THE EFFECT OF DELAYED COOLING AND DELAYED APPLICATION OF CA STORAGE OF MCINTOSH APPLES UNDER LOW AND HIGH ETHYLENE LEVELS

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The effectiveness of controlled atmosphere storage to delay ripening and preserve apples is markedly dependent on numerous and well documented factors (20). These include, among others, cultivar, level of O_2 and CO_2, humidity, temperature, maturity, and fruit calcium level. With the development of improved means to monitor and control the level of O_2 in the storage room has come the trend to store apples at 1 to 1.5% O_2 which can be dangerously close to the point of anaerobic fermentation. Increased awareness of the role of ethylene in ripening apples during controlled atmosphere storage (2,5,6,9) has also led to development and implementation of means to attenuate the synthesis or action of ethylene (1,2,3,13). Much research has been conducted in recent years employing low O_2 levels (9,11,15,16), pre-storage treatments with CO_2 (7,12), rapid CA (14), ethylene scrubbing (2,17), and hypobaric storage (1,9,10). It is generally recognized that apples for long term CA storage must be harvested and placed under CA before they gain capacity to produce ethylene autocatalytically (8). Moreover, during CA storage the ethylene level within the fruit must be kept below 1 ppm to effectively delay ripening (3). The studies reported here are an extension of the thorough investigations of Liu (17) on the effect of maturity, delayed cooling, delayed imposition of CA and ethylene removal on storage of McIntosh apples as grown in Michigan.

Materials and Methods

McIntosh were harvested twice from mature trees at the MSU Graham Experiment Station. The trees had received treatment with daminozide at 1000 ppm about 2 months before the first harvest. Fruits at the first harvest on Sept. 17 were preclimacteric and those at the second harvest on Sept. 24 were at the onset of the climacteric with respect to ethylene as determined by internal ethylene levels and by their readiness to ripen by the induced ethylene climacteric method. Fruits from each harvest were randomized into three treatment replicates of 60 fruits each in 20 l plastic containers and assigned to treatments. The containers were fitted with inlet and outlet tubulation. Fruits were stored at 3.3°C and subjected to a controlled atmosphere of 2% O_2 + 3% CO_2 + 95% N_2 prepared from liquified gases. The gas mixture was humidified and brought to the dew point at the storage temperature. The two ethylene levels were established by ventilating the containers with the gas atmosphere at a rate of 30 ml min^{-1} or 300 ml min^{-1} from a calibrated capillary flow rate control system. These ventilation rates provided about 2 and 0.2 atmosphere changes per hour, respectively. The experiment employed a total of 96 CA chambers and treatments were comprised of 2 maturities at harvest, 1 or 4 days cooling delay, 1,4,7, or 11 days delayed imposition of CA, 2 ethylene levels and 3 replicates. Additional samples were kept in air at 3.3^oC. The composition of the storage atmosphere and production of ethylene and CO_2 was monitored with electronic gas analyzers; CO_2 by infra-red, O_2 by paramagnetic; zirconia and O_2 electrode, and ethylene by gas chromatography.

At Harvest and Post-storage Evaluations

Internal ethylene content was determined by gas chromatography employing 10 individual fruits. Flesh firmness was determined for the same fruits with a Effegi penetrometer with an 11 mm diameter tip mounted in a drill press. Starch index was determined by I_2-KI test (19). The time of the autogenous ethylene climacteric was determined by the induced ethylene climacteric method employing three 10-fruit samples in sealed containers with dry lime. Fruits were examined after 0,2,4,6 and 8 months of storage. Freeze-dried samples were prepared at each sampling date and analyzed for ACC (1-aminocyclopropane-1-carboxylic acid) (18).

Results and Discussion

Maturity Assessment at Harvest: Flesh firmness measurements showed no difference between the harvests of 9/17 and 9/24 at harvest nor after 4 days holding after harvest at room temperature of about 68^oF. Starch index of 5 to 6 indicates a mid-to late stage of maturity for long-term CA storage. A range of 3 to 5 on the Ontario scale is considered by some operators to be appropriate for McIntosh for long term CA. Physiological maturity as judged by ethylene production capacity was determined to be ideal for long-term CA on about Sept. 20 for fruits not receiving daminozide and Sept. 27 for fruits which received daminozide. Internal ethylene levels were mostly less than 0.1 ppm at harvest on 9/17 and after 4 days at 20^oC. Median ethylene level at harvest on 9/24 was 0.13 ppm and the concentration ranged from .09 to 1.2 ppm. And after 4 days at 20^oC the range was 0.06 to 41 ppm. Based upon the starch index, maturity was judged to be mid-optimum or slightly late for long-term CA at 9/17 and late at 9/24. Based upon internal ethylene levels and capacity to produce ethylene, maturity was judged to ideal or slightly early for long-term CA on 9/17 and ideal for mid-to long term CA on 9/24 providing good CA technology application was administered properly and timely. Note that the median and range of internal ethylene levels are more indicative of physiological development of maturation chemistry than average or mean values because of the logrithmic aspect of auto-catalytic ethylene production as ripening potential is achieved.

Storage Studies: Results from CA storage studies with McIntosh after 2 months storage at 2% O_2 and 3% CO_2 at 3.3^oC are given in Tables 2 and 3. In Table 2 it is clear that it is much more important to avoid a delay in cooling the fruit than delaying imposition of CA. Fruits from either the

9/17 or 9/24 harvest and kept in low ethylene CA were maintained at less than 0.1 ppm ethylene in the storage atmosphere if cooled within 1 day after harvest even if there was an 11 day delay before establishing the CA atmosphere. Fruits that were not cooled for 4 days had to be under low ethylene CA conditions within 4 additional days to keep ethylene below 0.1 ppm after 2 months of storage. Fruits cooled within 1 day gained capacity to produce a significant amount of ethylene if CA conditions were not established by 7 days.

The consequence of delaying cooling and imposing CA conditions on fruit firmness immediately after only 2 months of storage is shown in Table 3 and this mirrors the results on ethylene production capacity. A four-day delay in cooling providing CA conditions were established within 4 days of harvest did not induce the development of softening. Delaying CA more than 4 days even if the fruit were cooled within 1 day induced the development of softening although this was not apparent from firmness measurements taken immediately after two months of storage; differences became quite apparent during subsequent holding for 7 days at room temperature (Table 3). And this was more evident if ethylene was not kept low during CA storage. The factor of fruit maturity is not very apparent in these data after only 2 months of storage.

The influence of delaying cooling and imposing CA conditions on flesh firmness of McIntosh after 8 months in high or low ethylene CA is given in Table 4. Ripening is quite effectively delayed but not arrested even in low ethylene CA. The internal ethylene levels of fruits regardless of prestorage treatments ranged from 11 to 24 ppm after 8 months storage. Higher flesh firmness values were observed after 8 months for fruits cooled immediately and under CA within 7 days or if cooling was delayed for 4 days if the atmosphere was established without delay. The data for flesh firmness after 8 months of storage correlates closely with the development of ethylene production capacity of the fruit during storage (Table 5).

Table 6 provides data for the same prestorage treatments for fruits stored hypobarically at 0.05 atmos. Hypobaric ventilation provides a means to ensure hyponormal ethylene levels in the storage chamber and within the internal atmosphere of the fruit. Reducing the storage pressure to 0.1 atmosphere should reduce the ethylene partial pressure within the fruit by a factor of 10 in comparison to the ethylene level in fruits at 1 atmos. providing the production rate remains constant. In fact, this is a conservative estimate since the ethylene production will be lowered as a result of the 10-fold reduction in oxygen partial pressure. Moreover, as the fruits' capacity to produce ethylene increases prior to hypobaric storage, either as a result of advanced maturity at harvest, delayed cooling or delayed imposition of hypobaric storage, the ability to attenuate ethylene action in fruit ripening would be expected to diminish. This is because restricting the oxygen supply and removing ethylene from the storage and fruit atmosphere cannot reduce ethylene levels below the threshold for activity. This can be seen clearly in the data of Table 6. After about 8 months of hypobaric storage at 0.05 atmos.

at 3.3°C ripening during storage as measured by flesh firmness progressed markedly if cooling was delayed more than 4 days or if hypobaric storage was delayed more than 7 days. And ripening changes proceeded earlier and more extensively in fruits which were more mature at harvest. It is important to note that the effect of maturity and pre-storage treatments cannot be assessed by following storage in air at 3.3°C even after as little as a 2 month storage duration. This is because storage in air does not effectively attenuate the ripening process. Fruits of both maturity levels and all pre-storage treatments quickly gain potential to ripen given adequate oxygen in air since ethylene is abundant in the fruit soon after storage begins. Judging from the flesh softening data of fruits stored hypobarically, attenuation of ethylene action diminished drastically if hypobaric conditions were delayed more than 7 days if the fruits were cooled immediately or more than 4 days if cooling was delayed for 4 days.

This data may be useful to set the limits of application of controlled atmosphere storage technology with the McIntosh cultivar. Thus, fruits which are about one week pre-climacteric with respect to ethylene may be considered suitable for low ethylene and low oxygen CA storage if cooled immediately and the atmosphere is established within 7 to 11 days of harvest for the earliest picked fruit. Fruits harvested at the onset of the ethylene climacteric may be stored safely if they are cooled quickly and have the atmosphere established within 7 days of harvest. The data in Table 4 support this.

Figure 1 summarizes the change in flesh firmness in relation to the delay in cooling and the delay of imposing low ethylene CA or hypobaric storage over the entire 8 month storage period. The data is for fruits of the first harvest which were preclimacteric with respect to ethylene at harvest. Flesh firmness is retained slightly better by hypobaric ventilation than by ventilation with ethylene-free atmosphere for both the fruits cooled immediately or after 4 days at 20°C. The deliterous effect of delayed cooling is evident after only 2 months of storage.

Accumulation of ethylene in the low ethylene CA storage chambers occurred earlier and to higher levels when cooling and imposing CA was delayed (Figure 2). Although high ventilation rates kept the ethylene in the CA chambers below 1 ppm for the duration of the storage period (Figure 2, B and D), the fruits began to produce ACC and ethylene earlier when cooling was delayed than if the fruits were cooled immediately after harvest (Figure 3). Development of ethylene production capacity reflected the increase in ACC content and this has been found in other studies.

In summary, delaying the cooling of 'McIntosh' apples for 4 days causes the fruit to develop capacity to produce ACC and ethylene and to soften during CA unless CA is established within four days of harvest. If fruits are cooled within 1 day of harvest and CA is established within 7 days, capacity to produce ACC and ethylene and to soften during subsequent CA storage is greatly diminished. Thus, markedly beneficial effects of controlled atmosphere storage on delaying ripening of McIntosh may be realized only through attenuation of ethylene synthesis and action. Our studies confirm and extend the results of Liu (17).

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TABLE 1. Maturity Parameters for McIntosh Controlled Atmosphere Storage Experiment - 1984.

Maturity Parameter		9/17			9/24		
	Harvest date*	9/17			9/24		
	Storage date		9/17	9/21		9/24	9/28
	Cooling delay (days)		0	4		0	4
Flesh firmness		15.1	15.4	15.2	15.0	15.1	15.4
Starch index**		4.6	5.5	6.0	5.3	5.8	6.6
Internal ethylene(ppm)							
	Median	.05	.04	.06	.13	.5	.14
	Range	.02-.09	.02-.5	.04-.2	.09-1.2	.2-5.	.06-41.
	Mean	.06	.09	.08	.25	1.1	5.4

* Harvest date of Sept. 20 was determined as onset of autogenous ethylene climacteric as judged by the induced ethylene climacteric of fruits not receiving Alar.
Sept. 27 was determined as onset of autogenous ethylene climacteric of fruits receiving daminozide treatment by the induced climacteric method.

** Starch index of Ontario Ministry of Agriculture; 1 = immature, 9 = 0 over mature

TABLE 2. Influence of delaying cooling and imposing CA* conditions on McIntosh fruits kept for 2 months in high or low ethylene CA on ethylene levels in the CA chambers.**

Cooling Delay (days)	CA Delay (days)	9/17 Harvest* High Ethylene	9/17 Harvest* Low Ethylene	9/24 Harvest High Ethylene	9/24 Harvest Low Ethylene
1	1	.11	.02	.06	.04
1	4	.20	.06	.12	.05
1	7	.11	.06	.11	.04
1	11	.68	.09	.59	.08
4	1	.06	.09	.35	.05
4	4	1.56	.09	.73	.11
4	7	0.98	.24	1.40	.18
4	11	3.34	.41	2.73	.31

* CA conditions were 2% O_2 + 3% CO_2 at 3.3°C.

** 20 l. chambers with 60 fruits were ventilated at 30 or 300ml/min. for high and low ethylene levels, respectively

*** Fruits were preclimacteric and at the beginning of the ethylene climacteric at Sept. 17 and 24th, respectively

TABLE 3. Influence of delaying cooling and imposing CA conditions* on flesh firmness (lbs) of McIntosh fruits in high or low ethylene CA for 2 months of storage and 7 days post-storage holding at room temperature ( ).**

		9/17 Harvest***		9/24 Harvest	
Cooling Delay (days)	CA Delay (days)	High Ethylene	Low Ethylene	High Ethylene	Low Ethylene
1	1	15.6(15.3)	15.5(15.5)	15.1(15.9)	15.4(15.3)
1	4	15.4(12.8)	15.6(13.4)	14.9(13.2)	14.9(14.9)
1	7	15.1(11.5)	15.4(10.4)	14.4(11.6)	14.7(11.8)
1	11	14.7(11.0)	15.0(10.9)	13.6(10.2)	14.0(10.6)
4	1	15.3(14.6)	15.2(14.2)	15.4(13.0)	15.4(14.4)
4	4	13.6(10.4)	13.9(11.2)	13.4(10.5)	13.1(10.3)
4	7	12.8(10.0)	12.1(10.1)	11.9(9.8)	11.6(9.7)
4	11	11.6(9.5)	11.5(9.6)	11.1(9.6)	10.7(9.5)

* CA conditions were 2% O_2 + 3% CO_2 at 3.3°C.

** 20 l. chambers with 60 fruits were ventilated at 30 or 300ml/min. for high and low ethylene levels, respectively

*** Flesh firmness at harvest was 15.1 and 15.0 lbs. on Sept. 17 and 24th, respectively

TABLE 4. The influence of delaying cooling and imposing CA conditions on flesh firmness (lbs) of McIntosh fruits kept for 8 months in high or low ethylene CA in the CA chambers and 7 days post-storage holding ( ).

Cooling Delay (days)	CA Delay (days)	9/17 Harvest*** High Ethylene	9/17 Harvest*** Low Ethylene	9/24 Harvest High Ethylene	9/24 Harvest Low Ethylene
1	1	13.8(11.2)	12.5(10.8)	13.9(10.7)	14.0(11.4)
1	4	13.5(11.1)	13.0(11.4)	13.6(10.7)	13.8(11.6)
1	7	13.3(11.4)	12.9(11.7)	12.4(11.5)	12.7(11.2)
1	11	12.2(11.2)	12.2(11.2)	11.8(10.6)	12.2(11.9)
4	1	14.1(11.5)	13.8(11.9)	13.9(11.7)	14.9(11.6)
4	4	12.1(10.8)	13.3(11.3)	11.8(10.9)	11.1(11.3)
4	7	11.0(10.1)	11.1(10.4)	11.0(10.0)	11.1(10.2)
4	11	10.5(9.7)	10.6(9.7)	9.8(9.4)	10.1(9.5)
Air Control		9.1(8.0)		8.7(8.1)	

* CA conditions were 2% O_2 + 3% CO_2 at 3.3°C.

** 20 l. chambers with 60 fruits were ventilated at 30 or 300ml/min. for high and low ethylene levels, respectively.

*** Fruits were preclimacteric and at the beginning of the ethylene climacteric at Sept. 17 and 24th, respectively.

Flesh firmness at harvest was 15.1 and 15.0 lbs. on Sept. 17 and 24th, respectively.

TABLE 5. The influence of delaying cooling and imposing CA conditions on C_2H_4 production of McIntosh fruits kept for 4 months in high or low ethylene CA in the CA chambers.

Cooling Delay	CA Delay	9/17 Harvest*** High Ethylene	9/17 Harvest*** Low Ethylene	9/24 Harvest High Ethylene	9/24 Harvest Low Ethylene
1	1	.70	.64	.10	.24
1	4	.96	.70	.38	.29
1	7	1.31	1.06	.88	.51
1	11	1.97	2.04	1.73	1.34
4	1	.32	.47	.41	.31
4	4	1.81	1.18	.85	1.21
4	7	2.30	2.87	2.03	2.15
4	11	3.54	3.45	2.91	2.70

* CA conditions were 2% O_2 + CO_2 at 3.3°C.

** 20 l. chambers with 60 fruits were ventilated at 30 or 300 ml/min. for high and low ethylene levels, respectively.

*** Fruits were preclimacteric and at the beginning of the ethylene climacteric at Sept. 17 and 24th, respectively.

TABLE 6. Influence of delaying cooling and imposing hypobaric storage at 0.05 atmos. on flesh firmness (lbs) of McIntosh fruits after 8 months storage at 3.3°C. and 7 days poststorage.

Cooling Delay (days)	Storage Delay (days)	9/17 Harvest 0	9/17 Harvest +7	9/24 Harvest 0	9/24 Harvest +7
1	1	14.2	13.0	14.2	12.2
1	4	14.6	13.2	13.1	12.7
1	7	14.3	12.4	12.2	11.6
1	11	13.6	12.5	11.8	11.5
4	1	13.2	12.9	14.1	12.5
4	4	13.7	11.4	13.1	11.9
4	7	11.9	10.8	11.3	11.1
4	11	11.4	10.6	10.6	9.7
2 months air storage					
1	1	10.5	9.5	10.0	9.1
4	1	9.2	8.1	9.7	8.8

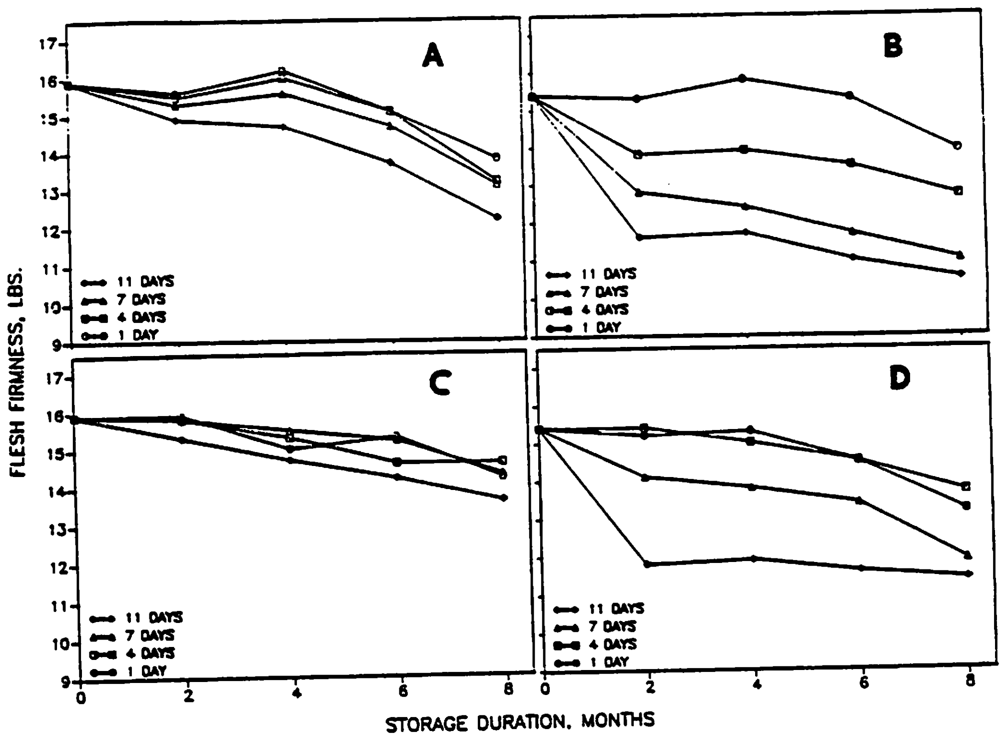

Figure 1. Changes in flesh firmness of McIntosh apples in relation to a delay of 1 to 11 days (A) and (B) before CA storage ($3\%CO_2:2\%O_2$) or (C) and (D) hypobaric storage (0.05 atmos.) at 3.3°C; (A) and (C) cooled immediately, (B) and (D) cooling delayed for 4 days.

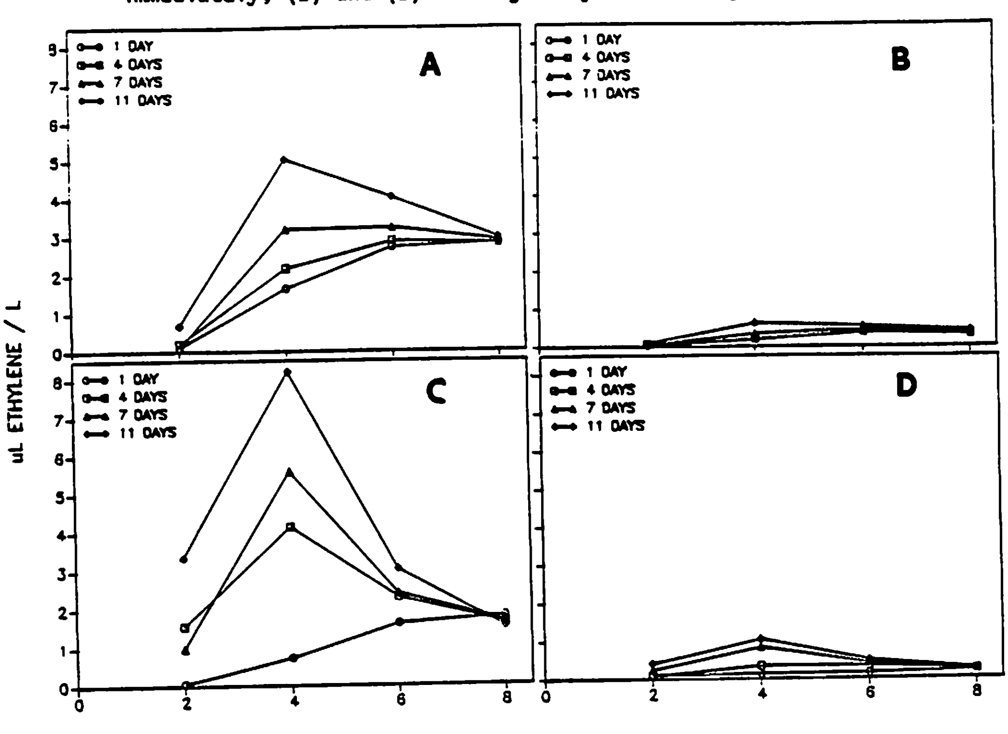

Figure 2. Changes in ethylene levels in chambers during CA storage of McIntosh apples. Apples cooled immediately and stored under high (A) or low (B) ethylene levels. Cooling delayed for 4 days and stored under high (C) or low (D) ethylene levels.

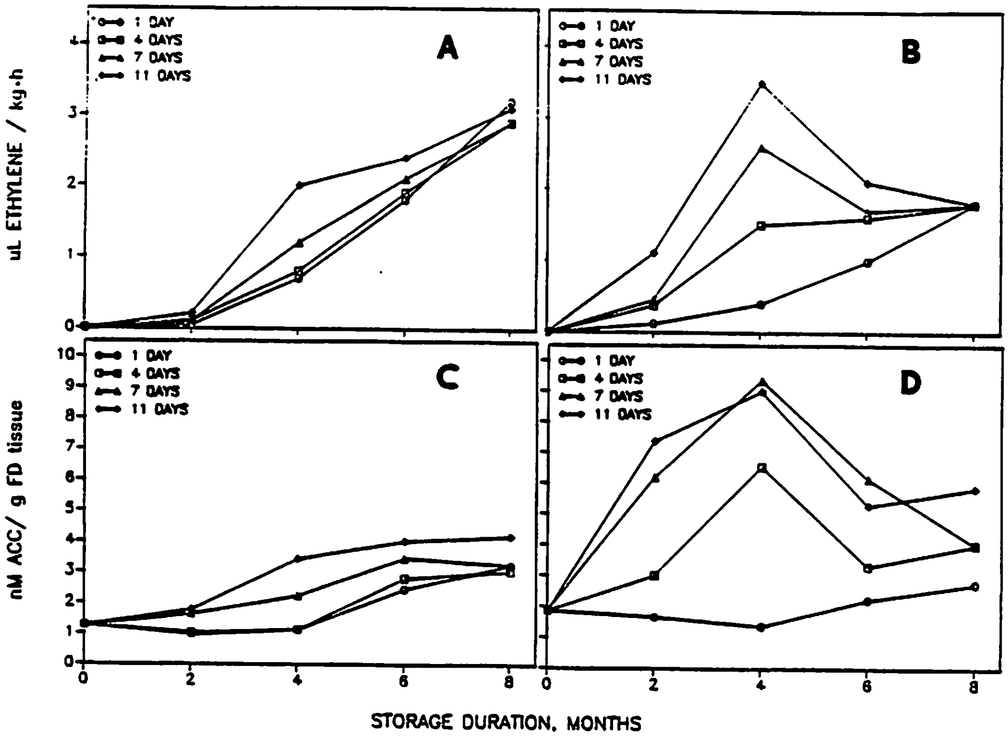

Figure 3. Changes in production of ethylene and 1-amino-cyclopropane-1-carboxylic acid (ACC) content in McIntosh apples stored in 3% CO_2:2% O_2. (A) and (C) apples cooled immediately and (B) and (D) cooling delayed 4 days before CA storage.