ethylene production by postclimacteric apples (Saniewski *et al*., 1987). Anderson (1989) suggested that such effects may be due to the use of phytotoxic levels of MJ. Our objective was to evaluate the response of an early-maturing summer apple variety, Summerred, to a relatively low concentration, short duration MJ treatment applied at various stages of fruit development. Specifically, effects of MJ treatment prior to and after onset of ripening on ethylene and other volatile production were examined.

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MATERIALS AND METHODS

Summerred apples (*Malus domestica* Borkh.) were harvested 91, 99, 106, and 113 days after full bloom (DAFB) from a research orchard near Wenatchee, WA. Sampling and analysis of internal ethylene concentration were performed as described previously (Williams and Patterson, 1962). Internal ethylene concentration was determined on the day of harvest. Ethylene production rate and emission of other volatile compounds were analyzed prior to and 1, 4, 7, 11, and 15 days after MJ treatment.

MJ Treatment. Three replicate samples (three apples each) of fruit after each harvest were placed into 4 L glass jars along with a filter paper wetted with 25 mg of MJ (Bedoukian Research, Danbury, CT). The filter paper was used to facilitate evaporation. The jars were tightly sealed, and the amount of atmospheric MJ in the jars at 20 °C was 2×10^{-7} mol L^{-1} of air (Franceschi and Grimes, 1991). After 12 h at 20 °C, the fruits were removed and subsequently ripened in air at 20 °C for 15 days.

Analysis of Headspace Volatiles. Volatiles were collected as previously described (Mattheis *et al*., 1991). Apples (three apples per each of three replicates) were placed into 4 L glass jars, and the jars were sealed using Teflon lids with two gas ports. Compressed air, purified via a column containing potassium permanganate, activated charcoal, calcium hydroxide, molecular sieve, and Tenax TA (Alltech Associates, Deerfield, IL), flowed into the jars at 100 mL min^{-1} for 4 h (to assure sampling under equilibrium conditions) at 20 °C prior to sampling. The volume of headspace collected from each replicate was 50−200 mL depending on fruit maturity. Volatile compounds in the outlet gas were adsorbed onto 50 mg of 30−50 mesh Tenax TA packed in glass tubing (17.5 cm × 0.4 cm i.d.).

Volatile compounds on the Tenax traps were desorbed at 250 °C for 3 min using a Tekmar 6000 aero trap desorber (Tekmar Co., Cincinnati, OH). The desorber was used with a cryofocusing module to condense the desorbed sample compounds in a cryogenically cooled trap. After the desorbed sample compounds were condensed, the cryofocusing module was flash heated to 250 °C under a stream of He carrier gas which carried the analytes into a Hewlett-Packard 5890A/5971A GC/MSD equipped with a DB-Wax column (J&W Scientific, 60 m × 0.25 mm i.d., 0.25 μm film thickness). Conditions for chromatography and mass spectrometry were reported previously (Mattheis *et al*., 1991). Spectra were recorded using Hewlett-Packard 59970C Chemstation software. Compound identification was made by comparison of spectra of sample compounds with those contained in the Wiley-NBS library and by comparing retention indices of

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Table 1. Internal Ethylene Concentration of Summerred Apples at Harvest[a]

days after full bloom	internal ethylene concn (μL L^{-1})
91	0.04 (0.00−0.1)
99	0.19 (0.08−0.42)
106	33.37 (0.57−157.68)
113	45.75 (10.96−101.44)

[a] Mean values were calculated from 20 apples. Values in parentheses indicate range of ethylene concentration detected.

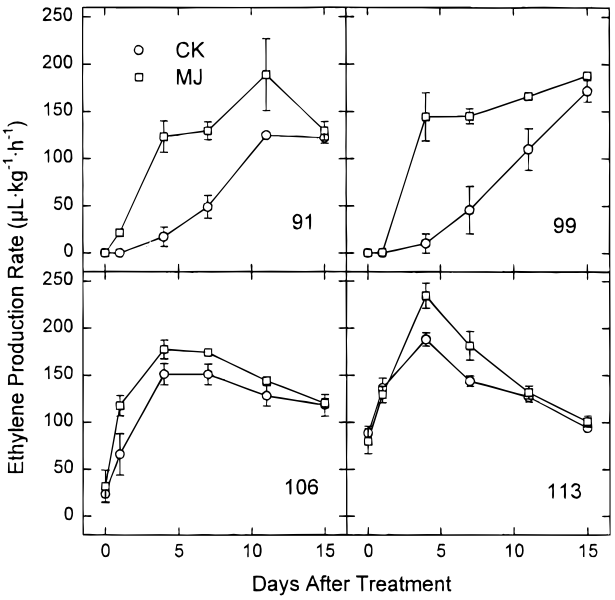

Figure 1. Ethylene production of Summerred apples following methyl jasmonate treatment. Apples were harvested 91, 99, 106, and 113 days after full bloom and held at 20 °C during treatment and ripening. MJ, methyl jasmonate; CK, untreated. Vertical bars represent SE of the mean.

sample compounds and standards (Aldrich, Milwaukee, WI). Quantification was performed using selected ion monitoring for base peaks, and quantitative values were calculated using response factors generated with standards. Volatile emission was expressed as nanograms per kilogram per hour.

Ethylene Production Rate. Fruit ethylene production was determined by collecting 1 mL gas samples from the jar outlet port prior to volatile collection on Tenax traps. Samples were analyzed using a Hewlett-Packard 5880 gas chromatograph equipped with a glass column (610 mm × 3.2 mm i.d.) packed with Porapak Q (80−100 mesh). Injector, oven, and flame ionization detector temperatures were 100, 50, and 200 °C, respectively. Gas flows for N_2 carrier, H_2, and air were 30, 30, and 300 mL min^{-1}, respectively. Ethylene production rate was expressed as microliters per kilogram per hour.

RESULTS AND DISCUSSION

Measurement of internal ethylene concentration at harvest indicated the onset of the climacteric occurred between 99 and 106 DAFB (Table 1). Ethylene production responses due to MJ exposure varied with fruit developmental stage (Figure 1). In preclimacteric fruit harvested 91 and 99 DAFB, MJ exposure stimulated ethylene production rate 7.2- and 14.4-fold, respectively, compared to controls 4 days after treatment. MJ stimulation of ethylene production was evident 1 day after exposure of apples harvested 91 DAFB. The difference in ethylene production rates between controls and MJ-treated fruits was no longer apparent in apples held for 15 days after MJ exposure. Saniewski *et al.* (1987) reported that 0.5% or 1% MJ−lanolin paste application to the surface of preclimacteric apples stimulated ethylene production, while identical treatment to postclimacteric apples inhibited ethylene production. No inhibitory effect was observed in our experiment, possibly due to use of a lower MJ concentration. We observed enhanced ripening-related apple peel yellowing on preclimacteric apples from the first two harvests after MJ treatment (data not shown). This observation agrees with that of Perez *et al.* (1993), who reported chlorophyll degradation and β-carotene synthesis in response to MJ treatment of Golden Delicious apples.

Table 2. Volatile Compounds Detected in Headspace Samples Collected from Summerred Apples[a]

peak	compound	retention index	amount (% total peak area)
1	2-propanol	931	0.08
2	ethanol	936	0.15
3	ethyl propanoate	953	0.05
4	pentanal	971	0.03
5	methyl butyrate	983	0.22
6	methyl 2-methylbutyrate	1009	0.02
7	ethyl butyrate	1037	1.44
8	1-propanol	1044	0.03
9	propyl propanoate	1045	0.03
10	ethyl 2-methylbutyrate	1054	0.08
11	butyl acetate	1074	0.02
12	hexanal	1079	0.23
13	2-methyl-1-propanol	1093	0.04
14	2-methylbutyl acetate	1123	0.02
15	ethyl pentanoate	1136	0.03
16	butyl propanoate	1143	0.40
17	1-butanol	1149	2.28
18	heptanal	1184	0.18
19	2-methyl-1-butanol	1210	0.29
20	butyl butyrate	1220	6.34
21	butyl 2-methylbutyrate	1235	0.80
22	ethyl hexanoate	1236	0.49
23	1-pentanol	1255	0.06
24	hexyl acetate	1275	0.09
25	2-methylbutyl 2-methylbutyrate	1283	0.02
26	octanal	1290	0.40
27	pentyl butyrate	1319	1.07
28	propyl hexanoate	1320	0.19
29	hexyl propanoate	1343	1.57
30	6-methyl-5-hepten-2-one	1343	0.01
31	1-hexanol	1360	4.02
32	nonanal	1398	0.83
33	butyl hexanoate	1418	4.02
34	hexyl butyrate	1420	37.39
35	hexyl 2-methylbutyrate	1433	9.44
36	ethyl octanoate	1437	0.02
37	acetic acid	1453	2.88
38	2-furancarboxaldehyde	1471	0.07
39	2-ethyl-1-hexanol	1497	0.03
40	decanal	1506	3.47
41	benzaldehyde	1539	0.11
42	hexyl hexanoate	1617	21.13

[a] Apples were harvested 113 days after full bloom. Compounds were collected using Tenax traps on the day of harvest and analyzed by GC-MSD. Amounts are means of 3 replicate 1kg (approx.) apple samples.

As many as 42 compounds were routinely identified in headspace samples from Summerred apples as esters, alcohols, aldehydes, ketones, or acids (Table 2). The major quantitative volatiles of Summerred during ripening included hexyl butyrate, butyl butyrate, ethyl butyrate, hexyl 2-methylbutyrate, hexyl hexanoate, butyl hexanoate, and 1-hexanol. Total ester production of Summerred apples harvested 91 and 99 DAFB was stimulated by the low concentration and short duration of MJ exposure (Figure 2). The effect on apples harvested 99 DAFB decreased as fruit ripened at 20 °C after treatment. MJ did not affect ester production in apples from the last two harvest dates, indicating that

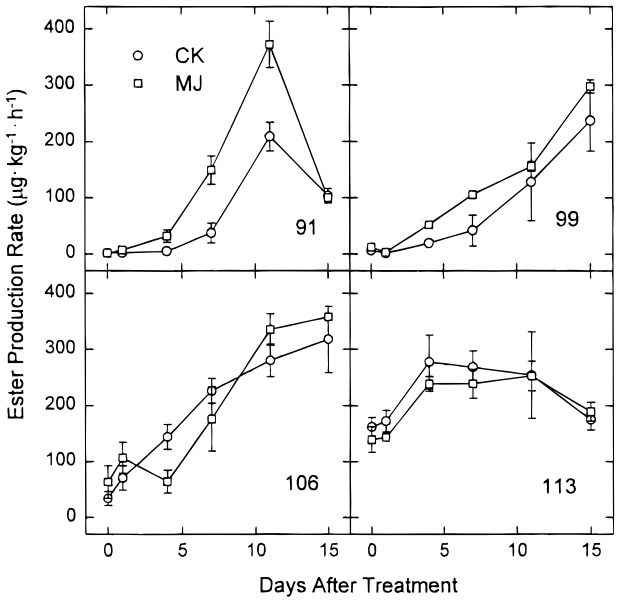

Figure 2. Total ester production of Summerred apples following methyl jasmonate treatment. Apples were harvested 91, 99, 106, and 113 days after full bloom and held at 20 °C during treatment and ripening. MJ, methyl jasmonate; CK, untreated. Vertical bars represent SE of the mean.

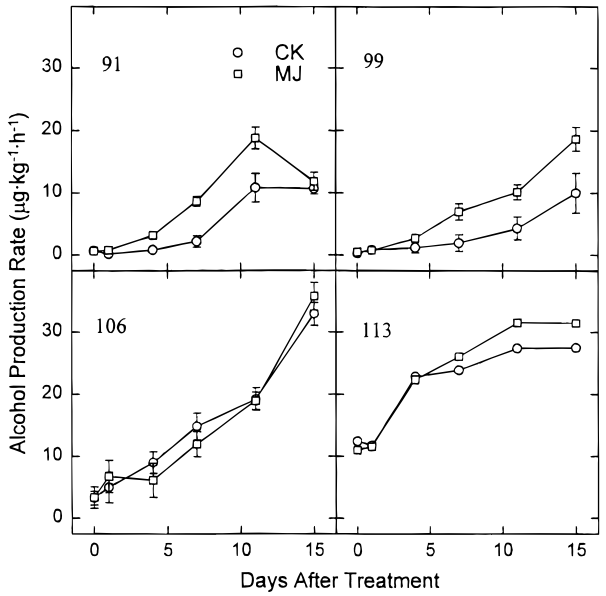

Figure 3. Total alcohol production of Summerred apples following methyl jasmonate treatment. Apples were harvested 91, 99, 106, and 113 days after full bloom and held at 20 °C during treatment and ripening. MJ, methyl jasmonate; CK, untreated. Vertical bars represent SE of the mean.

volatile production is related to developmental stage, and late-harvested fruit had already received the internal signal to ripen. MJ also stimulated total alcohol volatile production in fruit from 91 and 99 DAFB, but not in apples harvested 106 or 113 DAFB (Figure 3). MJ effects on acetic acid production were evident only for apples harvested 113 DAFB 11−15 days after treatment (Figure 4). Aldehyde production and ketone production were not influenced by MJ exposure regardless of apple harvest date (data not shown), suggesting that net production of these classes of volatiles is not ripening-related.

Many apple volatiles are acetate esters synthesized from alcohols and acetic acid as acetyl-CoA (Fellman and Mattheis, 1995) and comprise the majority of non-

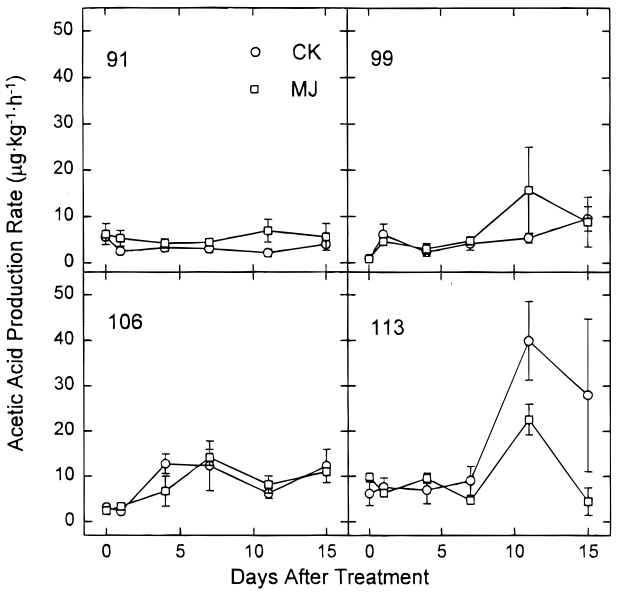

Figure 4. Acetic acid production of Summerred apples following methyl jasmonate treatment. Apples were harvested 91, 99, 106, and 113 days after full bloom and held at 20 °C during treatment and ripening. MJ, methyl jasmonate; CK, untreated. Vertical bars represent SE of the mean.

ethylene volatiles emanating from ripening apple fruit (Table 2). The amount of acetic acid emitted by Summerred apples is fairly constant during much of the development period characterized in this study (Figure 4) compared to the amount of alcohol (Figure 3), suggesting alcohol substrate availability is a limiting factor in volatile ester production. Comparison of the temporal relationship of alcohol and ester production (compare Figures 2 and 3) indicates that MJ may enhance acetate ester formation in treated fruits by increasing the quantities of alcohol available for subsequent reaction with acetyl-CoA. There was no qualitative difference in volatiles observed between controls and MJ-treated apples in this study.

Our results indicate that changes in apple volatile production in response to a low concentration MJ treatment are dependent on fruit developmental stage. Saniewski *et al.* (1987) reported similar results for apple ethylene production following exposure to a high concentration of MJ. We did not observe the same reduction in ethylene synthesis by postclimacteric apples they reported, perhaps due to our use of a lower MJ concentration and shorter treatment duration. Although our results indicate exposure to a physiological concentration of MJ enhanced ethylene and volatile production in preclimacteric apple fruit, the mechanism of MJ action remains unknown. A combination of MJ with an ethylene-releasing growth regulator (ethephon) led to a significant increase in tiller production when applied to spring barley leaves (Dathe, 1992). Neither MJ nor ethephon alone caused this effect, which suggests MJ increases plant sensitivity to ethylene. Apples stored in reduced oxygen atmospheres become less sensitive to ethylene (Bangerth, 1984); however, MJ treatment of Golden Delicious apples previously held in low-oxygen storage reduced ester volatile production (Olias *et al.*, 1992), indicating that the influence of MJ on the onset of apple ripening is complicated by developmental stage and previous storage conditions.

Many apple fruit volatiles are products of lipid and amino acid catabolism (Heath and Reineccius, 1986). MJ and JA stimulate lipase and protease activity in embryo

cultures derived from apple seeds (Ranjan and Lewak, 1992, 1995) and induce a lipoxygenase gene in *Arabidopsis*, tobacco, and cucumber (Melan *et al*., 1993; Avdiushko *et al*., 1995). It is possible that MJ stimulation of volatile synthesis in preclimacteric apples is a result of increased lipid and protein catabolic enzyme activities. The lack of response from postclimacteric fruit could be attributed to full development of ethylene sensitivity; therefore, any increase in substrate availability would not influence the esterification reaction rate already operating at maximum velocity. Some fatty acids are reported to increase ethylene sensitivity (Whitehead and Bosse, 1991), and exposure to MJ alters the fatty acid composition of tulip stems (Saniewski *et al*., 1992) and tomato fruit (Czapski *et al*., 1992). Apparently, JA and/or MJ participates in modulating initial events in apple ripening; however, the nature of the interaction between MJ and ethylene-related phenomenon remains to be elucidated.

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