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Effects of aminoethoxyvinylglycine on maturation, ripening, and storage of apples.

Wesley R. Autio
University of Massachusetts Amherst

EFFECTS OF AMINOETHOXYVINYLGLYCINE ON MATURATION, RIPENING, AND STORAGE OF APPLES

A Thesis Presented

By

WESLEY R. AUTIO

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WESLEY R. AUTIO

Approved as to style and content by:

William J. Bramlage, Chairperson of Committee

Richard A. Damon, Member

Duane W. Greene, Member

Allen V. Barker, Department Head
Department of Plant and Soil Sciences

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C H A P T E R I

INTRODUCTION

Ethylene is a plant hormone affecting many different responses (1). One of particular importance is the initiation of climacteric fruit ripening. Numerous studies (e.g., 28, 37, 38, 46) have shown that exogenous applications of ethylene to unripe fruit cause them to ripen. Burg and Burg (21, 22) showed that a reduction of internal ethylene using hypobaric conditions could indefinitely delay ripening. Liu (30) has also demonstrated that when ethylene concentration was kept below 1 ppm in controlled atmosphere storage, ripening of McIntosh apples was markedly slower than when ethylene concentration was not controlled. These observations suggest the possibility of increasing fruit storage life by preventing ethylene from initiating ripening.

Owens and coworkers (60) inhibited ethylene biosynthesis by 75% in sorghum seedlings and senescent apple tissue using the chemical rhizobitoxine. This material blocks ethylene biosynthesis by preventing the conversion of methionine to ethylene (60).

Several structural analogs of rhizobitoxine also inhibit ethylene biosynthesis (44), but most recent interest has focused on the aminoethoxy analog or aminoethoxyvinylglycine (AVG). Several studies (e.g., 10, 18, 53, 56, 70, 72, 79) have shown that AVG reduces ethylene production, and these suggest that AVG may be a useful chemical to retard ripening and increase the storage life of many fruits. Several parameters and problems need to be assessed before it may be considered for commercial use.

Bramlage et al. (18) monitored internal ethylene production after harvest of AVG-treated McIntosh, Spartan, and Spencer apples kept at room temperature. They found that the McIntosh fruit harvested 3 day after spraying with 500 ppm AVG slowly increased in ethylene production over a period of 30 days. The same results were obtained when fruit were harvested 17 days after spraying. Spartan fruit did not increase significantly in ethylene production following the 500 ppm AVG treatment, whether harvested 3 or 17 days after spraying. Spencer trees were sprayed with 0, 25, 125, 250, or 500 ppm AVG and harvested 2 weeks later. Fruit treated with 25 ppm reached control levels of ethylene production after a 1-week lag period at room temperature, whereas the fruit from the 125, 250, and 500 ppm treatments did not significantly increase in ethylene production.

After 2.5 months of storage at 0 C they found that all AVG-treated fruit had internal ethylene levels greater than 1 ppm and had ripened. Also, there was more browncore in AVG-treated than control fruit.

The study by Bramlage et al. (18) prompted 3 basic questions that will be examined in this thesis. First, do responses to AVG vary systematically among cultivars, i.e. are increasingly lower concentrations needed for an effect as cultivars mature progressively later in the season? Second, does AVG affect fruit maturation as well as delay fruit ripening, and was the browncore associated with AVG-treated fruit in storage (18) due to delayed maturity of fruit at harvest? Third, how do low temperature and atmospheric ethylene concentrations during storage affect AVG-treated fruit, and in

particular, does either factor trigger ripening of AVG-treated fruit during storage?

C H A P T E R II

LITERATURE SEARCH

Ethylene as a Plant Hormone

Many comprehensive writings have appeared concerning the effects of ethylene on plant tissue (e.g., 1, 19, 20, 40, 41). The following brief historical description was discussed by Abeles (1).

One of the first observations of a gas affecting plant physiology was by Girardin (31) in 1864. He noticed defoliation of shade trees near leaking illuminating gas lines. Similar reports appeared as the use of illuminating gas increased (1). Neljubov was the first to show that ethylene was the component of illulminating gas causing the response. In 1901 (54) he noticed that pea seedlings exposed to illuminating gas grew horizontally. In 1913 (55) he showed that ethylene was the active component by systematically testing each gas present in illuminating gas. Harvey (36) in 1915 confirmed these results and showed that ethylene was also the ingredient of smoke that caused plant growth effects. He also observed that ethylene could advance senescence of carnation.

Seivers and True (64) in 1912 used kerosene fumes to degreen lemons, and Denny (28) in 1924 proved that this also was an ethylene response. Advancement of fruit ripening is one of the most important aspects of ethylene biology.

The first suggestion that ethylene was produced by plants was Cousin's report of 1910 (27) which showed that a gas produced by oranges

could ripen bananas. In 1934 Ganes (29) proved that plants did in fact produce ethylene.

Ethylene Effects on Plant Growth and Development

Ethylene plays a large role in plant physiology, and many review articles have discussed this role thoroughly (e.g., 1, 19, 20, 40, 41). Areas where ethylene is important include (1): seedling germination; sprouting of tubers, corms, and bulbs; spore and pollen germination; prevention of cell elongation; decrease in cell division; swelling; adventitious root development; flowering of bromeliads and several other families; sex reversal in cucurbits; abscision; fruit ripening; and senescence. This discussion will be limited to ethylene's effect on fruit ripening.

Ethylene Effects on Fruit Ripening

Kidd and West (38) observed that apples and pears exhibit a rapid rise and peak in respiration rate during ripening. They termed these the climacteric rise and climacteric peak in respiration. Further study showed that respiration slows during fruit development to a preclimacteric minimum just prior to the climacteric rise. After the climacteric peak respiration rate slows in a postclimacteric fall. Color, texture, and chemical changes that lead to optimum edibility are linked to the climacteric (15). Many fruits follow this same pattern during ripening: e.g., avocado, banana, peach, blueberry, and tomato (51).

Ethylene's effect on ripening was established early (Seivers and True (64) and Denny (28)), and it was used commercially (1). The beginning of the climacteric rise is preceded by an increase in ethylene concentration (52). Burg and Burg (21, 22) showed that ethylene is essential for ripening and the climacteric to occur. Using hypobaric conditions they prevented ripening by preventing ethylene from accumulating in fruit.

Hackett et al. (35) proposed a 2 stage process whereby ethylene triggers ripening. First, autocatalytic production of ethylene is initiated. Second, ethylene affects the physiological reactions of ripening. A certain amount of ethylene is necessary to initiate the autocatalytic production of ethylene. This amount changes throughout the season, because the sensitivity of the tissue to ethylene increases as the season progresses. Until the tissue is responsive, physiological concentrations of ethylene will not initiate autocatalytic ethylene production and ripening. For apples near the climacteric 0.1 to 1.0 ppm internal ethylene is required to trigger ripening (35). Once triggered, ethylene production initially parallels the respiratory climacteric, but then it levels off or drops before death of the fruit.

Since ethylene is essential for ripening it would be possible to increase storage life by preventing ethylene accumulation (21, 22). This thesis deals with a chemical means of blocking ethylene production, but a discussion of ethylene biosynthesis is necessary first.

Ethylene Biosynthesis

Many studies (e.g., 12, 13, 24, 33, 42, 43, 62, 73, 75) have shown methionine to be the major precursor of ethylene in higher plant tissue. More specifically, carbons 3 and 4 of methionine become ethylene (24, 33, 42). Carbon 1 is released as CO_2 (2). Carbon 2 becomes formic acid (2). The nitrogen atom forms ammonia (2). The sulfur atom is conserved along with carbon 5 to resynthesize methionine (2, 24).

The first steps of the ethylene biosynthesis pathway are relatively well established. Using ^{35}S labeled methionine, Adams and Yang (2) showed that methionine is first converted to S-adenosylmethionine (SAM) and then split to give 5'-S-methyl-5'-thioadenosine (MTA) and an unknown compound which ultimately releases ethylene. MTA is quickly hydrolyzed to 5-S-methyl-5-thioribose (MTR) which is possibly used to resynthesize methionine (2).

Pyridoxyl phosphate was first thought to be important in ethylene biosynthesis, because inhibitors of pyridoxyl phosphate-mediated reactions inhibit ethylene biosynthesis (42, 59, 60). The splitting of SAM is the step blocked by these inhibitors (2).

Adams and Yang (3) used ^{14}C labeled methionine and showed that 1-aminocyclopropane-1-carboxylic acid (ACC) was the unknown compound formed when SAM split. They found that labeled methionine formed ACC in N_2 atmosphere, and labeled ACC readily formed ethylene in air. The formation of ethylene from ACC was not blocked by inhibitors of pyridoxyl phosphate mediated reactions. From these results and earlier observations, Adams and Yang proposed the pathway for ethylene

biosynthesis that appears in Figure 1.

The conversion of SAM to ACC has been well studied. *In vitro* studies (25) have shown it to be a rate limiting step of ethylene biosynthesis. Yu *et al*. (76) extracted from tomato an enzyme (ACC synthase) which catalysed this reaction. They reported that the enzyme has a Km = 20 μM with respect to SAM and is activated by 0.1 μM or higher pyridoxyl phosphate. It is competitively inhibited by aminoethoxyvinylglycine, Ki = 0.2 μM (17), and aminooxyacetic acid, Ki = 0.8 μM (76), which inhibit pyridoxyl phosphate-mediated reactions. This step in ethylene biosynthesis also involves auxin. Yu *et al*. (77) proposed that auxin induces enzyme synthesis.

The steps beyond ACC are essentially unknown, but they do require O_2 (13). The breakdown of ACC to ethylene also involves free radicals. Baker *et al*. (9) showed that pink and red tomatoes and avocados were not greatly affected by AVG, whereas climacteric apples and green tomatoes were. They also showed that free radical scavengers inhibit ethylene production in all tissues. Boller *et al*. (17) proposed that pink and red tomatoes were not affected by AVG because of a reserve of ACC synthesized prior to ripening. This would account for the results of Baker *et al*. (9) and suggests that free radicals are involved in the conversion of ACC to ethylene.

Many compounds affect ethylene in plants by either inhibiting its biosynthesis or by blocking its action. Several inhibitors will now be discussed.

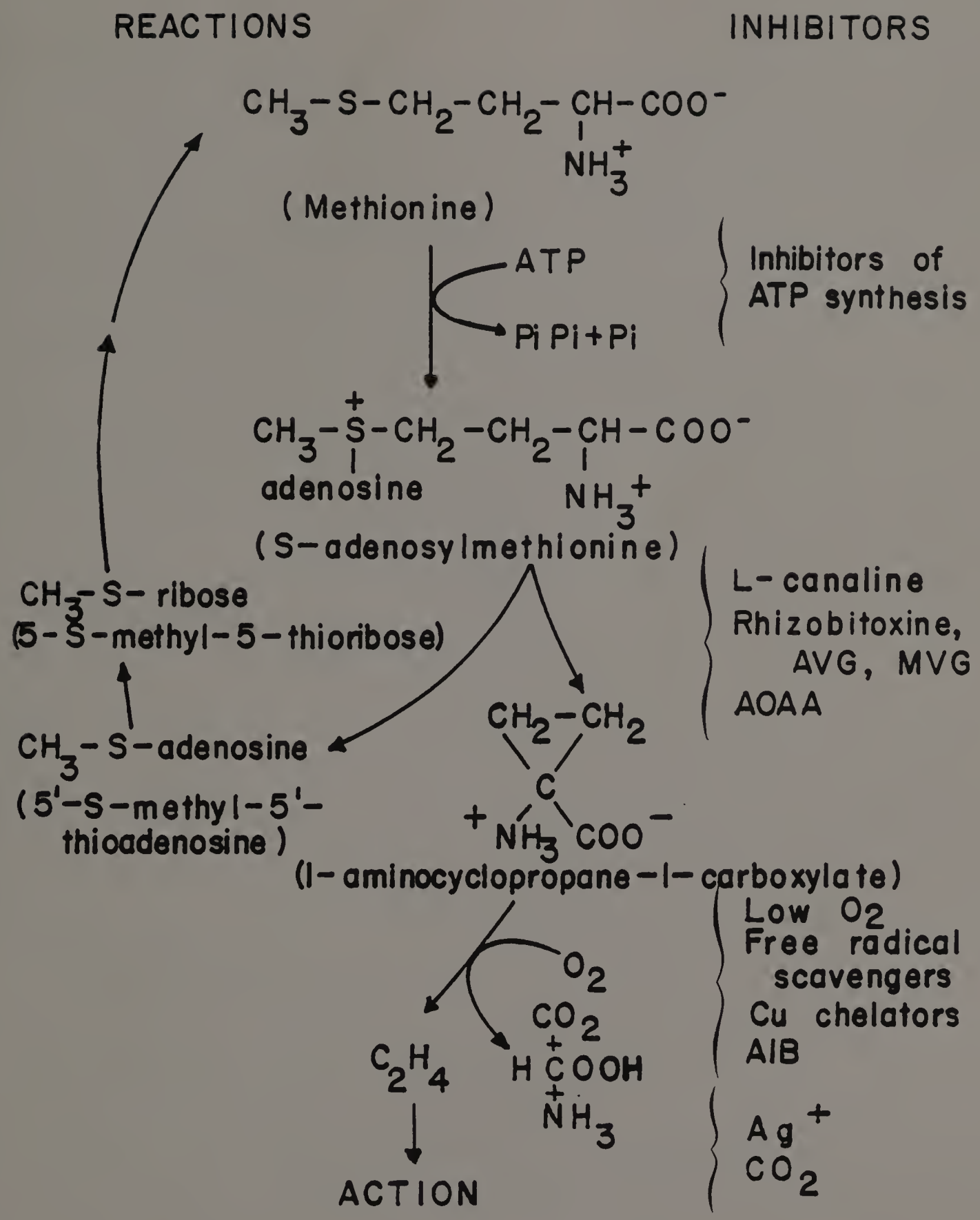


Figure 1. Condensed scheme of ethylene biosynthesis (38) with inhibitors and their points of action.

Inhibitors of Ethylene Biosynthesis and Action

Inhibition of the conversion of methionine to SAM.

The reaction involving the conversion of methionine to SAM requires the presence of ATP (74), so any compound which limits ATP synthesis can potentially inhibit ethylene biosynthesis. Appelbaum and coworkers (7) showed that several inhibitors of ATP synthesis did in fact inhibit ethylene synthesis.

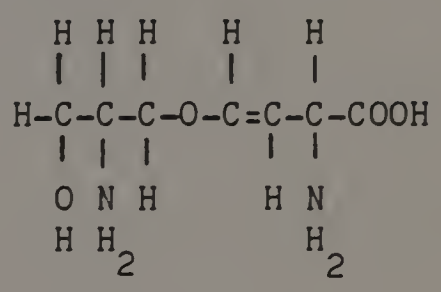
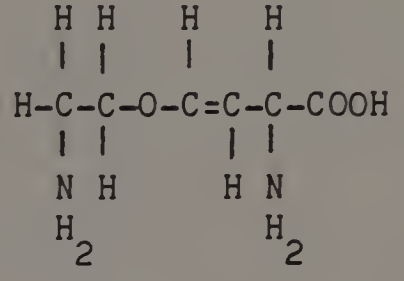
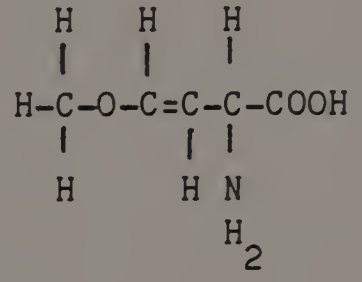
Inhibition of the conversion of SAM to ACC.

The conversion of SAM to ACC is mediated by the enzyme ACC synthase and requires the presence of pyridoxyl phosphate (40). Inhibitors of pyridoxyl phosphate-mediated reactions effectively inhibit ethylene biosynthesis by blocking this step (3).

One of the earliest studies using an inhibitor of pyridoxyl phosphate-mediated reactions was by Owens et al. (60). They showed that rhizobitoxine inhibited ethylene production in sorghum seedlings and senescent apple tissue by 75%. A series of structural analogs of rhizobitoxine (Table 1) also have similar properties (44). Aminoethoxyvinylglycine (AVG) is possibly the most important analog and has been included in many studies.

AVG has a profound effect on ethylene biosynthesis in many plant tissues. AVG can reduce the production of wound ethylene (34, 53), cause sex change of cucurbit flowers (8, 57, 58), inhibit bud growth after release from dormancy (79), and delay floral senescence (10). AVG can affect virtually any tissue in which ethylene is active. This study is concerned with AVG's effects on fruit ripening.

Table 1. Rhizobitoxine and its structural analogs.

Common name	Reference	Chemical name	Structure
Rhizobitoxine	40	L-2-amino-4-(2'-amino-3'-hydroxypropoxy)-trans-3-butenoic acid	
Aminoethoxy-vinylglycine	48	L-2-amino-4-(2-aminoethoxy)-trans-3-butenoic acid	
Methoxy-vinylglycine	48	L-2-amino-4-methoxy-trans-butenoic acid	

Wang and Mellenthin (70) treated pears with 1000 ppm AVG and found that it delayed the decrease in firmness, the increase in protein N, and the increase in soluble pectins. They also noted that a fast ripening cultivar was affected less than a slow ripening cultivar.

Baker et al. (9) treated green tomatoes with 68 μM AVG which inhibited ethylene production by 50 to 69 %. The same AVG concentration reduced ethylene production of pink and red tomatoes and of avocados by only 11 to 13 %. Boller et al. (17) later proposed that this result was due to a build up of ACC prior to treatment, so that AVG was less effective.

Bangerth (11) showed that tree sprays of 500 μM AVG on apples delayed ripening, reduced preharvest drop, increased fruit removal force, and reduced and delayed climacteric CO_2 production.

Ness and Romani (56) used vacuum infiltration techniques to treat pears with 1 to 5 μM AVG. It delayed ripening, reduced ethylene production, and reduced and delayed the respiratory climacteric. They also applied ethylene to AVG treated fruit and found that they ripened normally.

AVG would be expected to also have effects on the tree in the case of AVG tree-sprays. Williams (72) sprayed Delicious trees 2 weeks before harvest. The following season he found that AVG-treated trees had larger leaves, greater fruit set, more bud breaks, more spur and terminal shoot growth, and fruit with larger L/D ratios. Tree-sprays 2 weeks after bloom prevented June-drop, increased set, increased bud break, and increased branching. Green (32) found that AVG tree-sprays

on Delicious at bloom increased fruit set, reduced ethylene production of flowers, and increased L/D ratios.

Boller et al. (17) showed that AVG has a a Ki = 0.2 μM, and inhibits ACC synthase completely and reversibly.

Compounds with an aminooxy group (Table 2) are also effective inhibitors of pyridoxyl phosphate-mediated reactions and ethylene biosynthesis (4). Aminooxyacetic acid (AOAA) is one of the most effective and is comparable in activity to AVG (4). Carnation vase life is extended equally by 0.5 μM AOAA and 0.1 μM AVG (69).

Inhibition of the conversion of ACC to ethylene.

The exact steps involved in the conversion of ACC to ethylene are not known, but several different compounds inhibit this reaction. Oxygen is required for ethylene biosynthesis (13, 20) and is involved in ACC breakdown to ethylene (74), so a simple method of blocking this step is to reduce oxygen levels as in CA storage.

Lau and Yang (39) found that Co^{+2} inhibited ethylene production in mung bean hypocotyls and apple tissue, and Yu and Yang (78) showed that Co^{+2} inhibited the conversion of ACC to ethylene.

Baker and coworkers (9) suspected that free radicals were involved in the conversion of ACC to ethylene, so they treated tomato, avocado, and apple tissue with 1 μM benzoate and propyl gallate (free radical scavengers). Both compounds decreased ethylene production between 50 and 88 %.

Satoh and Esashi (63) used an analog of ACC, α-aminoisobutyric acid (AIB), and found that it competitively inhibited the conversion of ACC

Table 2. Aminooxy compounds and effective concentrations compared with AVG (68). "I 50" is the concentration (M) required for 50 % inhibition of ethylene production.

Compound	I 50 (M)		
	Mung bean hypocotyl	Apple plugs	Buckwheat hypocotyl
α-aminooxyacetic acid	6×10^{-6}	9×10^{-5}	4×10^{-6}
N-benzyloxycarbonyl-l-α-aminooxy-propionic acid	3×10^{-6}	-	-
Aminoethoxyvinylglycine	2×10^{-6}	-	1×10^{-6}

to ethylene in cocklebur cotyledons and etiolated pea stems. Appelbaum et al. (7) treated apple plugs, pea stems, and bean leaf discs with a range of structural analogs of ACC and found generally no effect. One analog, cyclopropane carboxylic acid (CCA), did reduce ethylene production but did not inhibit the conversion of ACC to ethylene competitively. In fact, CCA caused a reduction in ACC content. Short chain organic acids, such as acetic acid, propionic acid, and butyric acid appear to have a similar effect (7). The mechanism of this inhibition is as yet unknown, but steps prior to ACC appear to be affected.

Other inhibitors of the conversion of ACC to ethylene include Cu chelating agents (6). Apparently a Cu requiring enzyme is involved in this conversion.

Inhibition of ethylene action.

One of the earliest known inhibitors of ethylene action in plants was CO_2 (20). Mack in 1927 (47) was the first to show that CO_2 could block ethylene action. He found that CO_2 reduced ethylene's ability to blanch celery, and when CO_2 was absorbed by KOH ethylene action was increased. Burg and Burg (21, 23) observed that CO_2 competitively inhibited ethylene action.

Another inhibitor which has gained considerable attention in recent years is Ag^{+1}. Several studies have shown it to block ethylene action (e.g., 8, 14, 30, 32, 57, 61) applied either as silver nitrate or silver thiosulfate. It is proposed to substitute for Cu^{+2} at the site of ethylene action rendering the site inactive (14, 30).

Of the several inhibitors of ethylene action or biosynthesis, this study is involved with AVG, a competitive inhibitor of ACC synthase (17).

C H A P T E R III

MATERIALS AND METHODS

All experiments utilized mature apple trees grown on Malling (M) 7 rootstocks at the Horticultural Research Center, Belchertown, Massachusetts. Technical grade aminoethoxyvinylglycine (AVG) was supplied by Maag Agrochemicals, Vero Beach, Florida 32960. For all experiments AVG was dissolved in distilled water with 0.1% Triton B-1956 added as a spreader. Treatments were sprayed with a hand pump sprayer on leaves and fruit until runoff.

Experiment One

To determine the effects of a range of AVG concentrations on different cultivars, an experiment was designed utilizing 4 cultivars and 5 AVG concentrations. Five trees each of Early McIntosh, McIntosh, Cortland, and Delicious were chosen, and 5 limbs on each tree were selected for treatment. Each limb had about 25 fruit and was sprayed 1 week before harvest with 0, 125, 250, 500, or 1000 ppm AVG. The spray and harvest dates appear in Table 3.

To observe the effects of AVG on fruit ripening, internal ethylene was followed as an index. Four fruit were harvested from each limb and held at room temperature. Internal ethylene was monitored using the following method (18). A 21-gauge needle (with tip bent at 120) was attached to a 3 ml disposable, plastic syringe and inserted through the calyx of a fruit into the core area. About 1.5 ml of internal gas was removed, and the 21-gauge needle was replaced with a 26-gauge needle.

Table 3. Spray, harvest, and storage termination dates for each cultivar in Experiments One and Three.

Experiment	Cultivar	Spray date	Harvest date	Storage termination date
1	Early McIntosh	8- 8-80	8-15-80	-
	McIntosh	9- 8-80	9-15-80	-
	Cortland	9-16-80	9-23-80	-
	Delicious	9-26-80	10- 3-80	-
2	McIntosh	9- 8-80	9-16-80	2-24-81
	Delicious	9-27-80	10- 6-80	3-16-81

Samples were then reduced to 1 ml and injected into a Varian Series 2700 gas chromatograph equipped with a flame ionization detector and an activated alumina column to measure ethylene.

An analysis of variance was performed using the number of days for each fruit to reach 1 ppm internal ethylene, which was considered to be the number of days to initiate ripening (35), as the dependent variable. One ppm is a conservative estimate of the concentration of ethylene needed to trigger ripening. Cultivar means were separated with regression analysis using the number of days between bloom and harvest (the length of the growing season for each cultivar) as the independent variable. Treatment means were also separated using regression analysis with AVG concentration being the independent variable. Uneven numbers led to the use of the Least-squares and Maximum Likelihood General Purpose Program, developed by W. R. Harvey, to complete the analysis.

Experiment Two

To observe the effects of AVG on fruit maturation, an experiment was designed utilizing 7 Puritan trees and 4 limbs on each tree. Limbs were chosen by fruit load, each bearing about 75 fruit. Three limbs were sprayed with 500 ppm AVG on June 26, July 11, or July 28. The fourth limb was left untreated as a control. Fruit maturity was assessed by measuring fruit firmness, soluble solids, peel chlorophyll, flesh starch, and titratable acidity on 10 fruit samples harvested August 1, 4, 7, and 10 from each limb. To assess ripening 4 fruit were harvested from each limb on August 10 and held at room temperature. Internal ethylene was monitored as in Experiment One.

The firmness of each fruit was measured using a Magness-Taylor pressure tester (16). Diameter was also measured because of the inability to use fruit of similar size. Next, the fruit were peeled using a White Mountain Peeler, and 2 strips of peel from the middle of each fruit were frozen at -20 C for later chlorophyll analysis. Also, 2 strips of cortex from the middle of the fruit just below the peel were frozen for starch analysis. Opposite wedges were then removed from each fruit, and the wedges from the 10 fruit were combined and crushed to obtain a bulk juice sample. Five ml were titrated to pH 7 with 0.1 N NaOH to measure titratable acidity (5). The percent soluble solids was obtained for a juice sample using a hand refractometer.

Frozen peel samples were chopped, and 5 g were added to 150 ml of 80 % acetone saturated with Na_2CO_3 and macerated for 3 minutes in a blender. Ten ml were centrifuged, and absorbance was measured at 665 nm and 649 nm. Chlorophyll concentration in the supernate was calculated using the following equation (67):

Total Chlorophyll (μg/ml) = 6.45 (A665) + 17.72 (A649).

Chlorophyll content of the tissue was calculated with the following:

Chlorophyll (μg/gF.W.) = Total Chlorophyll (μg/ml) x 150 ml ÷ 5 g.

Frozen cortex samples were chopped and dried in a forced draft oven at 65 C for 10 days (71). Dried samples were ground in a Wiley Mill and passed through a 20 mesh screen. A perchloric acid digestion and anthrone test were used to measure starch content as described by McCready *et al*. (50).

Analysis of variance was performed on the ripening data using the number of days for each fruit to reach 1 ppm internal ethylene (the number of days to initiate ripening) as the dependent variable. These data along with the starch content, chlorophyll content, percent soluble solids, and titratable acidity were analyzed using the Biomedical Series analysis of variance program, BMD08V. Firmness data were analyzed with the Biomedical Series program, BMDP2V, using fruit diameter as a covariate. Control means were separated from the treatment means using a linear comparison, and the treatment means were separated using Duncan's New Multiple Range Test (p=0.05).

Experiment Three

To determine the effects of temperature and ethylene in the storage atmosphere on ripening of AVG-treated fruit, an experiment was designed including 4 storage conditions: 1) 0 C, low atmospheric ethylene (0,LE); 2) 3.3 C, low atmospheric ethylene (3.3,LE); 3) 3.3 C, high atmospheric ethylene (3.3,HE); and 4) 20 C, low atmospheric ethylene (20,LE). To assess the possibility of a chilling response, a low temperature sensitive cultivar (McIntosh) and a low temperature tolerant cultivar (Delicious) were chosen for study. Six trees of each cultivar were selected and 3 or 4 limbs on each tree (about 200 fruit per tree) were sprayed with 500 ppm AVG about 1 week before harvest. At harvest the fruit were divided among 4 boxes resulting in about 50 fruit per box. Spray and harvest dates appear in Table 3. Firmness of fruit from each tree was measured on a sample of 10 fruit with diameters of 6.9 to 7.1 cm (16). One box from each tree was placed in each storage condition.

In storage, low atmospheric ethylene was maintained below 0.014 ppm by adding to each box 150 g Purafil, an ethylene absorbing material obtained from Purafil, Inc., P.O.Box 80434, Chamblee, GA 30366. The Purafil was held in open paper bags on top of each box of fruit. High atmospheric ethylene was maintained by including 20 boxes of untreated apples in the storage room and by adding enough 100 % ethylene to the atmosphere to raise the concentration to about 10 ppm twice during the storage period.

McIntosh fruit were removed from storage on February 24, 1981, and Delicious fruit were removed on March 16, 1981. Both cultivars had been stored for 23 weeks at these removal dates. Internal ethylene was monitored on 4 fruit from each box as outlined in Experiment One. Ground color was scored on 10 McIntosh fruit from each box. For this, a Cornell color chart (66) was used, in which a score between 1 and 5 was given to each fruit, with 1 being light yellow-green and 5 being dark green. After 1 day at room temperature firmness was measured on 10 fruit from each box (16). After 1 and 3 weeks at room temperature the percentage decay and senescent breakdown were determined for all boxes, and after 3 weeks the percentage browncore was determined for all boxes of McIntosh fruit.

All statistical analyses of variance were completed using the Biomedical Series program, BMD08V. Means were separated with non-orthogonal comparisons. All percentages were transformed to arc sine before analysis.

C H A P T E R IV

RESULTS

Experiment One

Experiment One was designed to show the effects of a range of AVG concentrations on several cultivars ranging from early season to late season. Figures 2 through 5 graph the internal ethylene concentrations after harvest of fruit from each concentration within each cultivar. Table 4 summarizes these data by showing the time required to reach the ethylene peaks the and heights of these ethylene peaks. These data suggest that late cultivars, in general, have later and lower ethylene peaks and are more affected by AVG than are early cultivars.

Figures 6 through 9 graph the numbers of ripening fruit on each day after harvest. Table 5 summarizes these data by giving the number of days for 50 % of the fruit from each treatment to initiate ripening (reach 1 ppm internal ethylene). Again, these data show that late cultivars appeared to be more affected by AVG than early cultivars.

The number of days required for each of the fruit to initiate ripening was used as the dependent variable to statistically analyze the effects of AVG treatments (Appendix Table 8). Cultivar and AVG concentration means were separated with regression analysis. Figure 10 graphs the days to ripen against the length of season for each AVG concentration, and Figure 11 graphs the days to ripen against AVG concentration for each cultivar. A highly significant linear relationship existed between the length of season for a cultivar and the

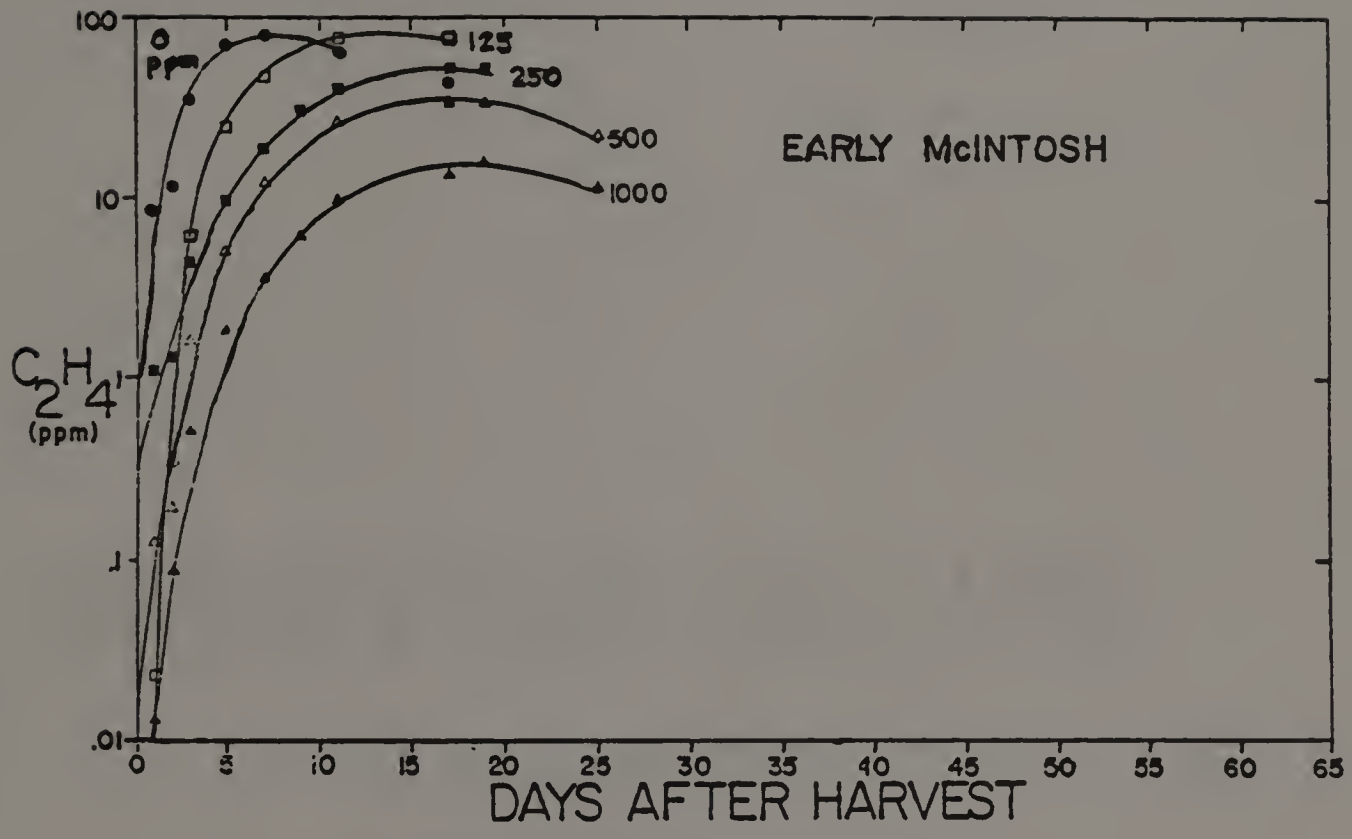


Figure 2. Internal ethylene concentrations of Early McIntosh apples kept at room temperature after harvest. Tree-limbs were sprayed with 0, 125, 250, 500, and 1000 ppm AVG 1 week prior to harvest.

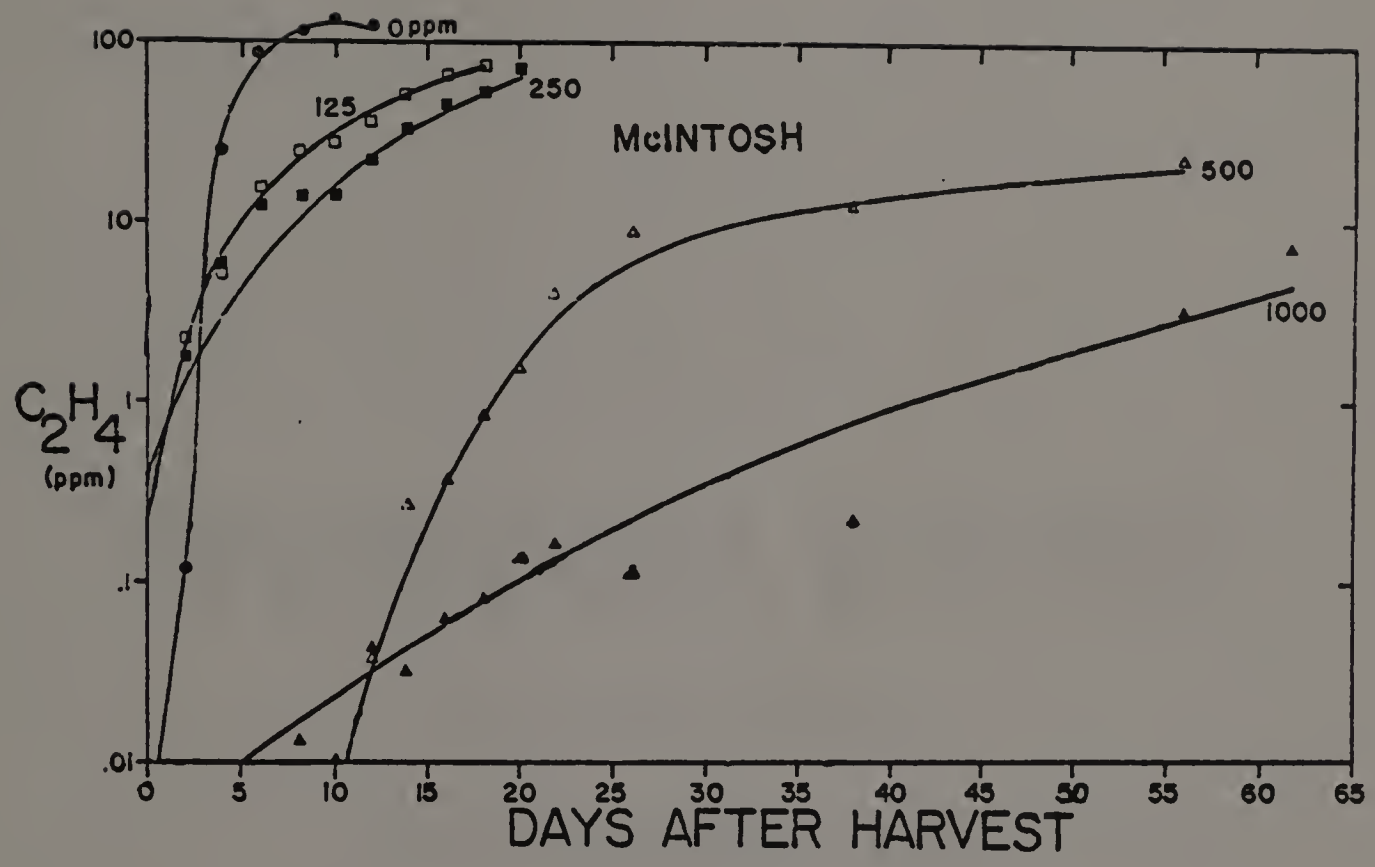


Figure 3. Internal ethylene concentrations of McIntosh apples kept at room temperature after harvest. Tree-limbs were sprayed with 0, 125, 250, 500, and 1000 ppm AVG 1 week prior to harvest.

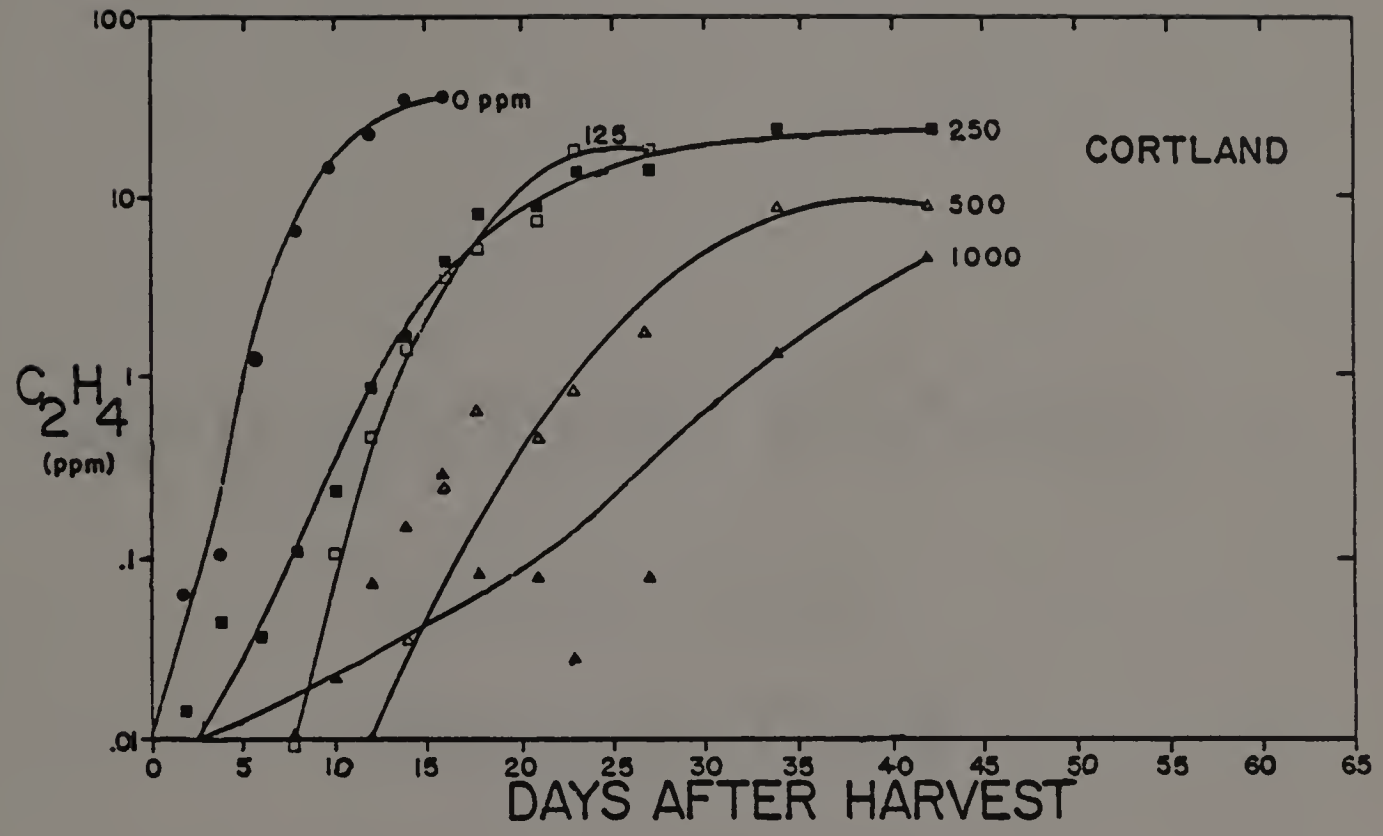


Figure 4. Internal ethylene concentrations of Cortland apples kept at room temperature after harvest. Tree-limbs were sprayed with 0, 125, 250, 500, and 1000 ppm AVG 1 week prior to harvest.

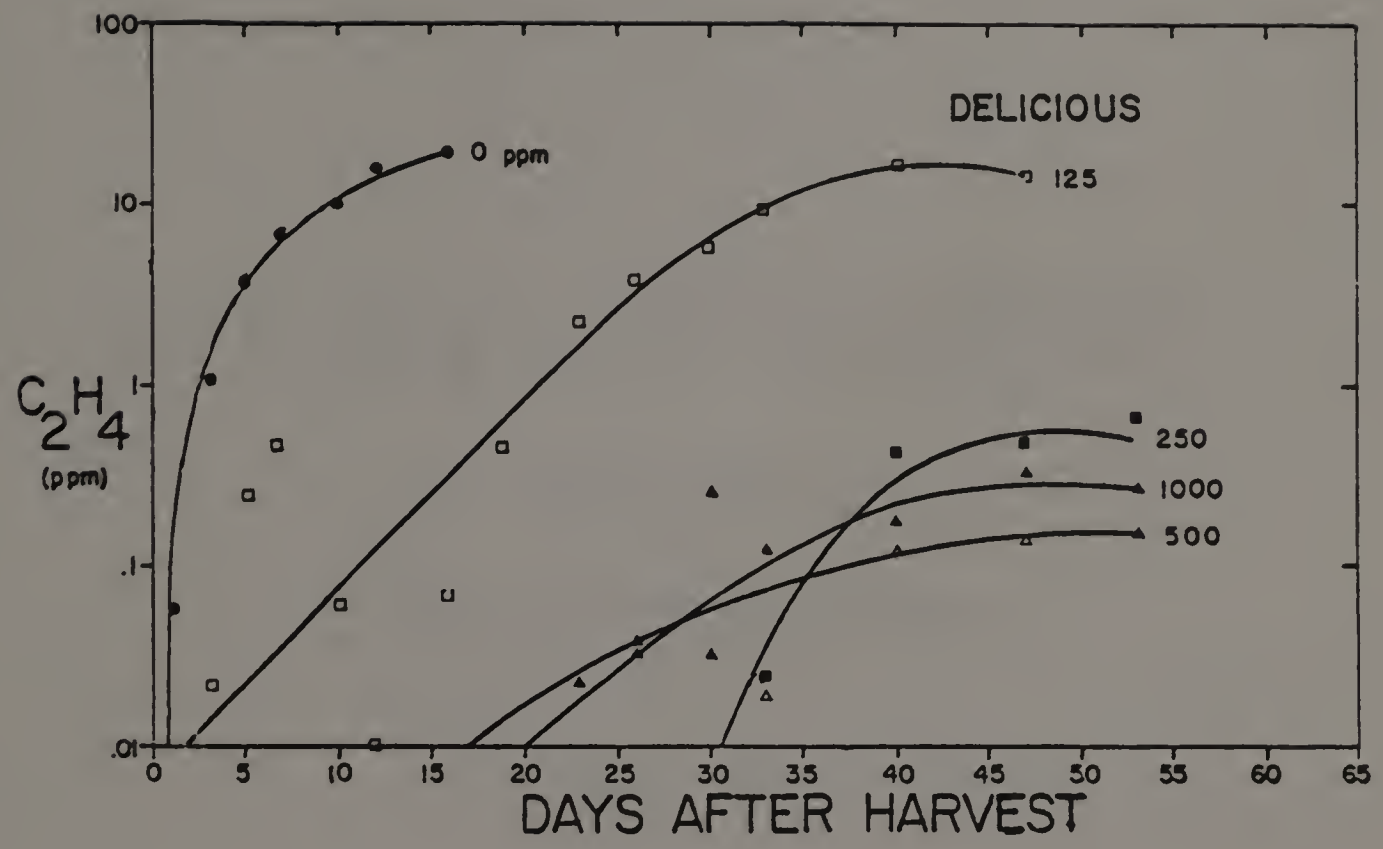


Figure 5. Internal ethylene concentrations of Delicious apples kept at room temperature after harvest. Tree-limbs were sprayed with 0, 125, 250, 500, and 1000 ppm AVG 1 week prior to harvest.

Table 4. Time of ethylene peaks and peak heights for the 4 cultivars at 5 concentrations of AVG.

Cultivar	AVG Concentration (ppm)	Time of peak (days after harvest)	Peak height (ppm)
Early McIntosh	0	8	90
	125	13	75
	250	17	55
	500	17	35
	1000	18	15
McIntosh	0	10	130
	125	20	90
	250	25	90
	500	50	23[z]
	1000	>62	-[z]
Cortland	0	18	42
	125	25	20
	250	41	25
	500	41	12[z]
	1000	>41	-[z]
Delicious	0	16	20
	125	43	15[z]
	250	>53	-[z]
	500	>53	-[z]
	1000	>53	-[z]

[z]No values are shown because these treatments did not reach an ethylene peak.

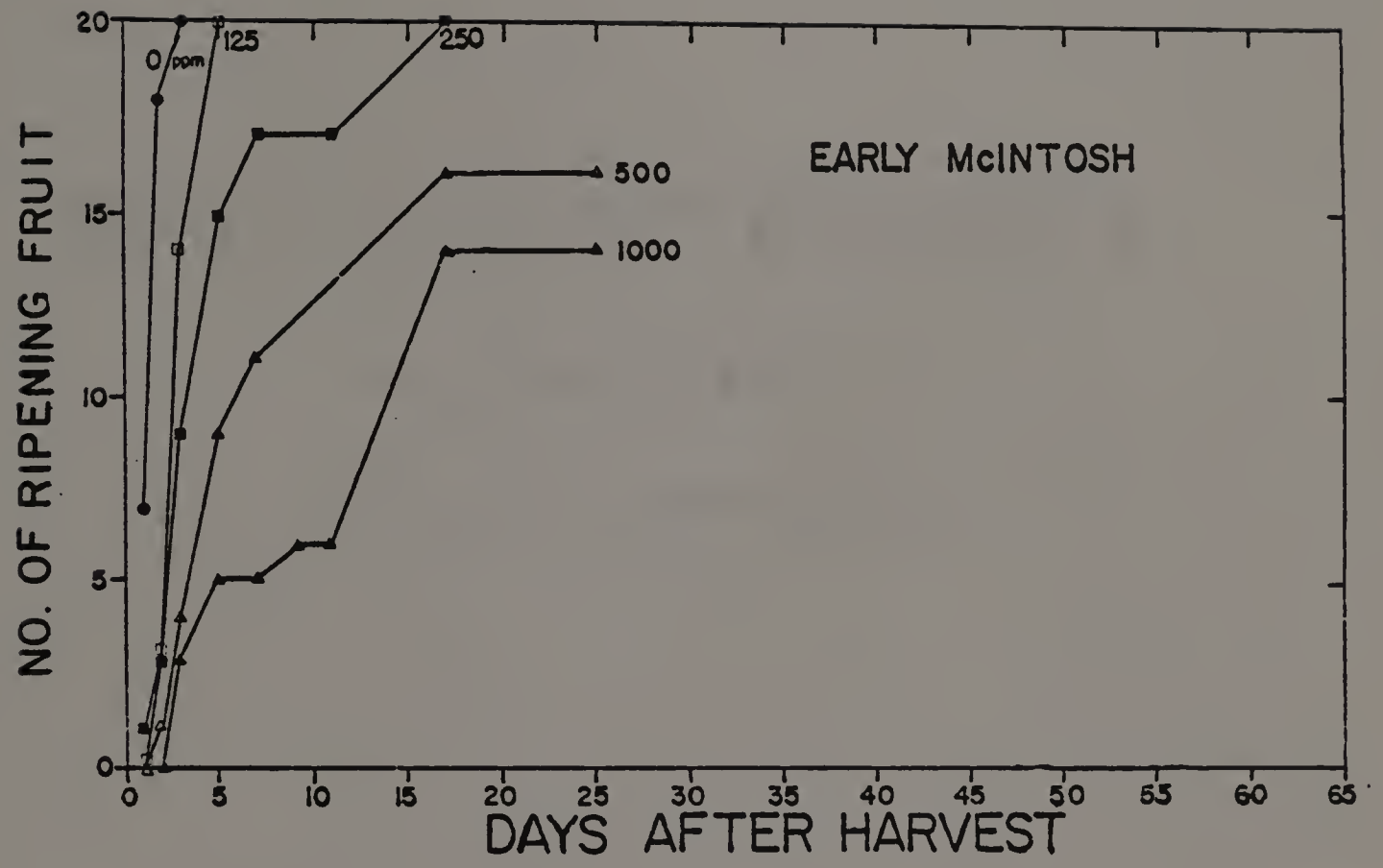


Figure 6. Cumulative ripening of Early McIntosh apples at room temperature after harvest, following applications of AVG 1 week prior to harvest. Fruit were judged to be ripening when internal ethylene concentrations reached 1 ppm.

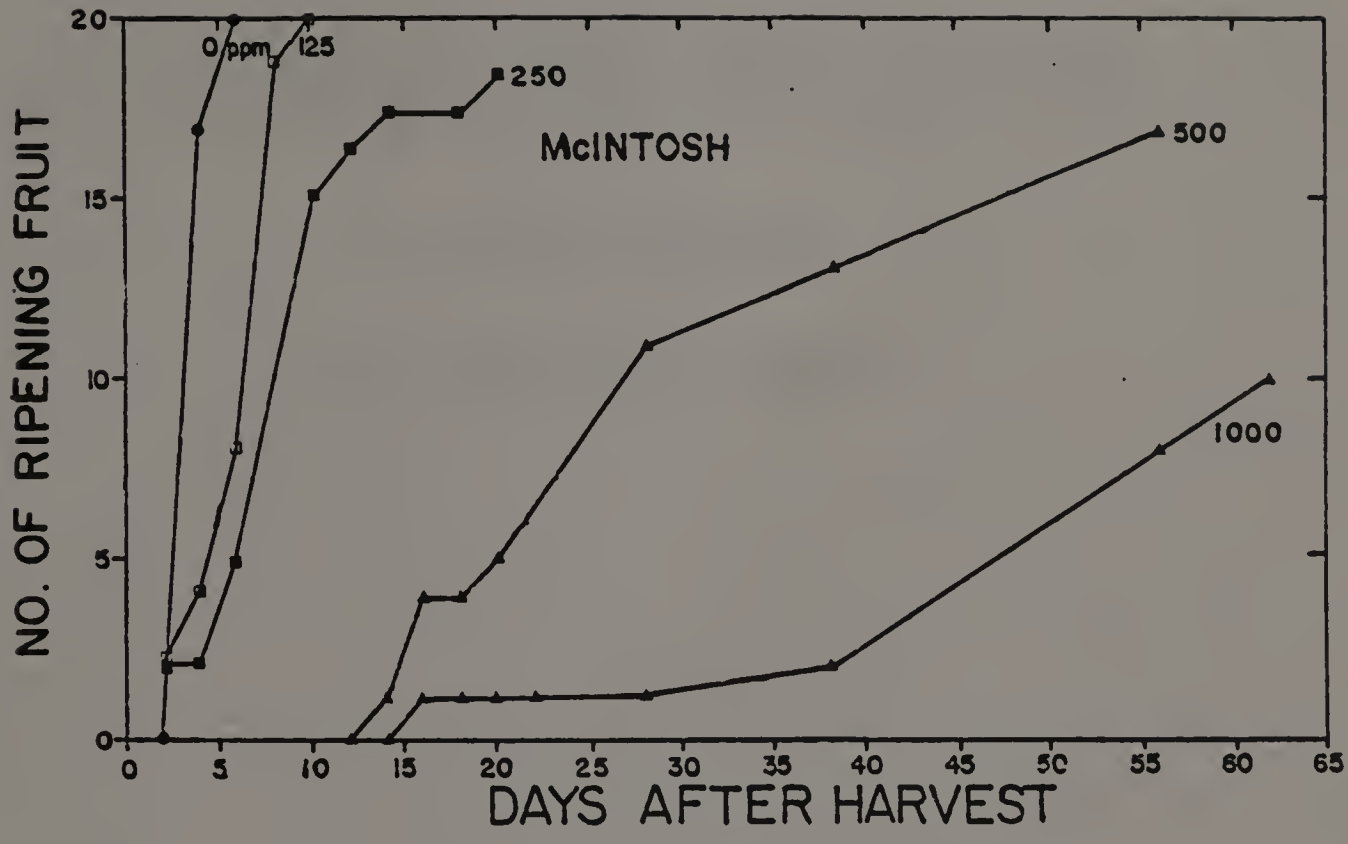


Figure 7. Cumulative ripening of McIntosh apples at room temperature after harvest, following applications of AVG 1 week prior to harvest. Fruit were judged to be ripening when internal ethylene concentrations reached 1 ppm.

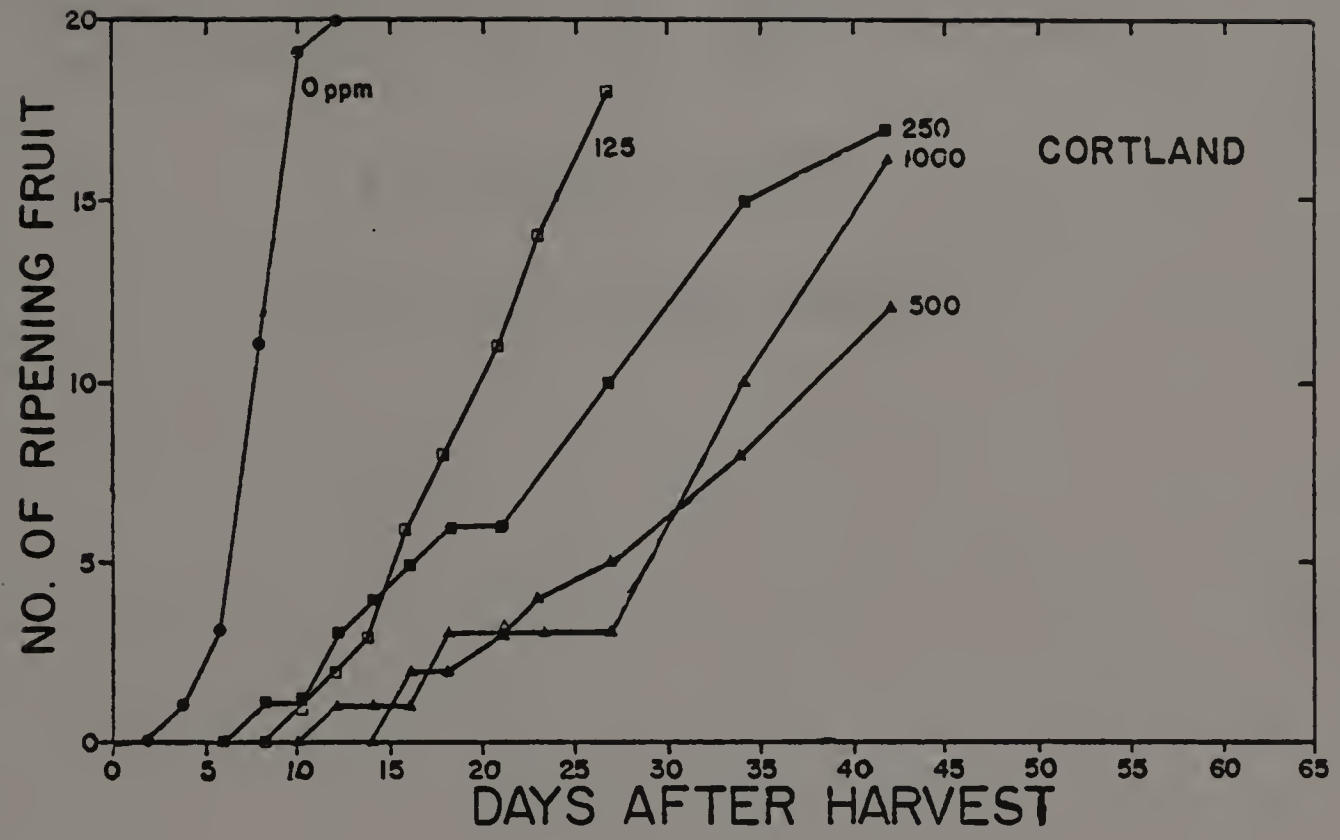


Figure 8. Cumulative ripening of Cortland apples at room temperature after harvest, following applications of AVG 1 week prior to harvest. Fruit were judged to be ripening when internal ethylene concentrations reached 1 ppm.

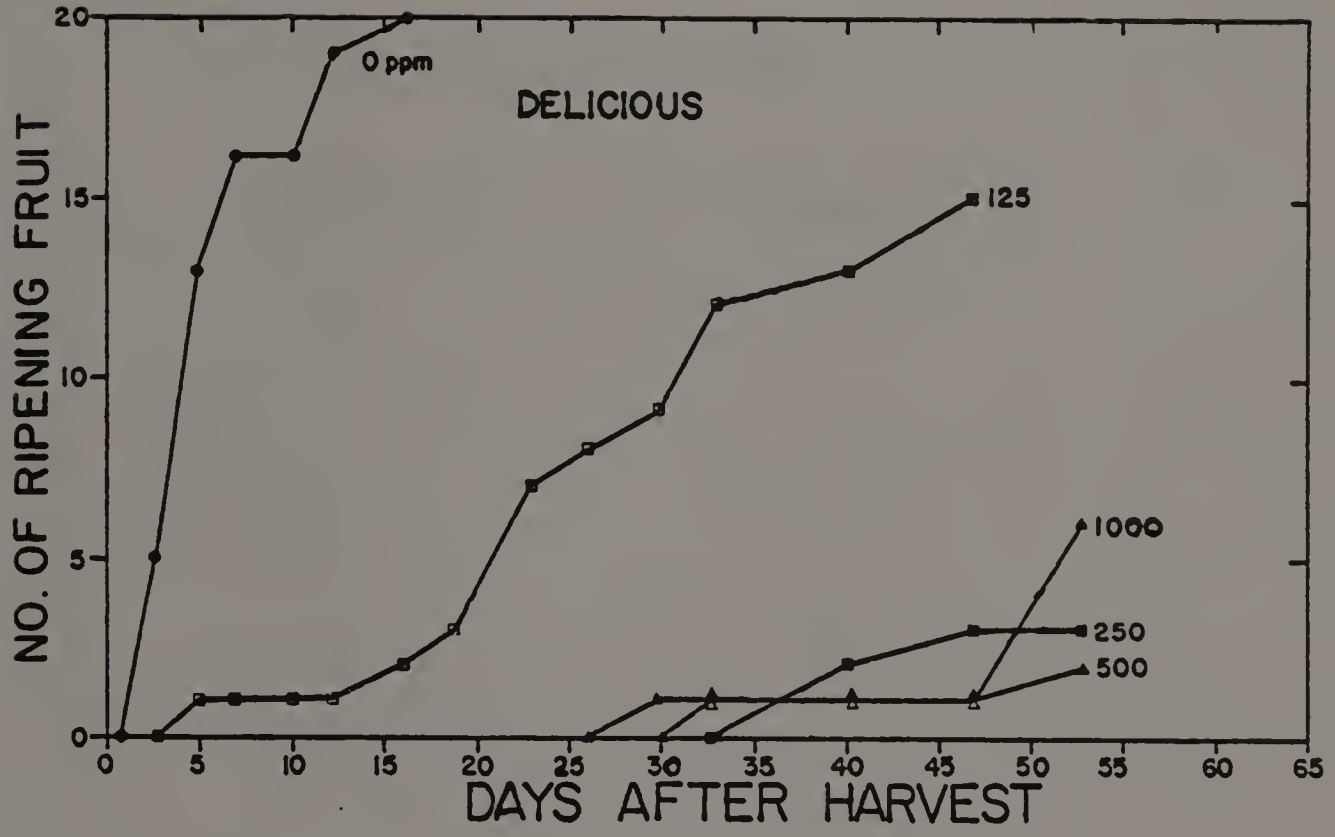


Figure 9. Cumulative ripening of Delicious apples at room temperature after harvest, following applications of AVG 1 week prior to harvest. Fruit were judged to be ripening when internal ethylene concentrations reached 1 ppm.

Table 5. The number of days at room temperature required for 50 % of the fruit in Experiment One to begin ripening (reach 1 ppm internal ethylene).

Cultivar	AVG concentration (ppm)	Days for 50 % to begin ripening
Early McIntosh	0	1.5
	125	2.5
	250	3
	500	6
	1000	14
McIntosh	0	3
	125	6
	250	8
	500	38
	1000	62
Cortland	0	7
	125	7
	250	19
	500	37
	1000	34
Delicious	0	4
	125	31
	250	-[z]
	500	-[z]
	1000	-[z]

[z]Less than 50 % of the fruit in these treatments were ripening after 53 days.

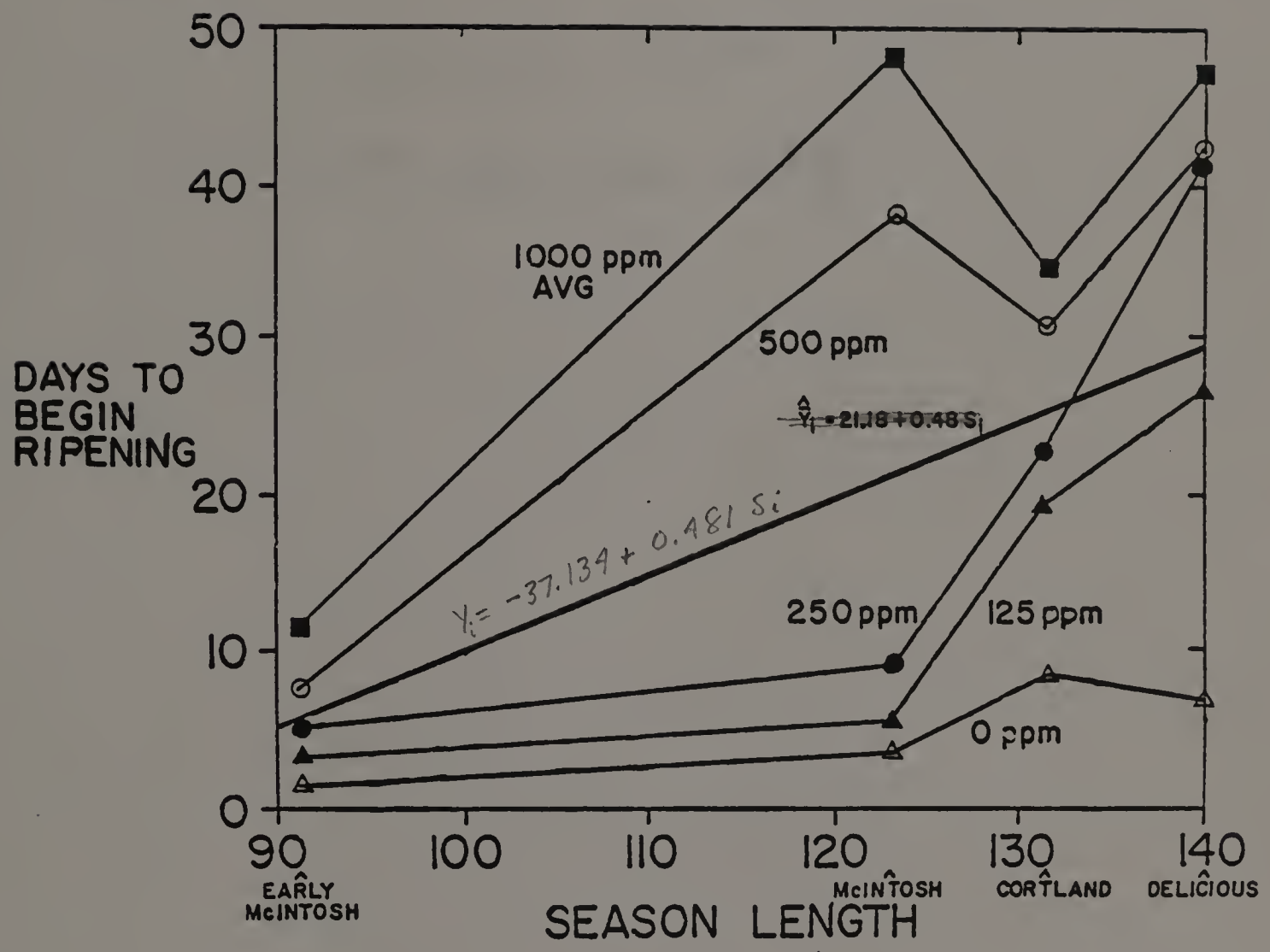


Figure 10. The number of days to begin ripening (reach 1 ppm internal ethylene) plotted against the length of season for each cultivar. The linear regression line is also plotted.

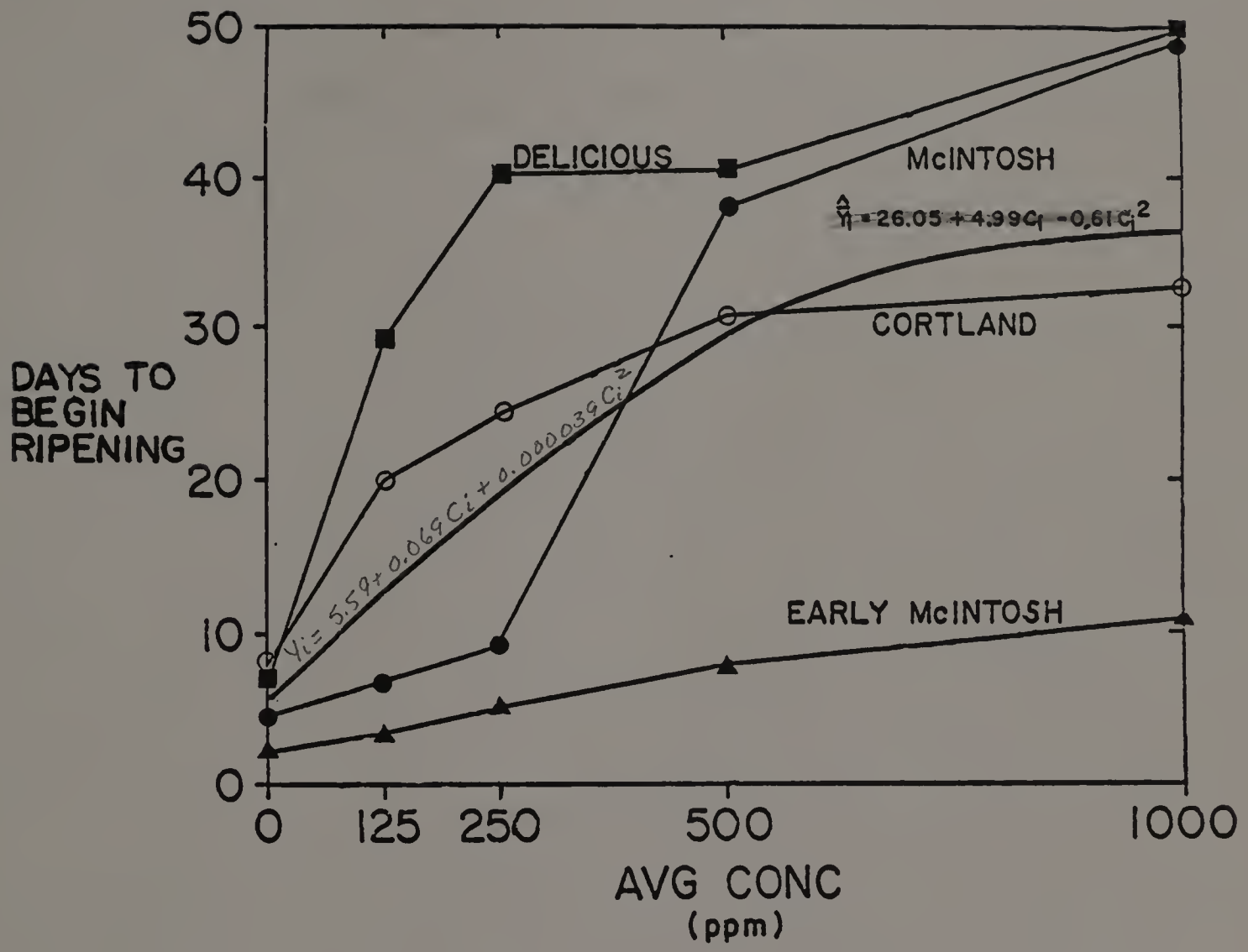


Figure 11. The number of days to begin ripening (reach 1 ppm internal ethylene) plotted against AVG concentration. The quadratic regression line is also plotted.

number of days to ripen. The regression line appears on Figure 10. A highly significant quadratic relationship existed between AVG concentration and the number of days to ripen. This regression line is graphed on Figure 11. A highly significant interaction between cultivar and AVG concentration existed, and this interaction is depicted by both Figures 10 and 11.

Experiment Two

To observe the effects of AVG on fruit maturation, limbs of Puritan trees were either treated with AVG at one of three different times before harvest or were left untreated. Figure 12 graphs the internal ethylene concentration after harvest of fruit from the different treatments. AVG treatments appeared to result in lower ethylene peak height in comparison to the control and appeared to delay the time of the ethylene peak. Appendix Table 9 shows the analysis of variance using the number of days to initiate ripening as the dependent variable. Highly significant differences existed among treatments. The treatment means are shown in Table 6, and a linear comparison shows that it took significantly longer for AVG-treated fruit to initiate ripening than it did for untreated fruit. The 3 AVG treatments were separated with Duncan's New Multiple Range Test which showed that the earliest treatment took significantly less time to initiate ripening than the two later treatments.

The analysis of covariance for firmness and analyses of variance for chlorophyll content, percent soluble solids, starch content, and

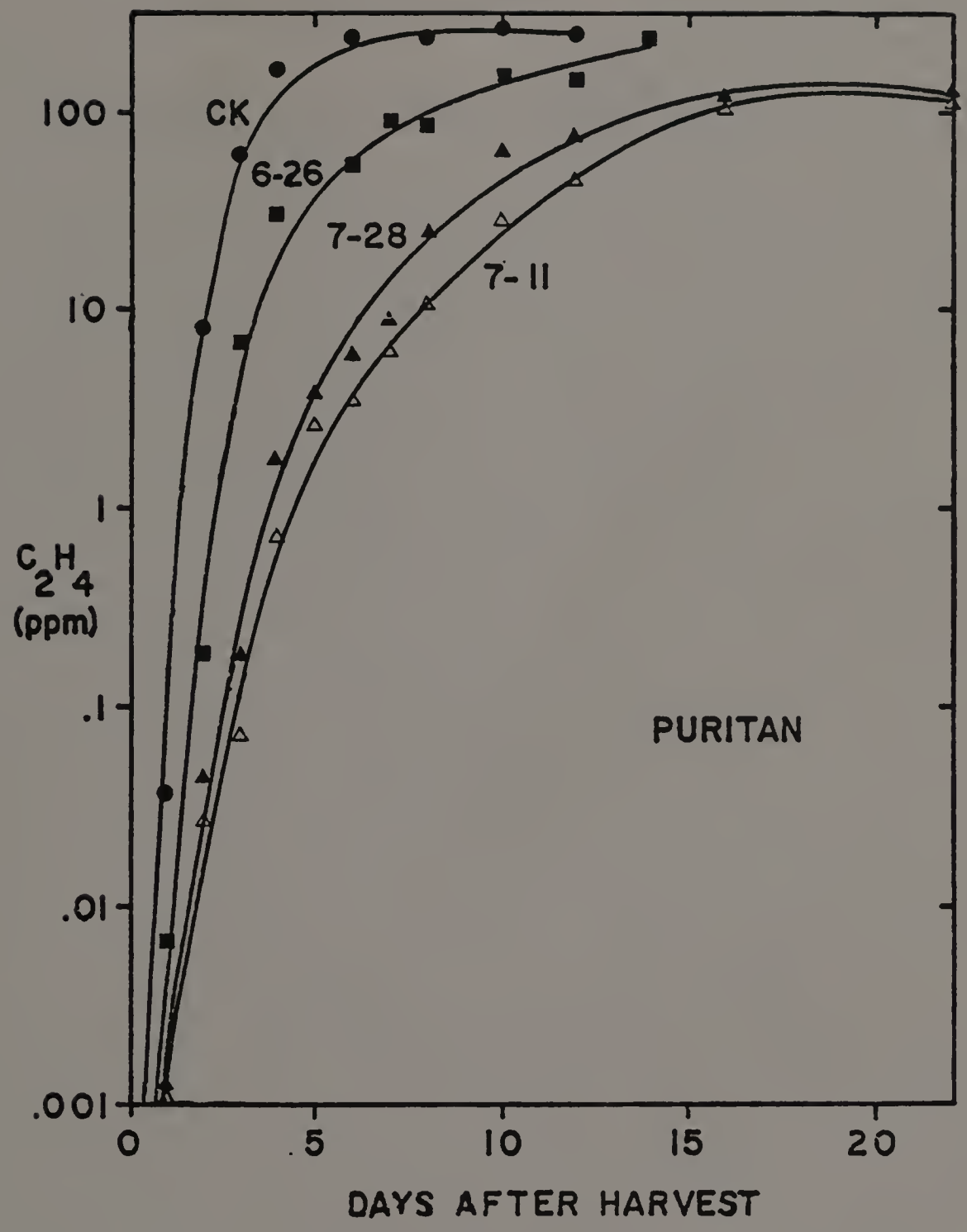


Figure 12. Mean internal ethylene concentrations of Puritan fruit kept at room temperature after harvest. Limbs were sprayed with 500 ppm AVG on the indicated date. Fruit were harvested on August 10.

Table 6. Means for maturity and ripening parameters of Puritan apples in Experiment Two.

Spray date	Days to begin ripening[z]	Firmness[y] (N/cm^2)	Chlorophyll (µg/g F.W.)	%Soluble Solids	Starch (µg/g)	Titratable acidity (meq/ml juice)
6-26	3.9 a	92[x]	93.7[x]	12.4[x]	71.6[x]	0.150[x]
7-11	6.4 b	91	94.6	12.5	63.5	0.148
7-28	6.2 b	92	90.0	11.6	72.2	0.148
Check	2.8	92	92.2	11.8	68.5	0.144

[z]For days to begin ripening a linear comparison of the Check and the treatments was highly significant. Treatments were then separated by Duncan's New Multiple Range Test at the 5 % level.

[y]Firmness means were adjusted by covariance to account for different fruit sizes.

[x]No significant differences existed for firmness, chlorophyll, soluble solids, starch, or titratable acidity.

titratable acidity appear in Appendix Tables 10 through 14. The means appear in Table 6. No significant differences existed among treatments for any of the measured parameters.

Experiment Three

To observe the effects of termperature and atmospheric ethylene on AVG-treated apples, fruit from 2 cultivars were stored in 4 different storage conditions: 1) 0 C, low atmospheric ethylene (0,LE); 2) 3.3 C, low atmospheric ethylene (3.3,LE); 3) 3.3 C, high atmospheric ethylene (3.3,HE); and 4) 20 C, low atmospheric ethylene (20,LE). However, no data were obtained from 20,LE, because shrivelling of the fruit prevented reliable measurements from being taken.

Analyses of variance for percentage decay, breakdown, and browncore along with fruit firmness and ground color appear in Appendix Tables 15 through 21, and means appear in Table 7. Low temperature (0 C) reduced softening of both McIntosh and Delicious and reduced decay after 3 weeks, breakdown after 1 and 3 weeks, and loss of green color of McIntosh fruit. Low ethylene reduced softening of both cultivars and reduced breakdown after 1 and 3 weeks for McIntosh fruit.

Figures 13 and 14 graph the level of internal ethylene for McIntosh and Delicious fruit after removal from storage. McIntosh fruit showed no significant differences between 3.3,LE and 3.3,HE, but 0,LE was initially lower than either one. All had ethylene levels well above that needed to trigger ripening. Delicious fruit were initially well below the triggering level of ethylene but rose slowly to reach about 0.8 ppm internal ethylene.

Table 7. Percents decay, breakdown, and browncore and ground color after storage and firmness before and after storage of AVG-treated fruit. Significance of a comparison is shown following data.

Measurements	Comparisons of Means 0,LE vs. 3.3,LE		3.3,LE vs. 3.3,HE	
		McIntosh		
Firmness (N/cm^2)[z]	51	48 **	48	44 **
Decay (%)				
1 wk	1.5	1.3 [x]	1.3	2.6 [x]
3 wk	2.5	9.0 *	9.0	8.7 *
Breakdown (%)				
1 wk	0.5	7.9 **	7.9	16.7 *
3 wk	2.7	15.0 **	15.0	25.4 *
Browncore (%)	63.1	68.9 [x]	68.9	61.4 [x]
Ground color	3.3	2.6 **	2.6	2.3 ns
		Delicious		
Firmness (N/cm^2)[y]	76	68 **	68	64 **
Decay (%)				
1 wk	1.2	0.4 [x]	0.4	2.2 [x]
3 ns wk	7.8	8.9 ns	8.9	9.3 ns
Breakdown (%)				
1 wk	0.8	0.4 ns	0.4	1.3 ns
3 wk	3.0	4.0 ns	4.0	4.7 ns

[z]Firmness at harvest for McIntosh was 81 N/cm , which was significantly different (1 %) from the values after storage.

[y]Firmness at harvest for Delicious was 93 N/cm , which was significantly different (1 %) from the values after storage.

[x]No comparisons made because analysis of variance showed no effect of storage condition.

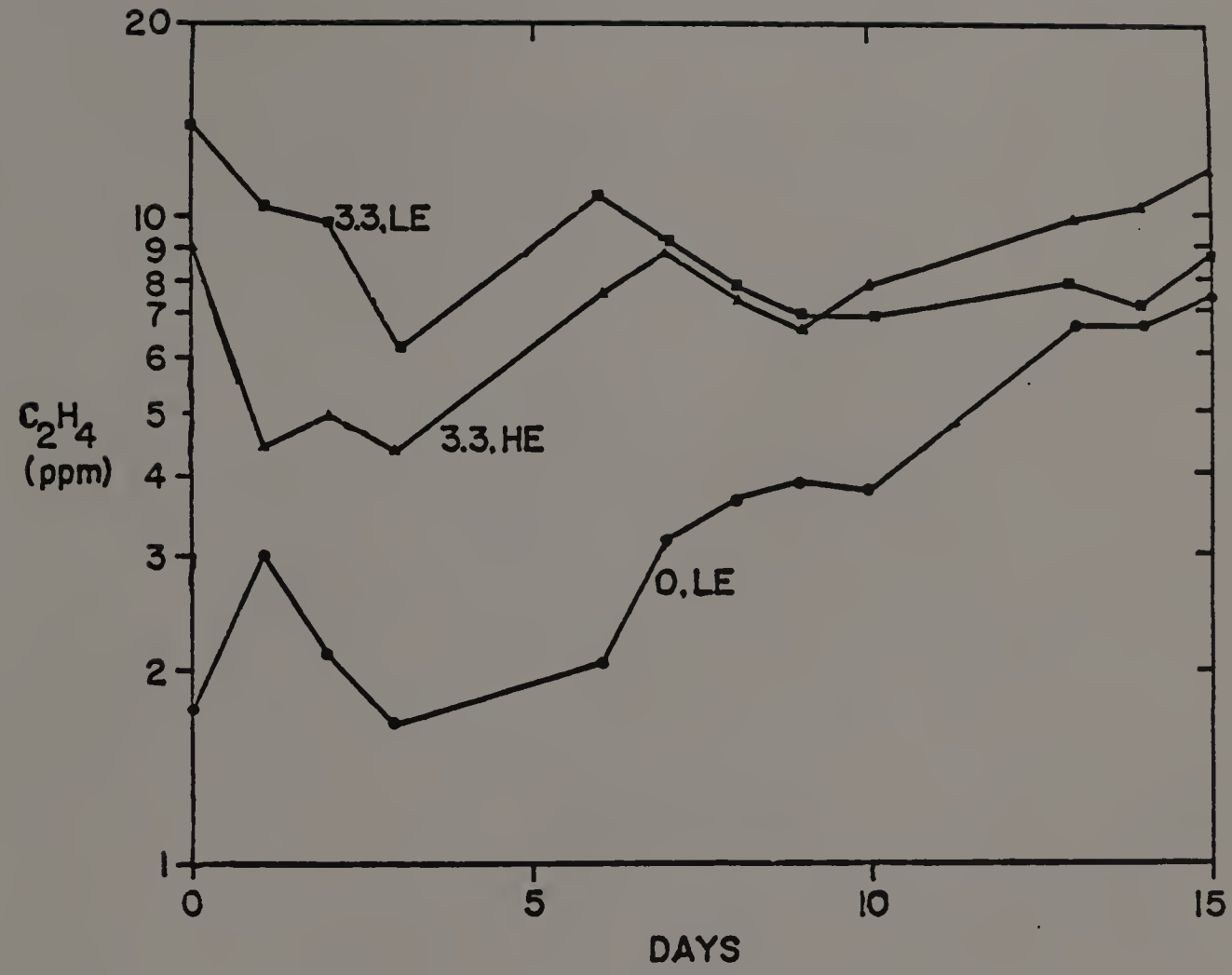


Figure 13. Mean internal ethylene concentrations of AVG-treated McIntosh fruit after removal from storage.

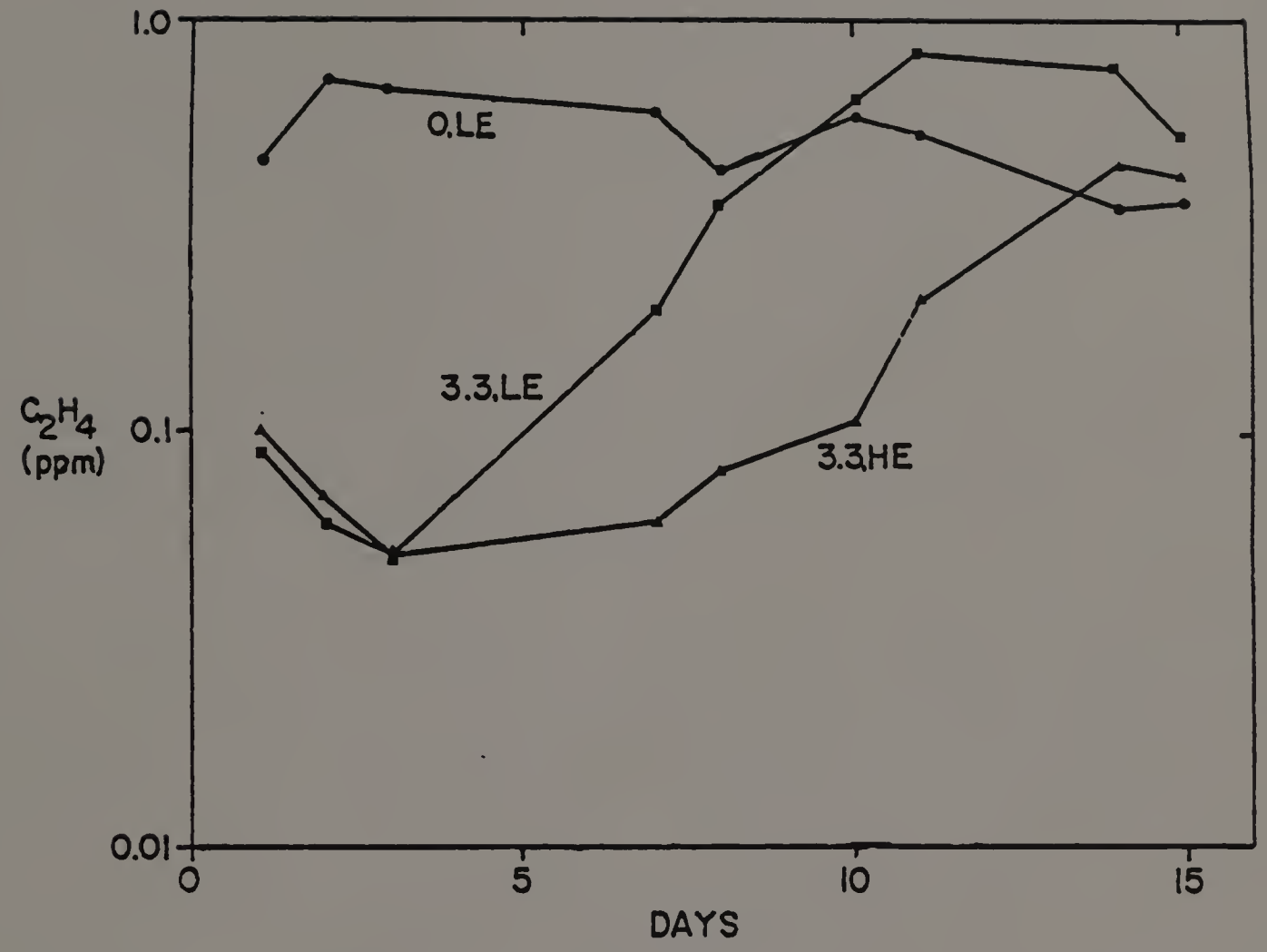


Figure 14. Mean internal ethylene concentrations of AVG-treated Delicious fruit after removal from storage.

C H A P T E R V

DISCUSSION

Experiment One

During the growing season developing fruit increase in sensitivity to ethylene to a point where autocatalytic ethylene production is initiated, and ripening is triggered (35). By inhibiting ethylene biosynthesis AVG can delay fruit ripening (Figure 1), and in this experiment it delayed apple ripening regardless of cultivar. However, late season cultivars were more affected by a given AVG concentration than were early season cultivars (Figure 10). There are several possible explanations for this difference in effect among cultivars.

The specific point at which AVG blocks ethylene biosynthesis is the conversion of SAM to ACC. AVG competitively and reversibly inhibits ACC synthase, the enzyme responsible for this reaction (17). Auxin may also be involved with the synthesis of ACC synthase (77). Early cultivars could simply have greater ACC synthase activity than late cultivars. Higher AVG concentrations would thus be necessary to delay ripening of early cultivars as much as late cultivars. Early cultivars may also have a higher turnover rate of ACC synthase, possibly due to higher auxin concentrations. This again would result in AVG being less effective.

Another possibility is that early cultivars may have already accumulated ACC prior to AVG treatment thus rendering AVG less effective. The results from Experiment Two may suggest that this is not

the case, in that early season AVG treatments of Puritan fruit did not have greater effects than treatment just before harvest. The conversion of methionine to SAM may also be important in reducing the effectiveness of AVG in early cultivars. Since the inhibition by AVG is competitive and reversible, increased levels of SAM would reduce its effectiveness.

Ripening of Early McIntosh, the earliest cultivar tested, was delayed 10 days by 1000 ppm AVG. While this delay was not nearly as great as that of Delicious, which was delayed more than 50 days, the effect on Early McIntosh may have more significance in marketing terms. Late cultivars normally ripen slowly and can be kept for relatively long periods of time, but early cultivars ripen very rapidly and have short postharvest lives. These cultivars are typically marketed immediately after harvest, and significantly delayed ripening could substantially reduce losses of quality and produce more orderly marketing.

Experiment Two

Bramlage *et al*. (18) reported an increased level of browncore in AVG-treated apples after storage. Since browncore characteristically develops more prevalently in immature fruit (65), this suggests that AVG may retard fruit maturation. While maturity cannot be precisely assessed, the parameters tested in this experiment (firmness, starch, chlorophyll, soluble solids, and titratable acidity) are usually considered to be maturity indices. The lack of a consistent effect on any of these parameters by AVG suggests that it did not affect the maturation processes of Puritan apples, although it clearly delayed

fruit ripening (Table 6). Chu et al. (26) obtained different results with a similar experiment using McIntosh trees. They found that AVG reduced starch degradation and increased firmness at harvest. These observations directly oppose our results, but the difference may be related to the cultivar being tested. Puritan is an early cultivar which ripens very rapidly, and the AVG applied may not have had sufficient effect to cause differences to appear in fruit maturation. A slower developing cultivar such as McIntosh may be more prone to a developmental effect.

Perhaps the same bases for ripening control by AVG that were discussed above apply also to maturation control. A test similar to Experiment Two should be made on later maturing cultivars such as McIntosh and Delicious.

Experiment Three

Experiment Three was designed to observe the effects of storage temperature and ethylene in the storage atmosphere on AVG-treated fruit from a cold sensitive and a cold tolerant cultivar. After storage there were significant differances in firmnness among treatments for both cultivars. Fruit stored in low atmospheric ethylene were firmer at 0 C than at 3.3 C, and fruit stored at 3.3 C were firmer in low atmospheric ethylene than in high atmospheric ethylene. After 1 and 3 weeks at room temperature McIntosh fruit stored in low atmospheric ethylene had sigificantly less senescent breakdown at 0 C than at 3.3 C, and at 3.3 C fruit in low atmospheric ethylene had significantly less senescent

breakdown than fruit in high atmospheric ethylene. Also, McIntosh fruit had greener ground color at 0 C than at 3.3 C in low atmospheric ethylene, and decay after 3 weeks was lower at 0 C. These data state, without question, that temperature and atmospheric ethylene both had significant effects on AVG treated fruit in storage. Ethylene had detrimental effects and low temperatues had beneficial effects.

The AVG-treated fruit acted similarly to what would be expected of untreated fruit under the same conditions. Low temperature slowed fruit senescence which led to less softening, less senescent breakdown, less decay, and slower chlorophyll degradation. High ethylene in the storage atmosphere acted as a trigger to induce ripening and advance senescence.

By the end of storage internal ethylene of AVG-treated McIntosh accumulated to levels sufficient to induce ripening regardless of storage temperature and atmospheric ethylene. This rules out the possibility of exogenous ethylene being the sole reason for McIntosh ripening during storage. Extrapolating from the work of Mattoo *et al.* (49), AVG is apparently only 75 % as effective at 0 C as at 3.3 C. However, cold temperature cannot account for the ripening since fruit ripened at both 0 C and at 3.3 C. Therefore, it appears that ripening of AVG-treated fruit in storage is at least in part due to endogenous ethylene buildup.

McIntosh fruit after harvest were less affected by a given concentration of AVG than Delicious fruit. Ethylene biosynthesis may not have been blocked sufficiently in McIntosh to prevent a slow, internal accumulation to a triggering level. Perhaps with longer

storage, the same pattern of response would have been seen for Delicious, which had not begun to ripen after the same storage time as McIntosh.

Low temperature injury perhaps may account for the ripening of McIntosh fruit. Wound ethylene, as would be produced by chilling, is at least in part synthesized via the same pathway as described earlier and can be blocked by AVG (33, 53). However, it is possible that some portion of the wound ethylene in apple is produced via a different pathway such as the linolenate pathway (1, 41). Chilled apples could accumulate ethylene which could then trigger ripening. Although, 3.3 C is not normally a chilling temperature for McIntosh, they did chill at this temperature because browncore developed, indeed to the same extent as it did in fruit stored at 0 C. Browncore is considered to be a symptom of chilling injury in McIntosh (65). It may be possible that AVG increased chilling sensitivity of McIntosh fruit so that even at 3.3 C fruit were chilled, leading to increased ethylene production and increased browncore. If so, this is both an interesting and significant response and bears further testing.

Implications of this Study

From these results and those of others the potential roles of AVG or another ethylene inhibitor in handling of apples can be envisioned. AVG certainly can delay ripening on the tree and have a stop-drop effect (68), and while the magnitude of delay has not been determined, for at least late-maturing cultivars it could be profound. After harvest and

without refrigeration the delays for late-maturing cultivars were profound; the delay from high concentration exceeded the limits of our tests in the absence of high concentrations of exogenous ethylene. Even for inherently fast-ripening, early maturing cultivars, ripening of harvested fruit was slowed substantially. AVG therefore might be a very good marketing tool, producing slower ripening of freshly harvested apples as they pass through the marketing channels, and an important benefit might be in marketing of early maturing cultivars that normally ripen very fast. AVG might also reduce energy consumption by reducing the need for refrigeration of apples destined for immediate marketing.

AVG would seem to have limited value for air-stored fruit, since an accumulation of ethylene in the storage atmosphere will override the AVG effect. However, if fruit are intended for only short storage in air, a significant effect might be obtained. Its usefulness in CA might be greater, assuming that preclimacteric fruit were placed under CA conditions before ripening was initiated. In a CA atmosphere AVG-treated fruit will probably start to ripen more slowly, thereby slowing the buildup of ethylene in CA-storage.

The effects of AVG are similar to the effects of Alar (daminozide) in a number of ways (68). Its effects in conjunction with Alar would be interesting to test, and it may be that AVG could contribute significantly to low-ethylene CA storage, especially in combination with Alar.

A potential concern is the apparent increase in browncore in AVG-treated fruit (18, 26). Development of browncore is usually avoided

by storage at 3.3 C, yet we obtained as much at this temperature as at 0 C (Table 7). If AVG increases cold-temperature sensitivity serious problems could be encountered.

AVG is no longer available for use in significant quantities and its safety has not been established. Nevertheless, other ethylene-inhibiting compounds are becoming available for testing, and the results with AVG should provide models for testing these materials.

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APPENDIX

Table 8. Analysis of variance for the number of days for fruit to begin ripening (reach 1 ppm internal ethylene) in Experiment One.

Source	df	SS	MS	F
C (cultivar)	3	21401.4	7133.8	145.5 **
linear	1	20801.0	20801.0	424.3 **
quadratic	1	137.9	137.9	2.8 ns
cubic	1	462.5	462.5	9.4 ns
T (treatment)	4	29876.9	7469.2	152.4 **
linear	1	26967.1	26967.1	550.1 **
quadratic	1	2823.6	2823.6	57.6 **
cubic	1	72.9	72.9	1.5 ns
quartic	1	13.3	13.3	0.3 ns
CT	12	13963.9	1163.7	23.7 **
W	285	13972.5	49.0	
Total	304	79214.7		

Table 9. Analysis of variance for the number of days for Puritan fruit to begin ripening (reach 1 ppm internal ethylene).

Source	df	SS	MS	F
A (treatment)	3	256.6	85.6	10.4 **
T (tree)	6	44.2	7.4	3.6 **
AT	18	147.4	8.2	4.1 **
F:AT (fruit)	84	169.7	2.0	
Total	11	617.7		

Table 10. Analysis of covariance for Puritan fruit firmness.

Source	df	SS	MS	F
Whole Plot				
A (treatment)	3	21.0	7.0	0.3 ns
T (trees)	6	321.7	53.6	16.8 **
AT	18	455.3	25.3	7.9 **
Total	27	798.0		
Split Plot				
S (sample date)	3	105.9	35.3	6.8 **
SA	9	41.3	4.6	0.9 ns
ST	18	92.8	5.2	1.6 *
SAT	54	268.2	5.0	1.6 **
F:SAT (fruit)	1007	3197.6	3.2	1.3 **
W	1120	2829.8	2.5	
Total	2211	6535.6		
Regression	1	2226.9	2226.9	703.1 **

Table 11. Analysis of variance for peel chlorophyll content of Puritan fruit.

Source	df	SS	MS	F
Whole Plot				
A (treatment)	3	88	29	0.1 ns
T (tree)	6	1869	312	1.5 ns
AT	18	3710	206	1.2 ns
Total	27	5667		
Split Plot				
S (sample date)	3	2366	789	2.4 ns
SA	9	1779	198	1.2 ns
ST	18	5859	325	1.9 ns
SAT	54	9271	172	
Total	84	19275		
Grand Total	111	24942		

Table 12. Analysis of variance for percent soluble solids of Puritan fruit.

Source	df	SS	MS	F
Whole Plot				
A (treatment)	3	16.4	5.5	1.7 ns
T (tree)	6	56.1	9.3	2.9 *
AT	18	57.5	3.2	0.6 ns
Total	27	130.0		
Split Plot				
S (sample date)	3	15.5	5.2	0.6 ns
SA	9	40.3	4.5	0.8 ns
ST	18	143.2	8.0	1.4 ns
SAT	54	301.1	5.6	
Total	84	500.1		
Grand Total	111	630.1		

Table 13. Analysis of variance for starch content of Puritan fruit.

Source	df	SS	MS	F
Whole Plot				
A (treatment)	3	1466	487	0.4 ns
T (trees)	6	2565	428	0.4 ns
AT	18	19933	1107	1.2 ns
Total	27	23964		
Split Plot				
S (sample date)	3	1198	399	0.4 ns
SA	9	7227	803	0.9 ns
ST	18	18542	1030	1.1 ns
SAT	54	49288	913	
Total	84	76255		
Grand Total	111	100219		

Table 14. Analysis of variance for the titratable acidity of juice from Puritan fruit.

Source	df	SS	MS	F
Whole Plot				
A (treatment)	3	1.20	0.40	1.1 ns
T (tree)	6	5.57	0.93	2.5 ns
AT	18	6.78	0.38	1.0 ns
Total	27	13.55		
Split Plot				
S (sample date)	3	10.86	3.62	5.6 **
SA	9	2.17	0.24	0.7 ns
ST	18	11.54	0.64	1.7 ns
SAT	54	19.86	0.37	
Total	84	44.43		
Grand Total	111	57.98		

Table 15. Analysis of variance for the percentage decay of McIntosh and Delicious fruit after 1 week at room temperature.

Source	df	SS	MS	F
Whole Plot				
C (cultivar)	1	33	33	0.9 ns
T:C (tree)	10	383	38	1.4 ns
Total	11	416		
Split Plot				
S (storage)	2	113	56	2.1 ns
SC	2	12	6	0.2 ns
ST:C	20	550	27	
Total	24	675		
Grand Total	35	1091		

Table 16. Analysis of variance for the percentage decay of McIntosh and Delicious fruit after 3 weeks at room temperature.

Source			df	SS	MS	F
Whole Plot						
C (cultivar)			1	131	131	2.5 ns
T:C (trees)			10	523	52	1.8 ns
Total			11	654		
Split Plot						
S (storage)			2	450	225	7.6 **
McIntosh	0,LE vs 3.3,LE		1	205	205	7.0 *
	3.3,LE vs 3.3,HE		1	11	11	0.4 ns
Delicious	0,LE vs 3.3,LE		1	51	51	1.7 ns
	3.3,LE vs 3.3,HE		1	16	16	0.6 ns
SC			2	31	16	0.5 ns
ST:C			20	589	29	
Total			24	1070		
Grand Total			35	1724		

Table 17. Analysis of variance for the percentage senescent breakdown of McIntosh and Delicious fruit after 1 week at room temperature.

Source	df	SS	MS	F
Whole Plot				
C (cultivar)	1	905	905	31.8 **
T:C (tree)	10	285	28	0.8 ns
Total	11	1190		
Split Plot				
S (storage)	2	683	342	10.0 **
McIntosh 0,LE vs 3.3,LE	1	424	424	12.4 **
3.3,LE vs 3.3,HE	1	206	206	6.0 *
Delicious 0.LE vs 3.3,LE	1	11	11	0.3 ns
3.3,LE vs 3.3,HE	1	28	28	0.8 ns
SC	2	580	290	8.5 **
ST:C	20	684	34	
Total	24	1947		
Grand Total	35	3137		

Table 18. Analysis of variance for the percentage senescent breakdown of McIntosh and Delicious fruit after 3 weeks at room temperature.

Source	df	SS	MS	F
Whole Plot				
C (cultivar)	1	585	585	9.8 **
T:C (trees)	10	594	59	1.9 ns
Total	11	1179		
Split Plot				
S (storage)	2	1146	573	18.5 **
McIntosh 0,LE vs 3.3,LE	1	539	539	17.4 **
3.3,LE vs 3.3,HE	1	200	200	6.5 *
Delicious 0,LE vs 3.3,LE	1	55	55	1.8 ns
3.3,LE vs 3.3,HE	1	6	6	0.2 ns
SC	2	383	191	6.2 **
ST:C	20	618	31	
Total	24	2147		
Grand Total	35	3326		

Table 19. Analysis of variance for the percentage browncore of McIntosh fruit.

Source	df	SS	MS	F
S (storage)	2	80	40	0.3 ns
T (tree)	5	1231	246	
ST	10	1444	144	
Total	17	2755		

Table 20. Analysis of variance for firmness data from Experiment Three.

Source	df	SS	MS	F
Whole Plot				
C (cultivar)	1	4200	4200	1575.5 **
T:C (tree)	10	27	3	2.4 *
Total	11	4227		
Split Plot				
S (storage)	3	7245	2415	1033.0 **
McIntosh Pre- vs others	1	4565	4565	1952.4 **
0,LE vs 3.3,LE	1	27	27	11.5 **
3.3,LE vs 3.3,HE	1	20	20	8.6 **
Delicious Pre- vs others	1	2361	2361	1009.8 **
0,LE vs 3.3,LE	1	187	187	80.0 **
3.3,LE vs 3.3,HE	1	64	64	27.4 **
SC	3	256	85	36.5 **
ST:C	30	70	2	2.1 **
F:CST (fruit)	432	486	1	1.8 **
W	480	293	1	
Total	948	8350		
Grand Total	959	12577		

Table 21. Analysis of variance for McIntosh ground color after storage.

Source	df	SS	MS	F
S (storage)	2	27	13.6	20.4 **
0,LE vs 3.3,LE	1	12	12.0	18.0 **
3.3,LE vs 3.3,HE	1	3	2.7	4.0 ns
T (tree)	5	5	1.0	2.1 ns
ST	10	7	0.6	1.4 ns
F:ST (fruit)	162	79	0.5	
Total	179	118		