# SOME FACTORS AFFECTING CONTROLLED ATMOSPHERE STORAGE DISORDERS OF JONATHAN APPLES

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William George Chace, Jr.

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Some Factors Affecting Controlled Atmosphere Storage Disorders of Jonathan Apples

presented by

WILLIAM GEORGE CHACE, JR.

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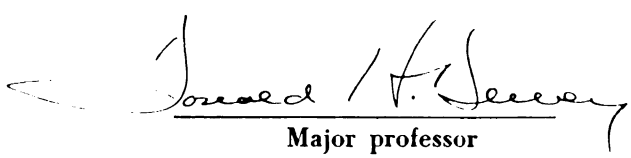

Major professor

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# SOME FACTORS AFFECTING CONTROLLED ATMOSPHERE STORAGE DISORDERS OF JONATHAN APPLES

By

WILLIAM GEORGE CHACE, Jr.

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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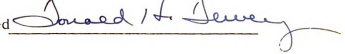
Approved 

WILLIAM GEORGE CHACE, Jr. ABSTRACT

The length of the storage period for the Jonathan variety of apple fruit usually is limited by the incidence of one or more storage disorders, such as soft scald, soggy breakdown, Jonathan spot and internal breakdown. Controlled atmosphere (CA) storage reduces the incidence of these disorders, but sometimes other injuries are encountered. Some of the factors influencing the development of CA disorders, such as voids, core browning and breakdown were investigated.

Prestorage treatments in 1957 included modification of the fruit/leaf ratio and enclosure of the fruit within a plastic film prior to harvest, maturity of the fruit at harvest, and holding the apples at 55° F for 10 days following harvest. Storage conditions employed were regular storage at 32° F and controlled atmospheres of 13 percent $CO_2$ - 3 percent $O_2$ and 5 percent $CO_2$ - 3 percent $O_2$ at 32 and 38° F for seven months. In 1958-59, the effects of maturity and fruit/leaf ratio of the fruit were studied in relation to regular and CA storage at 32° F.

Core browning, although previously reported as a CA disorder, occurred in regular storage as well as in CA storage in one of the two seasons studied. This disorder was characterized by a slight to severe brown discoloration of the pith tissue near the cambial line. The cells of the affected tissues showed a vacuole-like sac containing many particles surrounded by a brown fluid. The highest incidences of this disorder were associated with advanced fruit maturity, and a high concentration of $CO_2$ (13 percent) and a low temperature (32° F) during

storage. Defoliation of the limbs bearing the test fruit two months prior to harvest in 1958 did not significantly affect the development of core browning in the fruit during storage. Core browning appeared after 156 and 76 days in storage during the first and second years, respectively.

The development of voids or small spheroid air pockets in the pith and occasionally in the cortex of the fruit occurred only in controlled atmosphere storage. Factors associated with a high incidence of this disorder were large-sized fruits, the presence of water core in the apples at harvest, late picking, and storage in 13 percent carbon dioxide with 3 percent oxygen at 32° F. Defoliation of branches bearing the test fruit in 1958 significantly reduced the incidence of this disorder in CA storage. Similar fruits from branches which were not defoliated were of higher soluble solids content and were affected with water core. Voids developed in susceptible fruit after 125 or 176 days of storage.

Three types of fruit breakdown developed in these tests - internal breakdown in regular and CA storage, soggy breakdown in regular storage, and brown heart in CA storage. A high concentration of carbon dioxide (13 percent) and low temperature (32° F) were most conducive to the development of this disorder during storage. Over-maturity at harvest favored breakdown: whereas the other prestorage treatments had no consistent effect on its development. In 1957, fruit held in CA and regular storage at 32° F developed breakdown

after 176 days of storage and after 210 days when stored in controlled atmospheres at 38° F. Fruit held in CA and regular storage in 1958 developed breakdown after 210 days of storage.

Techniques were developed for the utilization of labeled carbon to possibly relate the distribution and movement of carbon dioxide in the fruit tissues to the development of CA disorders. These studies indicated that the properties of the fruit cells in respect to the accumulation and/or permeability to carbon dioxide are altered by CA storage since CA apples, following exposure to $C^{14}O_2$, evolved a greater total amount of $C^{14}O_2$ and at a higher rate than fruit from regular storage. Fruit previously stored in 13 percent carbon dioxide also evolved a greater total amount of $C^{14}O_2$ than fruit stored in 5 percent carbon dioxide. The greatest radioactivity accumulated in the seed cavities, cambial line, vascular bundles and pith tissues of the fruit. Also, the pith and cortex tissues, on a per gram basis, accumulated more $C^{14}$ in large fruit than in small fruit.

The recommended CA storage condition (5 percent $CO_2$ - 3 percent $O_2$ at 32° F) for Jonathan apples proved sound, since it gave the best retention of eating quality with a minimum danger of core browning and void formation. These experiments demonstrated that fruit of advanced maturity or affected with water core at harvest time should be avoided when selecting Jonathan apples for CA storage.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES CONT'D

LIST OF FIGURES

LIST OF FIGURES CONT'D

LIST OF FIGURES CONT'D

LIST OF FIGURES CONT'D

APPENDIX TABLES

APPENDIX TABLES CONT'D

# INTRODUCTION

The varied recommendations for extending the storage life of the Jonathan variety of apple can be largely attributed to the numerous physiological disorders associated with the storage of this fruit. The high incidence and severity of one or more disorders, such as soft scald, soggy breakdown, Jonathan spot and internal breakdown have limited the length of storage period for this variety. The importance of the Jonathan variety in Michigan, together with the need for a longer storage life of the fruit, led to controlled atmosphere experimentation with this variety in 1956 in Michigan. Previously, it had been reported that certain storage disorders could be reduced by altering the storage atmospheres, but also that other difficulties were then sometimes encountered. Controlled atmospheres (CA) have been proven to be suitable means of storing Jonathans in Michigan, and by 1959, the controlled atmosphere storage capacity was about 183,000 bushels of Jonathan apples, or 34.4 percent of the total CA storage capacity in the State.

The controlled atmosphere conditions utilized for commercial storage have not completely eliminated storage disorders. The amount and severity of CA injury has varied considerably from year to year, and from one lot of apples to another under the same storage conditions. Obviously, the inherent characteristics of the fruit are important; even though most of the difficulties in the past have been attributed to the storage conditions.

In view of the increasing importance of the controlled atmosphere method for the storage of Jonathans, this research was initiated to study the physiological behavior of the fruit to ascertain the possible factors influencing the development of CA injury. This was approached by inducing CA disorders by several storage conditions, by ascertaining the effects of pre-storage treatments on the induction of these disorders, and by study of the affected fruit tissues in relation to accumulation or deficiency of carbon dioxide and oxygen within the fruit.

The storage conditions employed in this study were normal air, the recommended controlled atmosphere conditions for Michigan Jonathans (5 percent carbon dioxide and 3 percent oxygen at 32° F), and controlled atmosphere conditions using high levels of carbon dioxide and higher temperatures which have tended to promote CA disorders. Since such preharvest treatments as defoliation, ringing, thinning and removing fruit from limbs bearing excess crops are known to alter the physiological behavior of the fruit, these treatments were included. Radioisotopic techniques were used to trace the distribution and pathways of carbon dioxide in the apples. For the latter, it was necessary to develop suitable techniques for the application and measurement of $C^{14}$ in apple fruit tissues.

# LITERATURE REVIEW

Functional or physiological disorders of Jonathan apples during storage have been the subject of much research. For the most part, these disorders have been attributed to a complexity of factors with few single influences specifically identified. Some of the factors known to affect the development of functional disorders in storage will be discussed in this review.

The effect of carbon dioxide in controlling the physiological disorders of Jonathan apples was probably first observed by the Australians in connection with overseas shipments in 1928-29 (Carne and Martin, 1935). Since then studies in New Zealand by Mandeno and Padfield (1953), in Victoria by Trout et al. (1940), and in Tasmania by Carne and Martin (1935, 1935a, 1938) and Carne et al. (1930, 1930a) have developed further advantages of storing Jonathan apples in controlled atmospheres. Control of Jonathan spot, reduced incidence of soft scald, preservation of eating quality, extended storage life and longer shelf life of the apples have been attributed in varying degrees to controlled atmosphere storage by the above authors as well as by Van Heile (1951) in the Netherlands, Rasmussen (1951) in Denmark, Phillips and Poapst (1952) in Canada, and by Dewey et al. (1957) in this country. These workers have also reported varying degrees of susceptibility of Jonathan apples to CA injuries.

The disorders encountered in the storage of Jonathan apples have included

Jonathan spot, soft scald, soggy breakdown and various types of senescent breakdown (Daley, 1924; Kidd and West, 1925; Plagge, 1925; Brooks et al., 1935; and Heald, 1933).

Jonathan spot has been a factor in preventing the long term regular storage of Jonathan apples. Plagge and Gerhardt (1930) found that 32° F was the best temperature for control of this disorder, but that low temperatures increased the incidence of soggy breakdown and soft scald. Jonathan spot has been controlled in CA storage at temperatures above 32° F by Mandeno and Padfield (1953), Ballinger (1955), Dewey et al. (1957), Plagge (1942), Vickery et al. (1951), Rasmussen (1951), Huelin and Tindale (1947), and Trout et al. (1947).

The control of other disorders by CA storage has been less successful. Soggy breakdown has been reported by Plagge et al. (1935) and Plagge and Maney (1937) as an internal breakdown of the cortical tissue caused by low temperatures. The best control of this disorder was storage at 36° F rather than at the lower temperatures (Plagge, 1926; and Kidd and West, 1925). Jonathan apples in 9 and 11 percent carbon dioxide and 11 and 9 percent oxygen at 32° F developed some soggy breakdown, however, concentrations of carbon dioxide below 9 percent reduced the incidence of this disorder (Plagge, 1942). Fruit had become predisposed by immediate storage at 32° F to the development of soggy breakdown, but was held practically free of this disorder as a result of prestorage exposures of carbon dioxide gas (Brooks and Harley, 1934).

Carne et al. (1930), Plagge and Maney (1928), and Phillips and Poapst (1952) consider "low temperature breakdown" (Kidd and West, 1925) identical to soggy breakdown (Plagge and Maney, 1928; Plagge et al., 1935). Carne and Martin (1935), Trout et al. (1940) and Huelin and Tindale (1947) reported the appearance of low temperature breakdown in Jonathan apples was not altered by the use of "gas storages". Low temperature breakdown increased in Jonathan apples with the maturity at picking time, and the concentration of carbon dioxide and the length of time in storage (Carne and Martin, 1935). Huelin and Tindale (1947) found that when this disorder was prevalent in regular storage it was also a serious problem in gas storage.

Soft scald appeared as a browning of the skin and flesh of the apple characterized by a sharp demarcation between the injured and healthy tissue (Plagge et al., 1935; Plagge and Maney, 1937; and Brooks and Harley, 1934). Soft scald develops at low temperatures and was controlled to some degree by temperatures of 36° F (Plagge and Maney, 1937; Brooks and Harley, 1934). Brooks and Harley (1934) found that exposure of fruit to a concentration of carbon dioxide of 20 percent or more before storing at 32° F will sometimes reduce the incidence of soft scald. Trout et al. (1940) showed that Jonathan apples stored in gas storage at 36 to 40° F controlled soft scald, as well as Jonathan spot. Mandeno and Padfield (1953) reported that deep scald (soft scald) occurred when the concentration of carbon dioxide reached 8 percent

or above at temperatures above 40° F. Soft scald did not appear when held in atmospheres of 5 percent carbon dioxide and 3 percent oxygen at 32° F and 38° F (Ballinger, 1955; and Dewey et al., 1957).

The description of brown heart by Kidd and West (1925) is similar to that of Plagge and Maney (1928, 1937) for soggy breakdown. The latter authors (1928), however, found that brown heart developed in the cortex, pith or in both areas, whereas soggy breakdown arose only in the cortex. Soggy breakdown tissue appeared also in more definite and regular patterns than brown heart. Brown heart may appear in storage at any time (Kidd and West, 1925), whereas soggy breakdown occurs only after a definite time interval in storage (Plagge and Maney, 1928). Brown heart has been associated with an excessive accumulation of carbon dioxide, but soggy breakdown did not appear to be associated with this condition (Plagge and Maney, 1928). Kidd and West (1925) showed that Jonathan apples and other English varieties were susceptible to brown heart at higher than normal levels of carbon dioxide. Carne and Martin (1935a) reported Jonathan apples less suscepible to brown heart than Sturmer and French Crab. These authors also stated that increasing the carbon dioxide levels increased the possibility of brown heart. Huelin and Tindale (1947) and Trout et al. (1940) showed that the more mature Jonathan apples were the more susceptible to brown heart. With concentrations higher than 7 percent carbon dioxide at 32° F brown heart occurred (Plagge, 1942).

The appearance of internal breakdown in controlled atmosphere storage apples has been reported by several workers (Kidd and West, 1928 and Dewey et al., 1957). Kidd and West (1928) found that within the temperature range of susceptibility of Bramley's Seedling apples, high levels of carbon dioxide tended to increase the amount of internal breakdown to a greater extent than the decreased levels of oxygen. Dewey et al. (1957) showed that storage of Jonathan apples in various levels of carbon dioxide and oxygen reduced the appearance of internal breakdown over fruit stored in normal air. They also noted that the least internal breakdown was found in apples stored in 5 percent carbon dioxide with 3 percent oxygen at 32° F. Internal breakdown in Jonathan apples stored at 2 1/2 percent carbon dioxide and 3 percent oxygen at 32° F was more prevalent than in regular storage at 35° F (Bunemann et al., 1959). Mandeno and Padfield (1953) reported that reduced oxygen and increased carbon dioxide increased the incidence of this disorder.

Brooks and Fisher (1926), Harley (1938) and Smock and Neubert (1950) reported that watercore apples have a high degree of susceptibility to internal breakdown. Watercore had a non-parasitic disorder that developed in the fruit on the trees when exposed to strong sunlight or allowed to become overmature (Brooks and Fisher, 1926). Fisher et al. (1930) found that watercore resulted in premature and non-uniform starch conversion within the fruit.

In addition to the many disorders that appear in regular storage, several

types of injury occurred as a result of storage under controlled atmosphere conditions.

Smock (1949) reported that New York-grown Jonathan apples stored in 5 percent carbon dioxide and 2 percent oxygen at 40° F were highly susceptible to internal carbon dioxide injury characterized by dry, brown, flaky areas or pockets in the flesh, particularly around the core. Ballinger (1955) found a similar type of injury as well as browning of the pith tissue and flesh browning which started at the epidermis and extended to various depths within the flesh. He attributed these disorders to the above-normal levels of carbon dioxide.

Smock (1946) suggested there were two separate types of internal browning injuries on McIntosh apples. One was due strictly to low temperature and the other resembled brown core, but was definitely due to high carbon dioxide. Kidd and West (1928) employed 1°, 5° and 10° C with various concentrations of carbon dioxide for Bramley's Seedling apples and found that alternating the atmosphere within the temperature range of susceptibility increased internal browning. Atmosphere alterations, when utilized at temperatures above the critical temperature for browning, reduced the occurrence of this physiological disorder. Barker and Kidd (1935) believed that carbon dioxide toxicity generally was greater at the lower temperatures than at higher temperatures.

Many workers, such as Kidd and West (1934), Haller and Lutz (1937),

Barker and Kidd (1935), Kidd and West (1926), have been unsuccessful in correlating respiration rate with the various storage disorders.

Kidd and West (1934) reported that low temperature breakdown (soggy breakdown) occurred during storage in English apples only when they were placed in storage while at the respiration peak. They did not believe this to be serious since the beneficial effects of carbon dioxide were slight for fruit placed in storage at this stage of maturity. Haller and Lutz (1937) found no correlation between the respiration rate and the occurrence of soft scald in Jonathan apples. Kidd and West (1926) found a positive correlation between the respiration rate of apples and softening and general storage life, but Ryall and Aldrich (1944) found that other factors may alter this relationship.

Cox's Orange Pippin apples were highly susceptible to carbon dioxide injury during the period immediately after the climacteric rise (Kidd and West, 1939). Fruit harvested three weeks early and treated with 1/500 ppm ethylene for 24 hours at 12° C before exposure to atmospheres containing carbon dioxide showed marked carbon dioxide injury (brown heart). These authors concluded that even immature apples, if stimulated by ethylene in such a manner as to cause premature climacteric rise, will become susceptible to carbon dioxide injury.

Many workers (Daley, 1924; Magness, 1929; Trout et al., 1940; Smock and Neubert, 1950; Carne and Martin, 1938) have reported a positive relation-

ship of size of fruit to the storage disorders of the fruit. Martin (1954) reported that light crop trees were more susceptible to breakdown in storage than the heavy crop trees, due to the larger average size of the cortical cells. Carne and Martin (1938) found a high positive correlation between the mean size of the fruit per tree and the incidence of disorders. Martin and Lewis (1952) indicated that in order to hold fruit in storage for longer periods, the size of the cell must be decreased and that the fruit size should be increased only by increasing the number of cells. Smith found that he late maturing varieties had a smaller number of cells per unit weight than earlier varieties. He associated the greater number of cells with lower respiration rate and good keeping qualities including fewer storage disorders.

Studies of seasonal variations in relation to storage quality have been limited and have given conflicting results. Seasonal variations in storage quality have been attributed to mean fruit size. No relation of maturity and breakdown was dependent on the season, but modified by fertilizer treatments seemed to have some influence.

Susceptibility to a given concentration of carbon dioxide may also depend upon the growing or climactic conditions in the orchard (Smock, 1944). Iowa-grown Jonathan apples have been shown by Plagge (1942) to tolerate 5 percent carbon dioxide at 40° F, while Smock and Van Doren (1941) showed that New York-grown Jonathan apples developed flesh browning when stored in the same corditions.

Histological and biochemical studies of the various types of carbon dioxide injuries have been limited. Although Bain (1956) found that browning in Granny Smith apples was associated with cells containing numerous chloroplasts, the browning did not appear to be caused by disorganization of the chloroplasts. The accumulation of succinic acid during controlled atmosphere storage was associated with carbon dioxide injury according to Hulme (1956). He believed that abnormal concentrations of this acid was toxic to the tissues.

Some insight into the biochemistry of the CA effects is given by Allentoff et al. (1954, 1954a) who exposed mature McIntosh apples to $C^{14}O_2$ for 18 hours in darkness. They found that the tissues incorporated $C^{14}$ in the malic, aspartic and glutamic acids, and alanine and serine of the nitrogenous fraction. Low radioactivity was found in sucrose. They found a linear relationship between the rate of carbon dioxide fixation and the concentration of external carbon dioxide. The fixation rate at harvest was concurrent with the respiratory climacteric. Greater variations after storage through December appeared to be due to the higher incidence of breakdown.

Rakitin et al. (1956) stored apples in 8 percent carbon dioxide labeled with radioactive $C^{14}$ at 38° to 46° F. Autoradiograms of apple slices showed that stem, calyx, skin, seed embryo, vascular bundles and cortical tissue contained the largest amount of radioactivity which apparently was due to inspired carbon dioxide from the atmosphere surrounding the fruit.

# MATERIALS AND METHODS

## Storage Arrangement - 1957-58

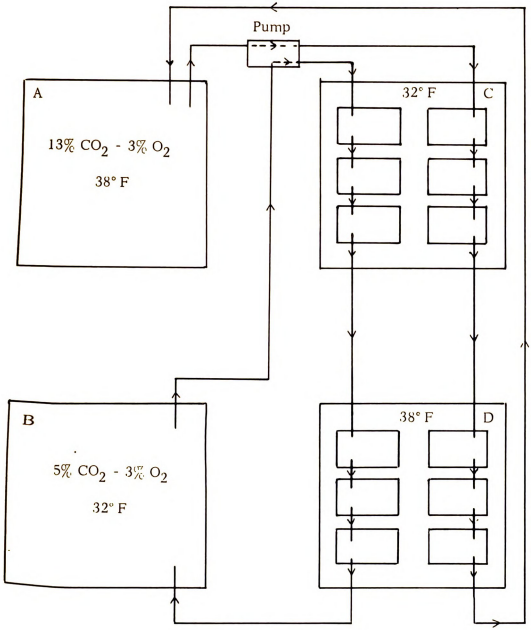
Two gastight, insulated and refrigerated chambers, each containing 38 bushels of Jonathan apples were used as master chambers to establish and maintain the desired atmospheres of 13 percent carbon dioxide - 3 percent oxygen and 5 percent carbon dioxide - 3 percent oxygen. The chamber with 13 percent carbon dioxide was held at 38° F, whereas the 5 percent carbon dioxide atmosphere was held at 32° F. Two refrigerated storage chambers were used to maintain the desired temperature of six two-bushel drums at 32° F, and six at 38° F.

Each atmosphere from the master chamber was circulated through the six two-bushel drums (Figure 1) by a diaphram type pump at the rate of 1.5 cubic feet per minute changing the atmospheres in the drums once every 27 minutes.

The atmospheres in the main chambers were analyzed daily. The atmospheres in the two-bushel drums were analyzed weekly. The oxygen level in the master chambers was adjusted by adding measured amounts of oxygen from compressed gas cylinders. The excess carbon dioxide was removed with a column-type carbon dioxide absorber (Angelini, 1956) using 160 grams of caustic soda (NaOH) per gallon of water as the absorbing solution.

Figure 1

A schematic and flow diagram of the storage facilities used in 1957-58. The atmosphere of the master CA chambers A and B were circulated through the small (2-bushel capacity) chambers in the refrigerated rooms C and D.


Pump
A
13% $CO_2$ - 3% $O_2$
38° F
32° F
C
B
5% $CO_2$ - 3% $O_2$
32° F
38° F
D

Pre-harvest Treatments - 1957-58

Forty-year-old Jonathan trees at the Michigan State University Horticulture Farm were selected as the source of fruit for the 1957-58 studies. A NE, NW, SE or SW quadrant of each tree was selected for each treatment, with a different quadrant of each of the four trees used for a given treatment.

1. Defoliation. Branches bearing representative fruit crops with leaf/fruit ratios of 30 to 50 leaves per fruit were completely defoliated back to the main scaffold approximately two months before harvest. Leaves were removed from enough branches of each tree to provide approximately one bushel of fruit from the defoliated area at harvest.

2. Ringing. Approximately two months before harvest, enough two to three-inch diameter limbs to provide one bushel of fruit per tree were ringed by removing a 1/4 inch strip of bark approximately one foot from the scaffold branches. The grooved area was scraped to insure that the cambium tissue was completely destroyed. The leaf/fruit ratio of the ringed branches was 40 to 50 leaves per fruit.

3. Thinning. Limbs bearing abnormally heavy crops (i.e., 3 to 5 apples per six inches of limb) were selected for the thinning treatments. Two months before harvest, fruits were removed so as to leave one apple for every six linear inches of the limb. The thinning increased the leaf/fruit ratio from 10 to 15 to approximately 40 to 60 leaves per fruit. One bushel from each of the four trees was harvested from the thinned areas.

4. Excess Crop. Limbs bearing a heavy crop in the same quadrants of the trees were used for the thinning treatments. The fruit was allowed to mature for harvest. These limbs bore three to five fruits for each six linear inches of the limb and the leaf/fruit ratio was 10 to 15 leaves per fruit.

5. Plastic Bag Fruit Covering. Approximately two weeks before harvest, five fruits from each tree were covered with white plastic film and five were covered with black plastic film. Thermocouples were inserted through the calyx end of the fruit into the seed cavity to measure the temperature. One copper-constantan thermocouple was placed in each of the two types of bags, one in a check fruit and one in air on each of the four trees. The temperatures were recorded at early morning and mid-day.

6. Checks. The fruit produced in the remaining quadrant of each tree served as control for all treatments. These fruits were stored in regular storage at 32 to 34° F in the Horticulture Building.

Time of Harvest Studies - 1957-58

Different stages of maturity were obtained by harvesting fruit September 20 and 25, and October 1 and 5. The optimum date for commercial harvest for storage purposes was estimated to occur about October 1, according to past history of harvest dates in this orchard and the condition of the fruit. Two bushels were harvested from each of the four trees on each date from the check quadrant of the trees used for the preharvest studies. The fruit

of each harvest was composited and randomized into five one-bushel samples. One bushel was stored in 5 percent carbon dioxide and 3 percent oxygen at 32° F, 5 percent carbon dioxide and 3 percent oxygen at 38° F, 13 percent carbon dioxide and 3 percent oxygen at 32° F, 13 percent carbon dioxide and 3 percent oxygen at 38° F, and regular storage at 32° F. The early harvested fruits were stored temporarily in sealed 55-gallon drums at the desired atmosphere and temperature until all harvests were completed. The atmospheres were artificially established and adjusted daily until the "master chambers" had established the desired atmospheres. When this atmosphere had been attained, the fruit was transferred to specially constructed two-bushel 15 x 17.5 x 40 inch drums according to the arrangement shown in Figure 1.

The temperature in each of the two master chambers was recorded daily from copper-constantan thermocouples located in front of the evaporator, one behind the evaporator and one in the fruit.

The air temperature within the drums was checked at intervals of one to two weeks with a thermocouple inserted to the center of the drum.

Comparable fruit samples were held in regular storage in small walk-in rooms regulated to a temperature of 32 to 34° F.

Storage Arrangement - 1958-59

The 38-bushel CA chamber holding the sample fruits from the defoliated and non-defoliated areas at 13 percent carbon dioxide - 3 percent oxygen at

32° F was used as a master chamber to establish the atmosphere for the time of harvest studies.

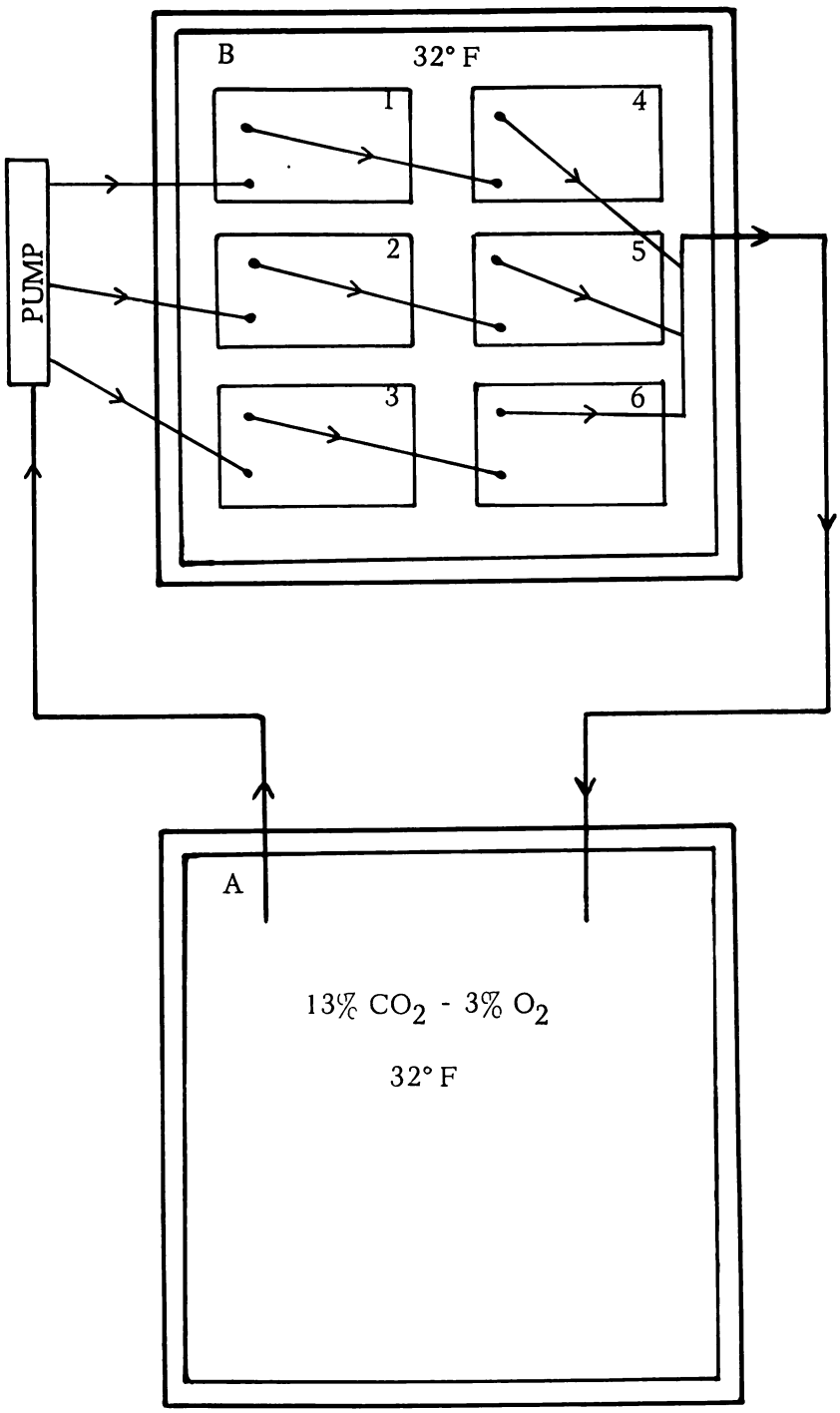
Six two-bushel drums (15 x 17. 5 x 40 inches) were maintained at 32° F in a small refrigerated storage. The atmosphere in the master chamber was circulated by a diaphragm type pump through plastic and rubber hose (1/4 inch inside diameter) to the six two-bushel drum, as shown in Figure 2. Each of the three diaphragm pumps supplied two drums at the rate of 0. 5 cubic foot per minute so as to change the atmosphere of each drum once every 25 minutes. The atmosphere of the master chamber was analyzed daily and maintained as described for the 1957-58 storage arrangement. The atmospheres in the two-bushel drums were analyzed weekly.

The temperatures of the master chamber and the six two-bushel drums were recorded daily with a recording potentiometer and copper-constantan thermocouples placed in the center of each drum, in front of the evaporator, behind the evaporator and in a fruit at the center of the stack of the master chamber.

The sample lots stored in 5 percent carbon dioxide with 3 percent oxygen were placed near a removable window of the metal door of the tilt-up storage building (Pflug et al., 1957). The window was constructed to allow quick removal of samples through the storage season, without serious disruption of the atmosphere of the storage room.

Figure 2

A schematic and flow diagram of the storage arrangements for 1958-59. The atmosphere of the master chamber A was circulated through the small (two-bushel capacity) chambers in the refrigerated chamber B.


B
32° F
1
4
2
5
3
6
PUMP
A
13% $CO_2$ - 3% $O_2$
32° F

The excess carbon dioxide was removed with a caustic soda absorber most of the season and by a water absorber during the final six weeks of storage. The oxygen level was adjusted by the addition of outside air from a pump and by metered amounts of nitrogen from a compressed gas cylinder.

Temperatures in the tilt-up storage buildings were recorded daily from six copper-constantan thermocouples in the fruit and two in the air which were connected to a recording potentiometer.

Defoliation Study - 1958-59

Defoliation studies in 1958 were made in commercial orchards located approximately five miles southwest of Sparta, Michigan. The orchards were selected for their similarity of climate and soil conditions and nutritional status so as to provide fruit of similar stages of maturity at a single harvest date.

Four of the five trees used in previous studies (Eunemann, 1958) were selected for uniformity of appearance and size of crop. The age of the trees ranged from 14 to 22 years. The orchard soil types in the area were silt loam and silty loam soil.

Two months before the estimated time of harvest, limbs in successive quadrants of each tree were completely defoliated. Three bushels of fruit from the non-defoliated and the defoliated areas of each tree were harvested October 5. The three bushels were composited and randomized into three

one-bushel samples; one was stored in 13 percent carbon dioxide and 3 percent oxygen, one in 5 percent carbon dioxide and 3 percent oxygen at 32° F, and one in regular storage at 32 to 33° F for approximately seven months. The fruit held in 5 percent carbon dioxide and 3 percent oxygen was stored in a 650-bushel capacity storage building (Pflug et al., 1957). The fruit stored in 13 percent carbon dioxide and 3 percent oxygen was held in a 38-bushel experimental controlled atmosphere storage in the Agriculture Engineering Building. The fruit held in regular storage was held in a walk-in storage in the Horticulture Building.

Time of Harvest Studies - 1958-59

The time of harvest study conducted in 1957 was repeated in 1958 using fruit of the same four trees. Two bushels were harvested from different quadrants of each tree, on September 20, September 30 and October 14. Each tree's fruit was composited at random into three two-bushel samples for placement in 13 percent carbon dioxide and 3 percent oxygen, 5 percent carbon dioxide and 3 percent oxygen, and regular storage at 32° F. The early harvested fruit was handled similarly to that in 1957-58. The fruit stored in 13 percent carbon dioxide and 3 percent oxygen was placed in a storage according to the arrangement shown in Figure 2. The fruit stored in 5 percent carbon dioxide and 3 percent oxygen was placed in the tilt-up CA storage building.

Sampling Size

Thirty fruits were taken at random for evaluation of the effects of time of harvest, ringing, thinning, excess crop and defoliation treatments at harvest and after 33, 90, 125, 176 and 211 days of storage. Fruit covered with plastic bags were observed at harvest and after 210 days in regular and controlled atmosphere storage. In 1958-59, samples of each time of harvest were observed at harvest and from all storage treatments after 76, 120, 156, 176 and 210 days in storage.

A 50-fruit sample was selected at harvest time from the defoliated and non-defoliated areas of each tree of the five locations for observations of fruit firmness, soluble solids, ground color and internal and external defects. Thirty-fruit samples were used for all observations after 210 days in regular and CA storage treatments.

Fruit Quality and Condition

The quality and condition of the fruit were evaluated both years, as follows:

Flesh firmness: Flesh firmness was determined with a Magness-Taylor pressure tester (Haller, 1941) equipped with a 7/16 plunger and applied at the pared surfaces of the blushed and non-blushed cheeks of each fruit. All readings were made by the author.

Ground color: The ground color was rated numerically by com-

parison with the McIntosh Color Chart (Southwick and Hurd, 1948) which ranges from a yellow color, with a rating of 1, to dark green color, having a rating of 5. Fruits with the entire surface red or striped red so as to mask the ground color were not measured.

Soluble solids: The juice pressed from the fruit during pressure testing was tested for soluble solids with a Zeiss-Opton hand refractometer calibrated in percent sugars.

Taste and texture: The fruit of all treatments were tasted and rated for flavor as highly acid, acid, sweet acid, sweet, lacking and alcoholic, and for texture as firm, crisp, melting and mealy. Fifteen fruits were tested to obtain an average judgment for each sample. Off-flavors were noted separately. All samples were evaluated by the author.

Exterior or skin blemishes: Disorders that had developed during the various storage treatments were observed and recorded from individual fruit inspections.

Interior disorders: Each apple tested for flesh firmness was cut in half laterally at least twice and examined for internal disorders that had developed during storage.

## Statistical Methods

The statistical methods employed to evaluate the results of the defoliation study of 1958, were in accordance with the methods prescribed by

Cochran and Cox (1950) and Snedecor (1946) for the split plot technique and correlation coefficient.

The results of evaluation of watercore, voids, core browning and breakdown were calculated on the basis of the percent fruit free of the disorder and these values were converted to the arc sine for further statistical computations. The actual values of percent soluble solids and pounds pressure of flesh firmness were used in all statistical comparisons.

Due to the limitations of the storage facilities, it was impossible to replicate within storage treatments. Fruit from each treatment (defoliated and non-defoliated) of each tree from each grower were kept as separate samples. They were placed in storage so that the fruit from the defoliated and non-defoliated limbs of each tree were in close proximity and therefore, used as replications within each storage treatment.

Microtechniques

Tissue from an apple of each tree of the five growers was placed into CRAF[1/] solution two days after harvest for comparison with the tissue of fruit stored in regular storage, 5 percent carbon dioxide and 3 percent oxygen and 13 percent carbon dioxide and 3 percent oxygen at 32° F for seven months.

1/ Solution A - chromic acid 1 g; glacial acetic acid 7 cc; distilled water 92 cc. Solution B - Formalin 30 cc; distilled water 70 cc.

A 1 x 1 cm sample of radial tissue was cut, impregnated in vacuo, and then fixed for 24 hours. The material was then transferred to 70 percent alcohol for holding until all materials were available for examination.

The dehydration, infiltration and embedding methods of Johansen (1940) were followed.

The sections were cut with a Spencer Rotary Microtome at 12 to 15 microns thickness, then prepared for staining as set forth by Johansen (1940). The Safranin and Fast Green staining procedure of Cross (1937) was used. The slides were mounted in piccarite and allowed to dry thoroughly before examination.

Samples of fruit from all storage conditions plus sections of tissues showing the various types of internal and external injury were prepared.

The areas of tissues which showed typical types of CA injury were examined and photographed. Both hand sections and sections made by using the freezing microtome technique were mounted (Sass, 1940; Johansen, 1940) for further intracellular inspections.

Isotopic Procedures with $C^{14}O_2$

The radioisotope of carbon ($C^{14}$) was used as $C^{14}O_2$ to determine the rates of carbon dioxide evolution upon removal from storage and carbon dioxide distribution in apple tissue during controlled atmosphere conditions.

Jonathan apples harvested from the university orchard on October 1, 1957 and September 30, 1958 stored in regular storage and controlled atmospheres

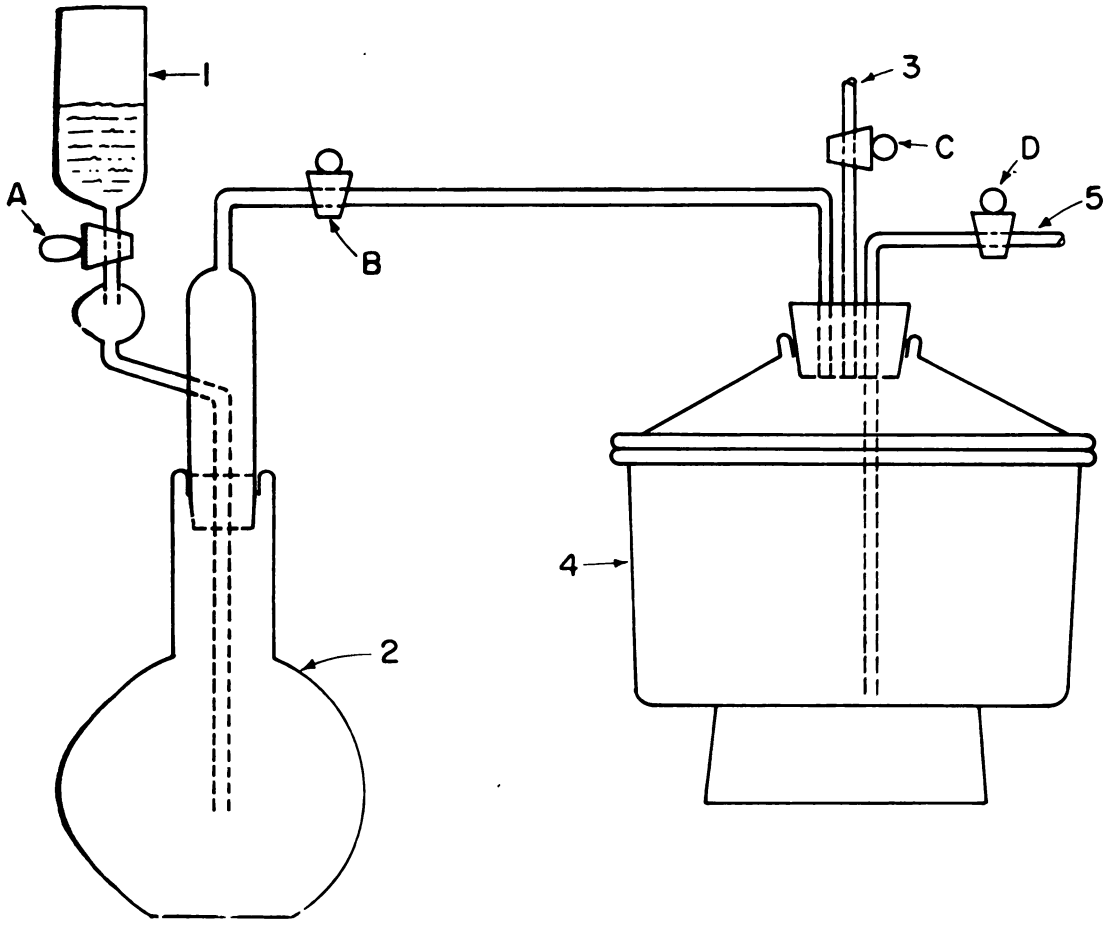
of 13 percent carbon dioxide - 3 percent oxygen, 2 1/2 percent carbon dioxide - 3 percent oxygen, and 5 percent carbon dioxide - 3 percent oxygen at 32° F and which were free from blemishes, 2 1/2 to 3 inches in diameter and graded U. S. Fancy were utilized in these studies.

Methods of Exposure to $C^{14}O_2$ - Procedure 1

A volume of fruit equal to approximately two liters (usually 12 fruits) was placed in a six-liter vacuum desiccator with a gas-tight seal. A weighed sample of $BaCO_2$ and $BaC^{14}O_2$, precalculated to produce the desired carbon dioxide concentration (see Appendix Table XVI) in the volume of free atmosphere in the desiccator, was placed in a dry reaction flask and connected to the desiccator containing the fruit. The apparatus was prepared by opening all stopcocks with the exception of "A" (See Figure 3). The outlet from stopcock "D" was then connected to a water aspirator and the maximum vacuum obtained, then "D" was turned to the closed position. The system was charged by adding an excess amount of 10 percent $H_3PO_4$ through "1" which was allowed to react with the $BaCO_3$ and $BaC^{14}O_2$ in the reaction flask "2" until the reaction had gone to completion. Water was added through the graduated burette into the reaction flask to sweep the $CO_2$ and $C^{14}O_2$ remaining in the reaction flask into the vacuum desiccator. A liquid was kept in the burette at all times to prevent the outside atmosphere from being swept into the system. A measured amount of oxygen was added to the desiccator through the aspirator tube (5)

Figure 3

A schematic diagram of the apparatus used for exposing apples to controlled atmospheres containing $C^{14}O_2$. A graduated burette "1" was used to add acid to the reaction flask "2", and also to add water to sweep the remaining $C^{14}O_2$ into the vacuum desiccator "4". Tube "3" was used as a connection to a mercury manometer. Connection to the water aspirator for drawing a vacuum in the system was made at "5".


1
A
B
2
3
C
D
5
4

to attain the desired oxygen level. Nitrogen was added to equalize the pressure and dilute the atmosphere when necessary. The atmosphere was analyzed daily with an Orsat analyzer.

The atmosphere was adjusted daily by adding a measured amount of water to the desiccator to displace a calculated amount of atmosphere. Measured amounts of oxygen and nitrogen were added to replace the water and adjust the atmosphere in the desiccator. Additional $C^{14}O_2$ was supplied as necessary according to Procedure 2, as described below.

Although this method was satisfactory for exposure of fruit to radioactive carbon dioxide, the precalculated atmosphere was not always attained. Complete evacuation of the system was impractical as there was a possibility of damage to the fruit tissues; therefore, it was difficult to precalculate the exact volume of normal air remaining in the system.

Procedure 2

The same apparatus was used as in Procedure 1. All stopcocks were in the open position and a water source was connected to tube "5" (see Figure 3). The manometer was disconnected from tube "3". Water filled the vacuum desiccator containing the fruit sample until it reached stopcock "C" and "B"; all stopcocks were then closed and the water source turned off. The manometer was reconnected and tubing was connected to point "5" to act as a siphon. Stopcocks "D", "C" and "B" were opened and some water allowed to

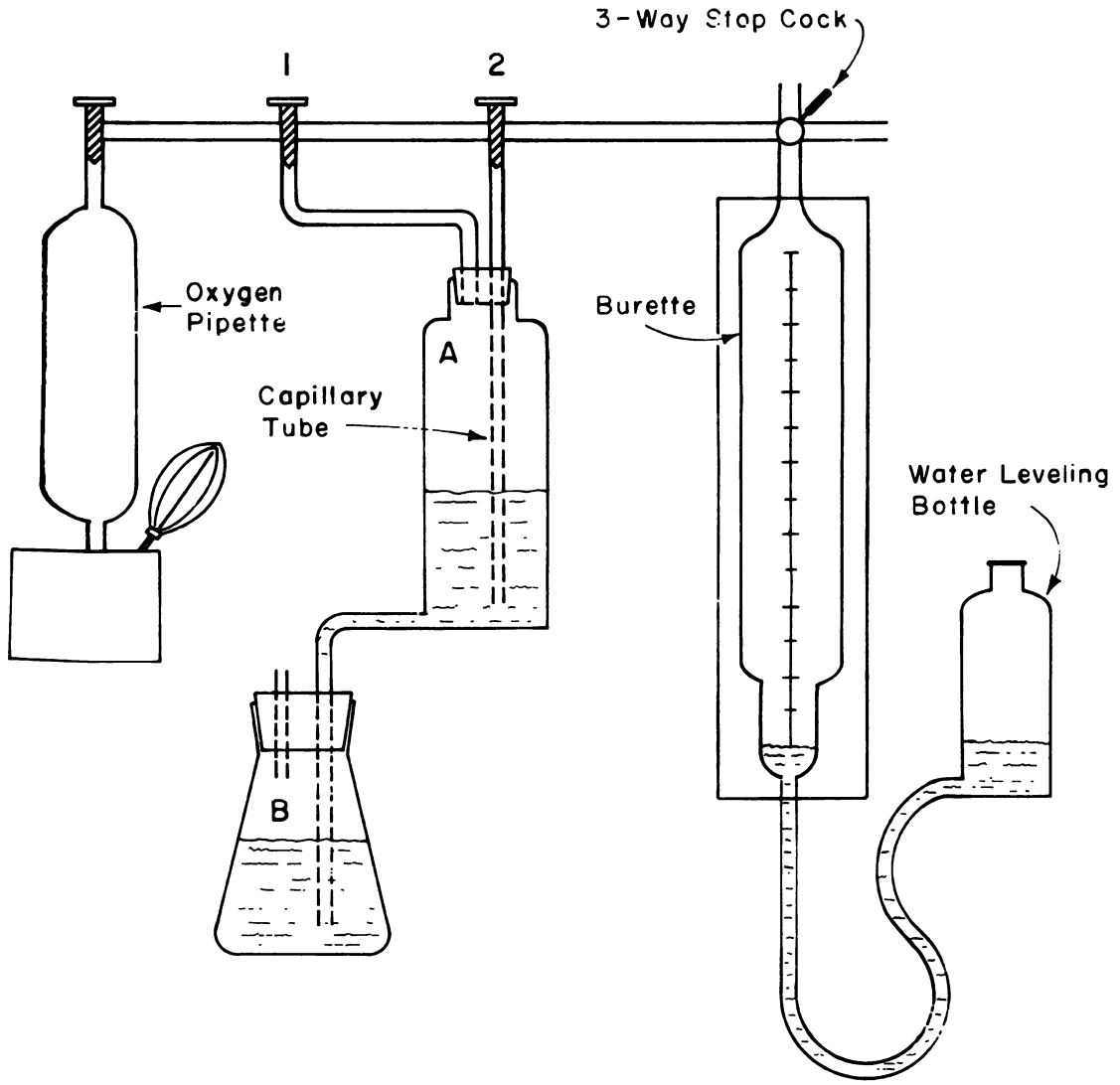
siphon off to build up a slight vacuum. Stopcock "C" was opened to draw all water out of the tube "3". A precalculated volume of 10 percent $H_3PO_4$ was added to an amount of $BaCO_3$ and $BaC^{14}O_3$ to give the desired level of labeled carbon dioxide. The carbon dioxide remaining in the reaction flask was swept out by adding water. The vacuum in the desiccator was controlled by stopcock "D". By measuring the volume of water siphoned off and knowing the volume of the reaction flask, exact levels of carbon dioxide and oxygen could be obtained.

An Orsat analyzer was modified, as shown in Figure 4, so that the amount of radioactivity of the atmosphere, as well as the percent carbon dioxide, could be determined. The carbon dioxide-containing atmosphere was forced from the burette through stopcock "2" into the capillary tube which provided a means of bubbling the atmosphere into the $CO_2$-absorbing solution contained in bottle "A". Bottle "A" was so constructed that as the atmosphere was bubbled in the solution could be forced out through the bottom into bottle "B". By closing stopcock "2" and opening stopcock "1" and lowering of the leveling bottle, the atmosphere was drawn out and replaced by the absorbing solution. Once the analysis was complete, all stopcocks were opened and the solution drained into bottle "B". Immediate analysis for the amount of radioactivity could then be made.

Upon completion of the experiment, water was siphoned into the desiccator to sweep the carbon dioxide atmosphere containing $C^{14}$ into two Fisher-

Figure 4

Modification of an Orsat analyzer to enable collection of the $C0_2$ absorbing solution containing $C^{14}0_2$. By filling the sampling line with water prior to connecting to desiccator, no dilution loss of sample was obtained. When flushing sample out of burette valve "2" is open, valve "1" closed. Upon returning the sample to the burette valve "2" is closed, valve "1" opened. Absorbing solution containing $C^{14}0_2$ is obtained by draining from container "A" into container "B".


3-Way Stop Cock
1
2
Oxygen Pipette
Capillary Tube
A
B
Burette
Water Leveling Bottle

Milligan gas washers in series attached to tube "3". The gas washers contained 250 ml of 0.1N NaOH.

Plating and Measurement of Radioactivity

Several procedures were used for plating and counting the radioactive samples utilizing the precipitated form of barium carbonate. Aronoff (1956) completely describes a technique using a glass filtering assembly which was found to be satisfactory, if comparatively large amounts of $BaC0_3$ and $BaC^{14}0_3$ precipitate were collected. When small amounts of $BaC0_3$ were plated, it was found that the $BaC0_3$ and $BaC^{14}0_3$ precipitate would pass through Watman No. 42 filter paper. This procedure was used for all 1957 and 1958 determinations.

In 1959, a slurry of $BaC0_3$ and $BaC^{14}0_3$ and 95 percent alcohol on a 2.5 cm diameter by 0.5 cm in depth stainless steel planchet was plated (Calvin et al. 1949) with this method repeatable results were easily obtained. The radioactive samples were counted by a Chicago Nuclear Model 47 gas flow counter[1/] with micro-mil window, and a Model 17 Scaling Units[1/] was used to record the number of particles detected by the gas flow counter.

Distribution of $C^{14}0_2$ in Peel, Core and Flesh

Eight Jonathan apples were removed for 2 1/2 percent carbon dioxide - 3 percent oxygen after five months and exposed by Procedure 1 (above) to 50

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[1/] Nuclear Instruments and Chemical Corporation, 223 West Avenue, Chicago 10, Illinois.

percent carbon dioxide labeled with (500 microcuries) of $C^{14}$ and 10 percent oxygen for 14 days at 75° F. After removal of the labeled carbon$^{14}$ dioxide normal air was passed through the desiccator at the rate of 210 cc per minute. The radioactive carbon dioxide given off by the fruit was collected in 0. 1N NaOH in 500 ml fritted gas washer. Periodic 10 ml samples were taken and measured for radioactivity as described.

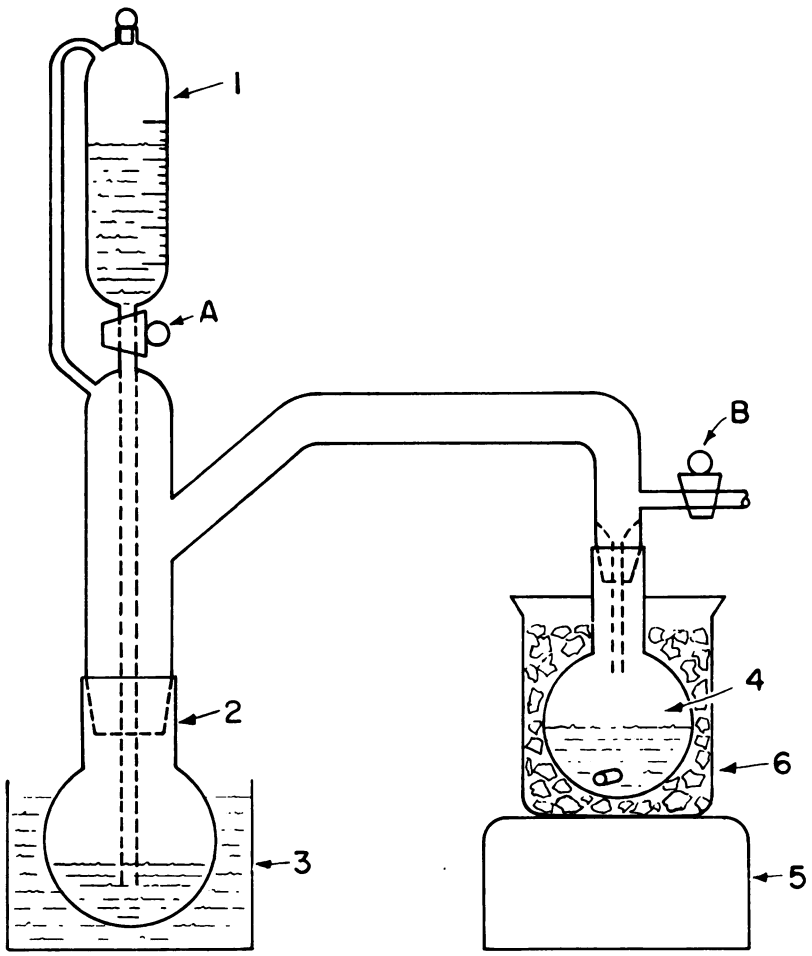
The remaining four fruits were peeled and cored mechanically. Peel, core and flesh were blended separately in Waring Blenders with 250 ml of 0. 1N NaOH for five minutes. These solutions were stored at 32° F for future processing.

This experiment was repeated using four Jonathan apples removed from 2 1/2 percent carbon dioxide - 3 percent oxygen after 5 1/2 months and exposed to 50 percent carbon dioxide containing 457 microcuries of $C^{14}$, and 10 percent oxygen for 14 days at 75° F. The procedure for handling the fruit was the same as above after the mechanical peeler had been adjusted so less of the flesh was included with the peel.

The blended solutions were reacted with 10 percent $H_3PO_4$ solution to release the carbon dioxide containing $C^{14}$ in the blended solution in the apparatus shown in Figure 5. The released $C^{14}O_2$ was collected in 50 mls of 0. 2N KOH. One milliliter samples were plated and counted, as previously described.

Figure 5

Reaction apparatus for measuring $C^{14}$ content of blended radioactive tissues. The blended solution was put into flask "2". 100 ml of 0.2N KOH was put in flask "4" with magnetic stirring rod. Beaker "6" was filled with ice water and beaker "3" was heated slowly by a Bunson burner. The graduate burette "1", with stopcock "A" in the off position, was filled with 11 percent phosphoric acid. All joints were sealed with stopcock grease and vacuum taken at "B". When blended solution in "2" started to boil, "B" was turned off and the phosphoric acid was added slowly. The magnetic stirrer "5" was started and the reaction allowed to continue for three hours.


1
A
B
2
3
4
6
5

## $CO_2$ Evolution Studies

After approximately six months in storage, eight McIntosh apples from 5 percent carbon dioxide - 3 percent oxygen at 38° F and regular storage at 32° F, and eight Jonathan apples from 2 1/2 percent carbon dioxide - 3 percent oxygen at 32° F and regular storage at 32° F were treated in a sealed 12-liter glass jar. The atmosphere was adjusted to 25 percent carbon dioxide and 10 percent oxygen using 10.4 milligrams of $BaC^{14}O_3$ to produce 433.3 microcuries of $C^{14}$ as described by Procedure 2 above. After 17 days of treatment the radioactive atmosphere was displaced with water and collected in fritted gas washers containing 500 ml of 0.1N NaOH.

The fruit samples were separated by variety and storage treatment and placed into separate 5-gallon jars and resealed. Air at the rate of 200 to 300 ml per minute was passed over the fruit and into a fritted gas washer containing 500 ml of 0.2N NaOH. Ten ml aliquots were removed after 1/3, 1, 1.5, 2.5, 9.25, 15, 24 and 39 hours. One milliliter samples were plated and dried for counting.

Four Jonathan apples removed from 2 1/2 percent carbon dioxide and 3 percent oxygen and regular storage at 32° F after six and one-half months storage and placed in 25 percent carbon dioxide containing 260 microcuries and 10 percent oxygen for 17 days, were also evaluated.

In another test, eight Jonathan apples stored four months in 13 percent

carbon dioxide - 3 percent oxygen at 32° F were placed in a two 4-liter container and sealed. The atmosphere in the container was adjusted to 25 percent carbon dioxide - 10 percent oxygen labeled with 166 microcuries of $C^{14}O_2$ (Procedure 2).

After seven days at 75° C for one jar and 32° C for the other jar the radioactive atmospheres were removed and collected in a fritted gas washer containing 0. 2N KOH. Two apples from each jar were left in the container at 75° F and the container resealed. An air flow of 200 to 300 cc of air per minute was passed over the fruit and washed with 500 ml of 0. 2N KOH. One ml samples were taken after 1, 3, 17, 23, 29 and 53 hours for radioactivity measurements.

Four Jonathan apples, removed from 13 percent carbon dioxide with 3 percent oxygen, 5 percent carbon dioxide with 3 percent oxygen and regular storage at 32° F after five months, were exposed to 25 percent carbon dioxide containing 166 microcuries of $C^{14}$, and 10 percent oxygen. After seven days of treatment the radioactive atmosphere was removed and the evolution of $C^{14}O_2$ from the three storage treatments measured at 1, 2, 6, 12, 20, 24 and 53 hours as previously described.

$CO_2$ Distribution in Fruit - Blotting Paper Technique

A 3/8 inch thick median cross sectional slice was removed from two fruits of each jar from the previous experiment. The slice was placed immediately between two pieces of blotting paper saturated with 0. 1N BaOH and placed in a

Buchner funnel. A slight suction was applied for five minutes. A stopper was placed over the seed cavity to prevent loss of suction. The blotting paper and fruit slices were separated and each placed between two five-pound metal plates. The plates containing the samples were dried in an oven at 150° F.

The dried samples were removed and marked off into 1/4 inch square grids and each grid was counted directly for activity.

$CO_2$ Distribution - Filter Paper Technique

Sample fruits were removed from 13 percent carbon dioxide - 3 percent oxygen after five months. Three fruits were sealed into each of 14 one-gallon containers in which the atmosphere was adjusted to 20 percent carbon dioxide and 10 percent oxygen using 2.9 milligrams of $BaC^{14}0_2$ as the source to provide 229.5 microcuries of $C^{14}$ labeled carbon. Single containers placed at 32° F and at 75° F for 1, 4, 24, 168 and 384 hours. After the desired exposure period, the fruit was removed from storage and the radioactive atmosphere flushed and absorbed as before.

Three cross sectional approximate median slices were taken from each fruit. One slice was immediately blended in a Waring Blender with 100 ml of 0.2N KOH for three minutes. After each slice the cut surfaces were placed on a 0.1N barium hydroxide soaked No. 2 Whatman filter paper. The filter paper and fruits were held while the next slice was taken. The cut surfaces of the slice and apple were immediately placed on No. 2 filter paper saturated in 0.1N

BaOH. The fruit slice between the two pieces of filter paper was then put in a Buchner funnel and a slight suction applied for three to five minutes. The filter paper and fruit slices were separated, placed between two pieces of botanical blotting paper and dried at 147° C under sufficient weight to ensure filter paper and fruit would be flat when dry.

The blended solution was placed in the reaction apparatus (Figure 5) and the evolved carbon dioxide collected in 100 ml of 0.2N KOH.

One set of dried filter paper and fruit slice samples were attached to botanical mounting boards (8 x 10 inches) and covered with Saran film. The mounted samples were placed in a light-tight box in contact with 8 x 10 inches Kodak Medical X-ray film, each sample being separated by 8 x 10 inches plastic board separators. Pressure was applied to the stack in the light-tight box to keep the film and mounted samples in close contact.

After 73 days, the film was removed and developed with Kodak developer and fixer. The development time was varied to bring out the grain in the autographs with a minimum of background fog. The developed film was washed for two hours in tap water and then dried at room temperature.

The second set of dried filter paper and fruit slices were treated with 10 percent phosphoric acid for two minutes and dried at 147° C. The samples were divided into 1/4 inch square grids and counted for radioactivity.

The filter paper method was considered a better technique than the blotting paper technique. Diffusion of the $BaC^{14}O_3$ away from the point of absorption was reduced to a minimum by this method.

# RESULTS

The data accumulated from the experiments for the two years in which these studies were conducted are tabulated in Appendix Tables I to XV. Portions of these data are presented in the text under the appropriate headings related to the storage disorders and their development.

## Core Browning

Core browning was characterized by various degrees of brown discoloration in some or all cells in the pith and occasionally in the cortical tissue. The injured tissue varied from light to dark brown in color and from dry and flaky to moist in texture depending on the severity of the injury (Figures 6 and 7). Although core browning development varied from fruit to fruit, it generally radiated from the vascular bundles and occurred around the cambial line in the pith tissue rather than in the tissue between the seed cavities (Figures 8 and 9).

Microscopic observations showed the presence of particles in the vacuole-like sacs of the browned tissue (Figure 5, A and B). These sacs contained a brown fluid surrounding the particles which was responsible for the brown color of the tissue. Normal appearing cells without brown discoloration of the vacuole-like sacs were also present in the browned tissue (Figure 10, A).

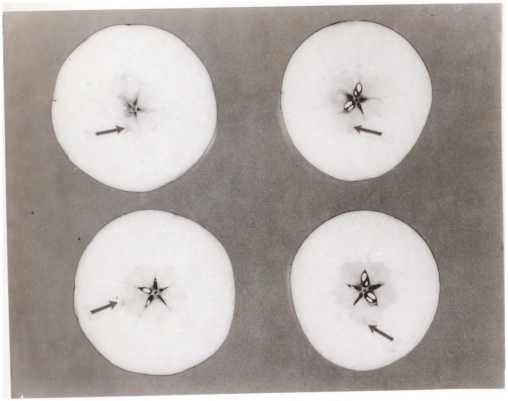
Data showing the effect of storage temperature upon the development of

Figure 6

Core browning of Jonathan apple fruit radiating from the primary vascular bundles (see arrows) into the pith tissue.

Figure 7

Severe core browning in which the affected pith tissues appeared dry and flaky in texture.



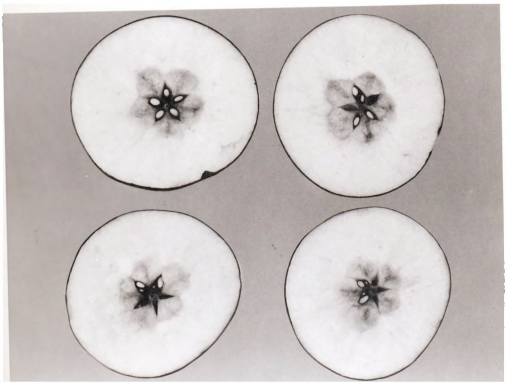


Figure 8

The cross sectional drawings of fruit A through D, the shaded portions represent the areas developing core browning during controlled atmosphere storage. Drawing D also depicts a void or air pocket present in the browned tissue.

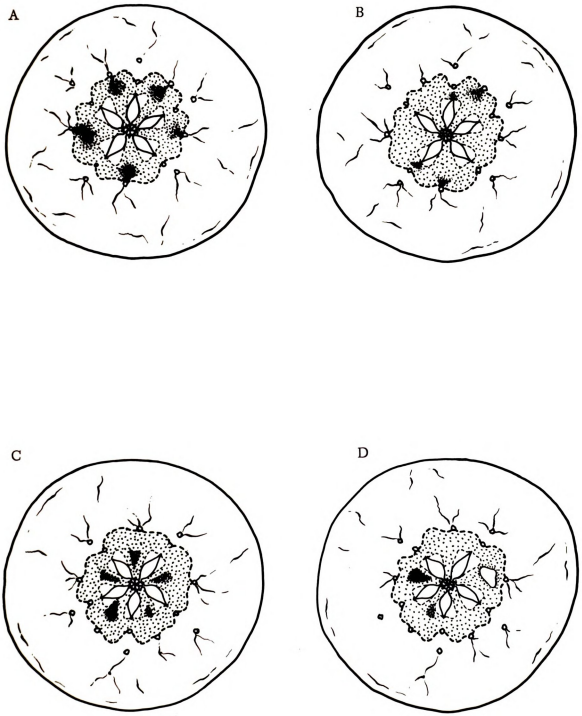
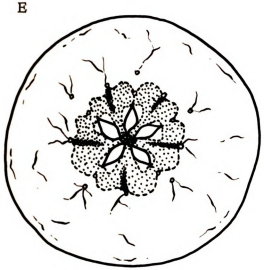

A
B
C
D

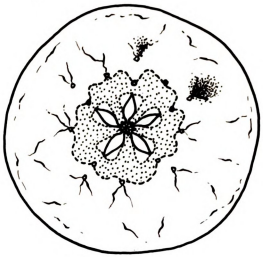
Figure 9

The shaded areas of the apple cross-sections E through H represent the regions affected by core browning. Usually only the pith tissues were involved, but occasionally core browning originated at the vascular bundles in the cortical tissue as in drawing F.

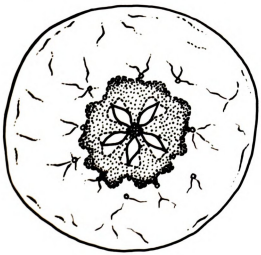
E



F



G



H

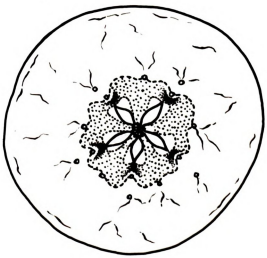
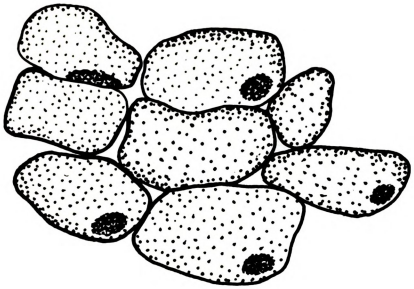


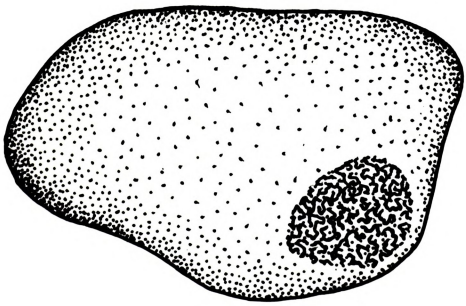
Figure 10

These drawings depict cells from the browned pith tissues of the fruit. The shaded areas within the cells represent vacuole-like sacs containing particles. Drawing A (approximately 30X) shows that not all cells in the affected area have these sacs. Some of these sacs contained a brown fluid surrounding the particles which was responsible for the brown color of the tissue. Drawing B (approximately 60X) is a single cell from the group of cells in A, showing the shape and position of the vacuole-like sacs within a cell.

A



B



core browning are summarized in Table 1. These data are the average percentage of fruit with core browning upon removal from controlled atmosphere storage at 32 and 38° F. Some core browning appeared in fruit held at 38° F, although 32° F was considerably more favorable for its development in controlled atmospheres.

Table 1

The Effect of Storage Temperature on the Development of Core Browning in Controlled Atmospheres

| | Percent Fruit with Core Browning | |
|---|---|---|
| | 32° F | 38° F |
| Experiment 1, Appendix I | 74.3 | 32.9 |
| Experiment 3, Appendix III | 60.0 | 5.0 |
| Average | 67.2 | 19.0 |

The effects of storage atmospheres upon core browning for the experiments from which comparisons are available are summarized in Table 2. The average values for storage at 32° F show the atmosphere of 13 percent carbon dioxide and 3 percent oxygen (51.8 percent) was more favorable for the development of this disorder than 5 percent carbon dioxide with 3 percent oxygen (49.0 percent) and both atmospheres yielded more core browning than storage in normal air (24.4 percent). The fruit in 1957 was considerably

Table 2

The Effect of Storage Atmospheres on the Development of Core Browning

| Experiment No. | See Appendix Table No. | Treatment | Year | Temperature (°F) | Air (%) | Storage Atmospheres 5% $CO_2$ 3% $CO_2$ (%) | Storage Atmospheres 13% $CO_2$ 13% $O_2$ (%) |
|---|---|---|---|---|---|---|---|
| 1 | I | Time of harvest | 1957 | | 80.5 | 66.0 | 82.5 |
| 3 | III | 10 days delay at 55° F in air | 1957 | | 0.0 | 40.0 | 80.0 |
| 4 | III | Ringing | 1957 | 32 | 41.5 | 90.0 | 47.0 |
| 2 | II | Time of harvest | 1958 | | 0.0 | 31.3 | 53.5 |
| | | Defoliation | 1958 | | 0.0 | 12.7 | 26.4 |
| Average | | | | | 24.4 | 48.0 | 57.8 |
| 1 | I | Time of harvest | 1957 | 38 | - | 24.7 | 41.0 |
| 3 | III | 10 days delay at 55° F in air | 1957 | | - | 0.0 | 10.0 |
| Average | | | | | - | 12.3 | 25.5 |

more susceptible to core browning than apples harvested in 1958. In the latter year, large amounts of core browning occurred in regular storage in two of these experiments, whereas none appeared in 1958.

The occurrence of core browning in some of the individual experiments varies notably from the average for storage treatments. For instance, fruit from ringed limbs showed more core browning when stored at 5 percent carbon dioxide and 3 percent oxygen (90 percent) than when stored in either air (41.5 percent) or 13 percent carbon dioxide and 3 percent oxygen (47 percent).

The controlled atmosphere with the highest carbon dioxide level (13 percent) produced more core browning than the atmosphere containing 5 percent carbon dioxide at a storage temperature of 38° F.

The defoliation studies of 1958 (Appendix Table IV) enabled a statistical comparison of core browning in relation to storage atmospheres at 32° F. Significantly less core browning occurred in apples in regular storage (0.0 percent) whereas those in 5 percent carbon dioxide and 3 percent oxygen had an average of 13.2 percent and those stored in 13 percent carbon dioxide and 3 percent oxygen averaged 26.1 percent. Highly significant interactions of atmospheres with growers and with defoliation were evident.

Time of fruit harvest was of some influence on core browning. According to the average values presented in Table 3, fruit harvested October 1, the date considered to be optimum for commercial harvest in 1957, developed the

Table 3

Mean Percentages of Jonathan Apples with Core Browning According to Time of Harvest and Subsequent Storage for 210 Days in Controlled Atmosphere Regular Storage

| | | Percent Fruit with Core Browning | | | | | |
|---|---|---|---|---|---|---|---|
| | | | 32° F | | 38° F | | |
| | Time of Harvest | Air | 5% $CO_2$ 3% $O_2$ | 13% $CO_2$ 3% $O_2$ | 5% $CO_2$ 3% $O_2$ | 13% $CO_2$ 3% $O_2$ | Ave. |
| | September 20, 1957 | 100 | 80 | 57 | 43 | 57 | 67.4 |
| Experiment 1 | September 25, 1957 | 80 | 73 | 100 | 20 | 57 | 65.9 |
| (See Appendix Table I) | October 1, 1957[a/] | 57 | 37 | 83 | 20 | 10 | 41.5 |
| | October 5, 1957 | 75 | 74 | 90 | 16 | 40 | 59.0 |
| | September 20, 1958 | 0 | 26 | 33 | - | - | 29.5 |
| Experiment 2 | September 30, 1958[a/] | 0 | 21 | 57 | - | - | 38.8 |
| (See Appendix Table II) | October 14, 1958 | 0 | 47 | 70 | - | - | 58.5 |

[a/] Considered optimum date for commercial harvest

least core browning in storage. This did not, however, hold true for all storage conditions. Namely, fruit picked on October 1 and stored in 13 percent carbon dioxide and 3 percent oxygen at 32° F developed 83 percent core browning, whereas fruit harvested on September 20 developed only 57 percent. Also, fruit harvested on October 5, stored in 5 percent carbon dioxide and 3 percent oxygen at 38° F yielded a lower percentage (16 percent) of core browning than fruit harvested on October 1 and stored under the same conditions (20 percent). The lowest average core browning in 1958-59 was for fruit picked at the earliest harvest date, but again there were exceptions according to the storage method and picking date. Fruit harvested on September 30, for instance, showed a lesser amount (21 percent) of this disorder than fruit harvested on September 20 (26 percent).

None of the prestorage treatments (Table 4) were of consistent effect on core browning development in the first year. Holding the fruit for 10 days at 55° F after harvest prior to placement in storage, prevented this disorder from occurring in regular storage, but gave quantities in controlled atmospheres similar to that of untreated fruit. Defoliation and thinning, on the other hand, increased this disorder in regular storage, but decreased it in controlled atmosphere storage. Defoliation, when tested more thoroughly in the following year, did not affect core browning. There was no significant difference between fruits from branches with and without their leaves removed approximately 60 days before harvest (see Appendix Table IV). The

Table 4

Percent Jonathan Apples with Core Browning. The Fruit Received Various Prestorage Treatments and were Subsequently Stored for 210 Days in Controlled Atmosphere and Regular Storage - 1957-58

| Experiment | See Appendix Table No. | Prestorage Treatment | Percent Fruit with Core Browning | | | | |
|---|---|---|---|---|---|---|---|
| | | | 32° F | | | 38° F | |
| | | | Air | 5% $CO_2$ 3% $O_2$ | 13% $CO_2$ 3% $O_2$ | 5% $CO_2$ 3% $O_2$ | 13% $CO_2$ 3% $O_2$ |
| 3 | III | 10 days delay at 55° F in air | 0.0 | 40.0 | 80.0 | 0.0 | 10.0 |
| 4 | III | Ringing | 41.5 | 90.0 | 47.0 | 0.0 | - |
| 5 | III | Defoliation | 100.0 | 27.0 | - | - | - |
| 6 | III | Thinning | 83.0 | 22.0 | - | - | 6.0 |
| 7 | III | Excess crop | 100.0 | 40.0 | - | - | 2.0 |
| 8 | III | Plastic bags | 0.0 | - | - | - | 0.0 |
| 1 | I | Untreated | 57.0 | 37.0 | 83.0 | 20.0 | 10.0 |

thinning test was not repeated the following year.

Periodic observations of the fruit condition were made during the storage season to determine the time of occurrence of core browning in storage (Table 5). The initial appearance of core browning in the 1957-58 storage season occurred after 90 days in fruit stored in 5 percent carbon dioxide and 3 percent oxygen at 32° F. Core browning first appeared in fruit stored at 32 and 38° F after 125 days. Generally core browning in all treatments for this year became more prevalent as the season progressed.

In 1958-59, the fruit stored in controlled atmospheres showed core browning after 76 days in storage. No core browning appeared in fruit held in regular storage at 32° F.

## Voids

Carbon dioxide injury often was evidenced by the formation of small spheroid air pockets or voids in the pith tissue and occasionally in the cortical tissue of Jonathan apples (Figure 11). These voids varied in size from barely visible (approximate size . 1 cm to 3 cm) to a complete hollowing of the pith tissue between the seed cavities (Figures 12 and 13). The tissues immediately surrounding the pockets were brown in color and spongy in texture and this condition sometimes extended into the surrounding areas, as shown in Figure 12. Compressed layers of cell walls from the collapsed cells, which previously occupied the voided areas, formed around the voids (Figures 14 and 15).

g developed in Controlled Atmosphere and Regular Storage

| | % $CO_2$ | % $O_2$ | Days in Storage 33 | 76 | 90 | 121 | 125 | 156 | 176 | 210 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Percent Fruit with Core Browning | | | | | | | |
| 1957-58 | | | | | | 32° F | | | | |
| | 5 | 3 | 0 | - | 1.5 | - | 4.1 | - | 65.0 | 65.8 |
| | 13 | 3 | 0 | - | 0 | - | 45.7 | - | 80.0 | 85.7 |
| | Air | | 0 | - | 0 | - | 4.1 | - | 53.7 | 78.0 |
| | | | | | | 38° F | | | | |
| | 5 | 3 | 0 | - | 0 | - | 7.6 | - | 13.9 | 24.8 |
| | 13 | 3 | 0 | - | 0 | - | 2.5 | | 30.0 | 33.2 |
| 1958-59 | | | | | | 32° F | | | | |
| | 5 | 3 | - | 16.1 | - | 38.4 | - | 32.6 | 10.8 | 28.8 |
| | 13 | 3 | - | 35.7 | - | 41.9 | - | 44.0 | 42.8 | 53.4 |
| | Air | | - | 0.0 | - | 0.0 | - | 0.0 | 0.0 | 0.0 |

Figure 11

Longitudinal view of a Jonathan apple having small voids in the pith tissue.

Figure 12

A medial cross-section of a Jonathan apple from CA storage with severe voids.

Figure 13

A medial cross-section of a Jonathan apple showing a void in tissue which was also affected with brown heart.

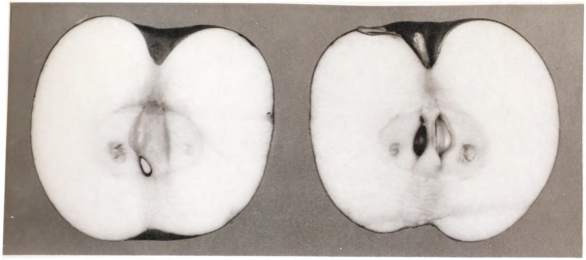
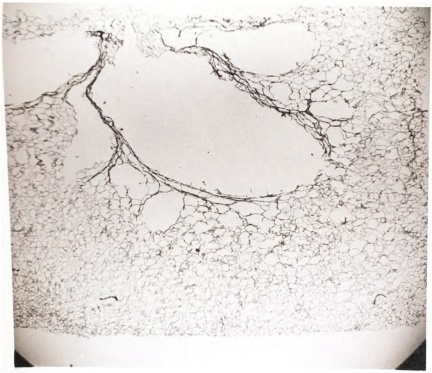


Figure 14

Photomicrographs (X 10) showing voids within the pith tissue of a Jonathan apple. Note the smaller voids formed around the large void in the upper photograph.



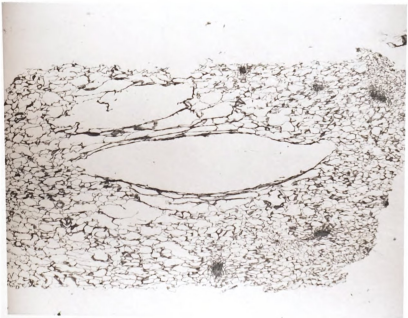


Figure 15

Photomicrograph (X 100) of the cells at one side of a void in the pith tissue of a Jonathan apple. The walls of at least six cells close together at the left side of the photograph formed the side of the void.

apsed cells were free of contents other than an occasional sac
ng plastid-like particles.

effects of storage temperature upon the development of voids in
ed atmosphere storage (Table 6) are shown as the average percen-
ccurring in apples of comparable experiments when stored at 32 and
The storage temperature of 32° F was considerably more favorable
formation of voids than 38° F.

Table 6

ffects of Storage Temperature on the Development of Voids in Fruit Stored in Controlled Atmospheres

| | Percent of Fruit with Voids | |
|---|---|---|
| | 32° F | 38° F |
| iment 1, Appendix I | 43.6 | 5.6 |
| riment 3, Appendix III | 60.0 | 5.0 |
| Average | 51.8 | 5.3 |

Data from comparable experiments are summarized in Table 7 showing
effects of storage atmospheres upon the development of voids. The
es, which are the average percentages of fruit with voids formed in
rage temperatures of 32 and 38° F, consistently show that the storage
nospheres of 13 percent carbon dioxide and 3 percent oxygen were more

| Experiment No. | See Appendix Table No. | Treatment | Year | Temperature (° F) | Storage Atmospheres | | |
|---|---|---|---|---|---|---|---|
| | | | | | Air (%) | 5% $CO_2$ 3% $O_2$ (%) | 13% $CO_2$ 3% $O_2$ (%) |
| 1 | I | Time of harvest | 1957 | 32 | 0.0 | 26.6 | 61.7 |
| 3 | III | 10 days delay at 55° F in air | 1957 | 32 | 0.0 | 40.0 | 80.0 |
| 4 | III | Ringing | 1957 | 32 | 0.0 | 0.0 | 47.7 |
| 2 | II | Time of harvest | 1958 | 32 | 0.0 | 0.3 | 5.2 |
| | | Defoliation | 1958 | 32 | 0.0 | 0.0 | 4.0 |
| Average for 32° F Temperature | | | | | 0.0 | 13.4 | 39.6 |
| 1 | I | Time of harvest | 1957 | 38 | - | 3.0 | 8.2 |
| 3 | III | 10 days delay at 55° F in air | 1957 | 38 | - | 0.0 | 10.0 |
| Average for 38° F Temperature | | | | | | 1.5 | 9.1 |

for the development of voids than 5 percent carbon dioxide with 3
xygen. Voids never develop in fruit stored in normal air at 32° F.
of the 1957 crop was more susceptible to the formation of voids
es harvested in 1958.

atistical comparison of void formation in relation to storage atmos-
t 32° F was made in 1958 for the defoliation experiment; the data of
re shown in Appendix Table V. Apples stored in 5 percent carbon
with 3 percent oxygen and in normal air showed little or no void de-
ent. Voids developed in 3.4 percent of the fruit stored in 13 percent
dioxide with 3 percent oxygen, and this amount was significantly greater
r the other storage atmospheres.

me of harvest was of some influence on the susceptibility of fruit to the
pment of voids in storage. According to the data in Table 8, fruit har-
September 25 in the 1957 storage test developed the least amounts of
in CA storage. The earliest harvested apples (September 20) in 1958
the only fruits which remained free of voids in CA storage. Generally,
uit became more susceptible to the development of voids as it became
mature. Although this occurred for the average of each storage season,
not hold true for all storage conditions. For example, fruit harvested
ctober 1, 1957 showed more voids when held at 13 percent carbon dioxide
°F (25 percent) than fruit harvested on October 5, 1957, and stored
r the same conditions (8 percent).

Mean Percentages of Jonathan Apples with Voids after Storage
for 210 Days in Controlled Atmosphere and Regular Storage

| | Time of Harvest | Percent Fruits with Voids | | | | | |
|---|---|---|---|---|---|---|---|
| | | | 32° F | | 38° F | | |
| | | Air | 5% $CO_2$ 3% $O_2$ | 13% $CO_2$ 3% $O_2$ | 5% $CO_2$ 3% $O_2$ | 13% $CO_2$ 3% $O_2$ | Average |
| Experiment 1 (See Appendix Table I) | September 20, 1957 | 0 | 3 | 33 | 0 | 0 | 7.2 |
| | September 25, 1957 | 0 | 0 | 10 | 0 | 0 | 2.0 |
| | October 1, 1957[a/] | 0 | 30 | 100 | 6 | 25 | 32.5 |
| | October 5, 1957 | 0 | 73.5 | 100 | 6 | 8 | 37.5 |
| Experiment 2 (See Appendix Table II) | September 20, 1958 | 0 | 0 | 0 | - | - | 0.0 |
| | September 30, 1958[a/] | 0 | 0 | 1.7 | - | - | 0.6 |
| | October 14, 1958 | 0 | 0.8 | 14.1 | - | - | 3.0 |

[a/] Considered optimum date for commercial harvest.

he prestorage treatments listed in Table 9 showed no conformable effects
development of voids. Holding apples for 10 days at 55° F prior to place-
in storage prevented this disorder from occurring in 5 percent carbon
de at 38° F, but gave quantities about equal to the non-treated fruit when
n the other storage conditions. Thinning and excess crop were asso-
d with few or no voids in 5 percent carbon dioxide with 3 percent oxygen
° F. Defoliation greatly increased the incidence of voids over that of
on-treated fruit when stored in 5 percent carbon dioxide and 3 percent
en at 32° F. When a more thorough defoliation study was conducted in
, however, fruit from the defoliated braches responded in an opposite
ner in respect to the development of voids when stored in CA conditions
Appendix Table V). Fruit from non-defoliated branches had an average
e of 2.2 percent voids, which was significantly greater than the amount
s than 0.1 percent) of voided-fruits which had been picked from defoliated
nches.

Intermittent observations of void development during the storage season
summarized in Table 10. Voids first appeared in the 1957-58 storage
son at 125 days in fruit stored in 13 percent carbon dioxide with 3 percent
gen at 32° F. The initial appearance of voids in fruit stored in 5 percent
rbon dioxide and 3 percent oxygen at 32° F occurred at the inspection made
176 days. No voids were noted in fruit stored in CA conditions at 38° F

Storage for 210 Days in Controlled Atmosphere and Regular Storage (1967-68)

| Experiment No. | See Appendix Table No. | Prestorage Treatment | Percent Fruit with Voids | | | | |
|---|---|---|---|---|---|---|---|
| | | | 32° F | | | 38° F | |
| | | | Air (%) | 5% $CO_2$ 3% $O_2$ (%) | 13% $CO_2$ 3% $O_2$ (%) | 5% $CO_2$ 3% $O_2$ (%) | 13% $CO_2$ 3% $O_2$ (%) |
| 3 | III | 10 days delat at 55° F in air | 0 | 40 | 80 | 0 | 10 |
| 4 | III | Ringing | 0 | 0 | 47 | 37 | - |
| 5 | III | Defoliation | 0 | 87 | - | - | - |
| 6 | III | Thinning | 0 | 0 | - | - | 2 |
| 7 | III | Excess crop | 0 | 0 | - | - | 0 |
| 8 | III | Plastic bags | 0 | - | - | - | 25 |
| 1 | I | Untreated | 0 | 30 | 100 | 6 | 25 |

Percent Fruit with Voids Developed in Controlled Atmosphere and Regular Storage

| | | | Days in Storage | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 33 | 76 | 90 | 121 | 125 | 156 | 176 | 210 |
| | $\%CO_2$ | $\%O_2$ | Percent Fruit with Voids | | | | | | | |
| 1957-58 | | | 32° F | | | | | | | |
| | 5 | 3 | 0 | - | 0 | - | 0 | - | 19 | 26.6 |
| | 13 | 3 | 0 | - | 0 | - | 29.2 | - | 66.5 | 60.7 |
| | Air | | 0 | - | 0 | - | 0 | - | 0 | 0 |
| | | | 38° F | | | | | | | |
| | 5 | 3 | 0 | - | 0 | - | 0 | - | 0 | 3 |
| | 13 | 3 | 0 | - | 0 | - | 0 | - | 0 | 8.7 |
| 1958-59 | | | 32° F | | | | | | | |
| | 5 | 3 | - | 0 | - | 0 | - | 0 | 0.2 | 0.2 |
| | 13 | 3 | - | 0 | - | 0 | - | 1.8 | 2.7 | 5.2 |
| | Air | | - | 0 | - | 0 | - | 0 | 0 | 0 |

il 210 days. Generally, voids became more prevalent as both seasons gressed.

In the 1958-59 storage season, the fruit stored in 13 percent carbon xide with 3 percent oxygen showed voids after 156 days in storage. ds first appeared in fruit stored in 5 percent carbon dioxide and 3 percent gen at the inspection made at 176 days of storage.

Information on the occurrence of voids according to fruit size is avail- from the defoliation study made in 1958 (Experiment 9). One hundred ts from each of four trees of the five growers were used to establish an rage fruit size per grower. These average fruit sizes were found to fall four categories according to grower. Upon removal from seven months age in 13 percent carbon dioxide with 3 percent oxygen at 32° F, 30 fruits the four trees of the five growers were inspected for the development ids. According to the data in Table 11, the larger sized fruits were e susceptible to the development of voids than the smaller fruits. This rvation was confirmed in fruit of the other experiments.

Table 11

Occurrence of Voids in Various Sized Fruits Stored in 13 Percent Carbon Dioxide with 3 Percent Oxygen at 32° F for Seven Months

| meter of Fruits (Inches) | 2-1/4 | 2-1/2 | 2-3/4 | 3 |
|---|---|---|---|---|
| ntage fruits with voids | 4 | 11.5 | 9.7 | 17.0 |

Breakdown

ıree types of breakdown developed in the Jonathan apples stored under
rage conditions of this study.

ternal breakdown was characterized by browning of the vascular bundles
cortical tissue followed by a collapse and browning of the cortical tissue
l the affected vascular bundles (Figure 16). In the later stages, the
:d tissues became soft, dry and crumbly, and light to dark brown in
Internal breakdown in the advanced stages was evidenced externally
ull waxy appearance of the epidermis over the affected areas. This
er developed in controlled atmosphere and in regular storage.

oggy breakdown developed in the inner part of the cortical tissue as
, soft and spongy areas that were sharply delimited from the normal
. The affected and adjacent tissue often developed an alcoholic or fer-
d flavor. Soggy breakdown was not evident externally until large areas
tical tissue had become affected; then, the skin showed a brown dis-
ıtion and the flesh seemed spongy when a slight pressure was applied.
disorder developed only in regular storage.

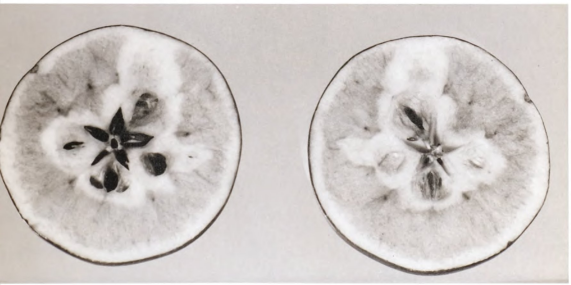
rown heart, on the other hand, developed only in controlled atmosphere
ions. This disorder developed in the pith, or cortical tissue alone, or
h tissues of the same fruit and often on numerous patches of brown
y tissue that were sharply defined from the normal tissue (Figure 17).

Figure 16

The darkened areas in the pith and cortical tissue of this Jonathan apple are injured as a result of internal breakdown. Note the voids in the pith tissue within the affected tissue.

Figure 17

The shaded tissues within the pith and cortex of these fruits are affected with brown heart. The large air pockets or voids in the pith tissue were not associated with brown heart.



ılly a zone of the peripheral tissue remained sound; similar to that in
ɜy breakdown.

Because of the difficulty of distinguishing either the very early stages or latter stages, which were often invaded by secondary pathogens; these sorders are reported as the combined percentages of breakdown present.

The effects of storage temperature on the development of breakdown in ɔntrolled atmosphere storage (Table 12) are shown as the average per-entages of fruit with breakdown upon removal from storage after seven ɴonths in CA storage at temperatures of 32 and 38° F. Both experiments ɨncluded over-ripe fruit which were susceptible to breakdown in storage. Small amounts of breakdown appeared in fruit held at 38° F, whereas, large amounts occurred in storage at 32° F.

Table 12

The Effect of Storage Temperature on the Development of Breakdown in Controlled Atmosphere Storage

| | % Fruit with Breakdown | |
|---|---|---|
| | 32° F | 38° F |
| Experiment 1, Appendix I | 29.0 | 2.2 |
| Experiment 3, Appendix III | 58.0 | 1.5 |

Data showing the effect of storage atmospheres upon the development of breakdown for experiments from which comparisons are available, are

summarized in Table 13. The average values for storage at 32° F show that the storage atmosphere of 13 percent carbon dioxide with 3 percent oxygen were more favorable for the development of breakdown than an atmosphere containing 5 percent carbon dioxide or normal air. Storage in normal air yielded slightly greater amounts of breakdown than fruit stored at 5 percent carbon dioxide at 32° F. The average percentage of breakdown at 38° F was relatively small within both CA atmospheres. Fruit in 1957 was considerably more susceptible to breakdown than apples harvested in 1958.

Breakdown was affected by circumstances other than storage temperature and atmosphere. Fruit from the 1957 time of harvest studies and fruit delayed 10 days at 55° F prior to storage, for example, showed more breakdown when stored at 5 percent carbon dioxide with 3 percent oxygen at 32° F than in normal air, whereas all other experiments stored in 32° F developed more breakdown when stored in normal air than in 5 percent carbon dioxide with 3 percent oxygen.

Statistical comparison of breakdown in fruit of the defoliation studies in 1958 (Appendix Table VI) showed there was no significant effects of storage atmospheres, orchards, defoliation or their interactions.

The effect of time of harvest on breakdown of fruit in storage is presented in Table 14. The greatest amount of breakdown developed in storage occurred in the more mature fruit (harvested on October 1 and 5 in 1957 and on October

Table 13

The Effect of Storage Atmospheres on the Development of Breakdown

| Experiment No. | See Appendix Table No. | Treatment | Year | Temp. (°F) | Storage Atmosphered | | |
|---|---|---|---|---|---|---|---|
| | | | | | Air (%) | 5% $CO_2$ 3% $O_2$ (%) | 13% $CO_2$ 13% $O_2$ (%) |
| 1 | I | Time of harvest | 1957 | 32 | 3.2 | 8.0 | 50.0 |
| 3 | III | 10 days delay in 55° in air | 1957 | 32 | 13.0 | 16.0 | 100.0 |
| 4 | III | Ringing | 1957 | 32 | 16.0 | 0.0 | 73.0 |
| 2 | II | Time of harvest | 1958 | 32 | 3.3 | 0.0 | 3.2 |
| | | Defoliation | 1958 | 32 | 4.0 | 1.4 | 1.3 |
| | | Average | | | 7.6 | 5.1 | 45.5 |
| 1 | I | Time of harvest | 1957 | 38 | - | 2.2 | 2.2 |
| 3 | III | 10 days delay at 55° F in air | 1957 | 38 | - | 0.0 | 3.0 |
| | | Average | | | - | 1.1 | 2.6 |

Table 14

Mean Percentages of Jonathan Apples with Breakdown According to Time of Harvest and Subsequent Storage for Seven Months in Controlled Atmosphere and Regular Storage

| | Time of Harvest | Percent Fruit with Breakdown | | | | | |
|---|---|---|---|---|---|---|---|
| | | 32° F | | | 38° F | | |
| | | Air | 5% $CO_2$ 3% $O_2$ | 13% $CO_2$ 3% $O_2$ | 5% $CO_2$ 3% $O_2$ | 13% $CO_2$ 3% $O_2$ | Average |
| | September 20, 1957 | 0 | 0 | 0 | 0 | 0 | 0 |
| Experiment 1 (See Appendix Table I) | September 25, 1957 | 0 | 0 | 0 | 3 | 0 | 0.6 |
| | October 1, 1957[a] | 0 | 16 | 100 | 3 | 6 | 25.0 |
| | October 5, 1957 | 13 | 16 | 100 | 0 | 3 | 26.4 |
| Experiment 2 (See Appendix Table II) | September 20, 1958 | 0 | 0 | 0 | - | - | 0 |
| | September 30, 1958[a] | 0 | 0 | 0 | - | - | 0 |
| | October 14, 1958 | 13.2 | 0 | 13.0 | - | - | 8.7 |

[a] Considered optimum date for commercial harvest.

14 in 1958). Breakdown development, when stored in normal air at 32° F appeared only in the fruit picked on October 5 in 1957, and October 14 in 1958.

The percentages of breakdown occurring as a result of the various pre-storage treatments applied in 1957 appear in Table 15. No consistent effect of treatment occurred. Holding the fruit for 10 days at 55° F after harvest and prior to placement in storage, for example, increased the development of breakdown in regular storage, but gave quantities in CA storage similar to that of the non-treated fruit. Ringing, on the other hand, prevented this disorder from occurring in 5 percent carbon dioxide and 3 percent oxygen at 32° F, but yielded 30 percent breakdown when stored in the same atmosphere at 38° F. Thinning and excess crop decreased the development of breakdown in storage. The average value of breakdown from the plastic bag treatment markedly increased the development of breakdown in normal air at 32° F and 13 percent carbon dioxide and 3 percent oxygen at 38° F. Defoliation increased slightly the amount of breakdown when held in normal air and 5 percent carbon dioxide with 3 percent oxygen over that formed by non-treated fruit in comparable storage treatments.

Fruit condition was inspected periodically during the storage season to determine the time of occurrence of breakdown in storage. According to the data in Table 16, breakdown appeared initially after 176 days in all storages

Table 15

Percent Breakdown in Jonathan Apples from Various Prestorage Treatments Subsequently Stored for Seven Months in Controlled Atmosphere and Regular Storage (1957-58)

| Experiment | See Appendix Table No. | Prestorage Treatment | Percent Fruit with Voids | | | | |
|---|---|---|---|---|---|---|---|
| | | | 32° F | | | 38° F | |
| | | | Air (%) | 5% $CO_2$ 3% $O_2$ (%) | 13% $CO_2$ 3% $O_2$ (%) | 5% $CO_2$ 3% $O_2$ (%) | 13% $CO_2$ 3% $O_2$ (%) |
| 3 | III | 10 days delay at 55° F in air | 13 | 16 | 100 | 0 | 3 |
| 4 | III | Ringing | 16 | 0 | 73 | 30 | - |
| 5 | III | Defoliation | 10 | 20 | - | - | - |
| 6 | III | Thinning | 3 | 0 | - | - | 4 |
| 7 | III | Excess crop | 3 | 3 | - | - | 3 |
| 8 | III | Plastic bags | 25 | - | - | - | 75 |
| 1 | I | Untreated | 0 | 16 | 100 | 3 | 6 |

Table 16

Periodic Observations on the Percent Breakdown Developed in Controlled Atmosphere and Regular Storage

| | | | Days in Storage | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 33 | 76 | 90 | 121 | 125 | 156 | 176 | 210 |
| | $\%CO_2$ | $\% O_2$ | Percent Fruit with Breakdown | | | | | | | |
| 1957-58 | | | | | | | 32° F | | | |
| | 5 | 3 | 0 | - | 0 | - | 0 | - | 1.2 | 8.0 |
| | 13 | 3 | 0 | - | 0 | - | 0 | - | 25.0 | 50.0 |
| | Air | | 0 | - | 0 | - | 0 | - | 2.5 | 3.2 |
| | | | | | | | 38° F | | | |
| | 5 | 3 | 0 | - | 0 | - | 0 | - | 0 | 1.5 |
| | 13 | 3 | 0 | - | 0 | - | 0 | - | 0 | 2.2 |
| 1958-59 | | | | | | | 32° F | | | |
| | 5 | 3 | - | - | - | 0 | - | 0 | 0 | 0 |
| | 13 | 3 | - | - | - | 0 | - | 0 | 0 | 4.3 |
| | Air | | - | - | - | 0 | - | 0 | 0 | 4.4 |

at 32° F during 1957. No breakdown occurred at 38° F in 1957, or at 32° F in 1958 until the inspection made at 210 days of storage.

## Other Symptoms of Controlled Atmosphere Injury

Several types of injury were observed on fruit which has been stored in controlled atmospheres. One of these appeared as a yellowish-brown, depressed and wrinkled area of the epidermal layers quite similar in shape to the injury caused on fruit in regular storage by soft scald (Figure 18). Less than 0. 1 percent of the fruit stored in controlled atmospheres in either year of this study was affected. This disorder usually appeared on the most mature fruit, and was seen more often on fruit stored in 13 percent carbon dioxide than in the atmosphere containing 5 percent carbon dioxide.

Another external injury was characterized by a blister-like disorder of the epidermal layers similar in size and shape to soft scald. Usually the blisters were slightly raised and sharply delimited from the normal tissue. The tissue below the epidermal layers were soft, brown and moist.

Browned and blister-like indented areas that extended in irregular patterns over the fruit were more similar to soft scald than the injury described immediately above (Figure 19). This disorder developed on fruit from one orchard employed for Experiment 14 in 1958 when stored in 5 percent carbon dioxide and 3 percent oxygen at 32° F. It also appeared occasionally on large fruit from trees bearing light crops when stored in controlled atmos-

Figure 18

Epidermal injury of the Jonathan apple stored in controlled atmosphere conditions of 13 percent $CO_2$ - 3 percent $O_2$ at 32° F.

Figure 19

Injury of epidermal and subepidermal tissues of the Jonathan apples stored in 5 percent carbon dioxide and 3 percent oxygen.

pheres. Comparable fruit developed a high percentage of soft scald (37.7 percent) when held in regular storage at 32° F.

A dark brown, water-soaked area occurred in the pith tissue (Figure 21) of one lot of fruit. These apples had inadvertently been exposed to an atmosphere of 15 percent carbon dioxide and 1.3 percent oxygen for approximately seven days. The normal and affected tissue had a musty and alcoholic flavor when this injury appeared.

## Water Core

Water core had developed in some of the larger and more mature fruits at the time they were harvested for experimental storage. Since there were no external symptoms of the disorder it was detected only upon cutting the fruit. Internally it appeared usually around the cortical and primary vascular bundles of the fruit. This condition generally disappeared during controlled atmosphere and regular storage, but occasionally it was still present at the end of the storage season (Figure 20).

In 1957, relatively large quantities (47 percent) of the fruit harvested from the defoliated branches had water core; whereas non-defoliated branches yielded fruit relatively free of water core. The fruit from the defoliated branches developed a high percentage (87 percent) of voids during seven months of storage in 5 percent carbon dioxide and 3 percent oxygen at 32° F.

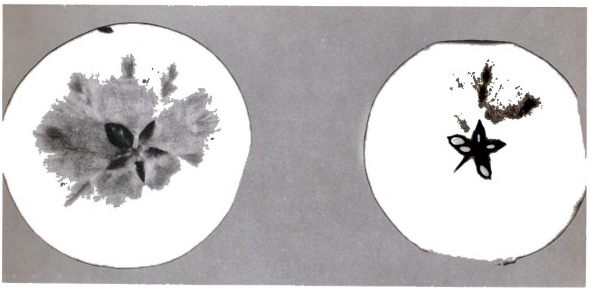
Further studies of the effect of defoliation on water core development

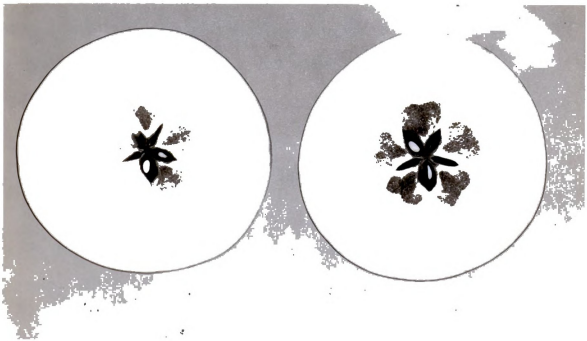
Figure 20

The glassy, water-soaked areas of water core that persisted through seven months of CA storage are the darkest wedge-shaped discolorations around the vascular bundles. The larger and less darkened areas are affected with brown heart (which was not particularly associated with the presence of water core).

Figure 21

The dark areas in these apples are brown and water-soaked pith tissue of apples exposed to 15 percent carbon dioxide with 1.3 percent oxygen for seven days at 32° F.





the following year gave opposite results. Apples from the non-defoliated branches yielded 34. 9 percent water core, an amount significantly greater than the amount of water core in the fruit from the defoliated areas (4. 9 percent). These data, together with their statistical evaluations are given in Appendix Table VII.

## Fruit Condition and Quality

The average flesh firmness of Jonathan apples as affected by prestorage treatments, time of harvest, defoliation and storage atmospheres and temperature, is shown in Appendix Tables VIII through XI.

The time of harvest somewhat influenced flesh firmness changes during storage. According to the average values presented in Appendix Table VIII, fruit harvest on October 1 and 5 in 1957 softened 2. 8 and 2. 7 pounds respectively, whereas earlier harvested fruit softened 2. 2 and 2. 4 pounds. Fruit harvested on October 14, the last harvest date in 1958, showed the greatest loss (5. 8 pounds), and the middle harvested fruit the least (3. 5 pounds). Flesh softening was more severe in 1958 than in 1957.

Storage temperatures and atmospheres also affected the changes in flesh firmness. In 1957, fruit stored in 13 percent carbon dioxide with 3 percent oxygen at 38° F had the greatest average loss (3. 8 pounds), whereas in 1958 fruit stored in normal air at 32° F yielded the greatest change (5. 3) pounds.

Fruit stored in 5 percent carbon dioxide with 3 percent oxygen had the best average retention of flesh firmness in both years.

The flesh firmness changes in fruit due to prestorage treatments are shown in Appendix Table IX. Small differences in flesh firmness occurred between the prestorage treatments and the non-treated fruit at harvest and upon removal from CA and regular storage. Fruit held for ten days in 55° F in air lost 2.0 pounds prior to placement in CA and regular storage, but softened only a slightly more during the storage period than the non-treated fruit.

A statistical comparison of flesh firmness in 1958 (Appendix Table X) showed there was no significant effect at harvest of orchards or defoliation treatment. When compared after storage at 32° F, it was found (Appendix Table XI) that apples stored in 13 percent carbon dioxide with 3 percent oxygen were significantly firmer (14.92 pounds) of flesh than fruit stored in 5 percent carbon dioxide with 3 percent oxygen (12.90 pounds) or normal air (12.3 pounds). The flesh firmness of fruit stored in 5 percent carbon dioxide with 3 percent oxygen and in normal air was similar.

The soluble solid contents of fruit as affected by prestorage treatments, time of harvest, defoliation and storage atmospheres and temperatures are presented in Appendix Tables XII through XV.

According to the time of harvest data for 1958 (Appendix Table XII),

soluble solids increased from 12 percent at the first harvest to 13.5 percent at the second harvest and then leveled off. Small changes in soluble solids appeared within each harvest date as a result of storage atmospheres.

Usually little change in soluble solids occurred during the storage conditions of this study due to the prestorage treatments (Appendix Table XIII). The soluble solids in Jonathan apples from the non-defoliated branches had a significantly higher percentage soluble solids (13.45 percent) than fruit from defoliated branches (12.45 percent), see Appendix Table XIV. After seven months in CA and regular storage, fruit from the non-defoliated areas still had a significantly higher percentage soluble solids than fruit from the defoliated areas. Also fruit stored in 13% carbon dioxide with 3 percent oxygen had a significantly higher percent soluble solids than fruit stored in 5 percent carbon dioxide with 3 percent oxygen or from fruit in normal air.

The number of fruits having complete red coloring, which masked the ground color, or striped red coloring, which partially masked the ground color, varied considerably from one treatment to another. Since this variation made it impossible to accurately rate the ground color, these data were not presented.

Fruit quality was judged by the author on the basis of the flavor and texture. The fruit harvested for the maturity studies had wide variation in fruit quality. The early harvested fruit was firm-crisp in texture and

acid in flavor at harvest; when removed from CA and regular storage, it was firm in texture, but lacking in flavor. Fruit picked on the optimum date for commercial harvest was generally firm and sweet-acid at harvest. After regular storage, it was slightly mealy and had very little flavor, whereas that from controlled atmosphere storage at 32° F or 38° F maintained much of the quality. The most mature fruit, especially fruit harvested on October 14, 1958, was melting and sweet and considered to be of high eating quality prior to storage. These apples after regular storage were melting with most of the large fruits mealy and sweet in flavor. Similar fruit stored in 5 percent carbon dioxide with 3 percent oxygen was firm in texture, but lacking in flavor.

Fruit delayed for ten days at 55° F following harvest was of melting texture and sweet flavor prior to storage. All of these became mealy in regular storage, whereas in CA storage only the larger fruits became mealy. Fruit receiving the other prestorage treatments was generally of similar quality to the fruit picked on the optimum date for commercial harvest.

The development of core browning and voids in storage did not affect the flavor and texture of the fruit. Apples with internal breakdown were generally mealy and dry with a musty flavor. Soggy breakdown, when severe, imparted an alcoholic or fermented taste to the fruit, whereas brown heart gave a musty flavor to the fruit.

## Labeled Carbon Dioxide Studies

Much of the experimentation utilizing the radioisotope of carbon$^{14}$ was devoted to developing techniques of application and measurement that would provide evidence for the distribution and movement of carbon dioxide in the fruit tissues.

The uptake of labeled carbon dioxide by whole Jonathan apples which had been previously stored in controlled atmospheres was measured by recovery of $C^{14}$ from the tissues. The quantities of $C^{14}$ recovered in the peel, flesh and core of fruit exposed to an atmosphere of 50 percent carbon dioxide and 10 percent oxygen at 75° F labeled with $C^{14}O_2$ (500 microcuries) for 14 days are given in Table 17. The fruit employed in the first trial (apples A through D) yielded great irregularities in the radioactivity per gram of peel, flesh and core. These variations were reduced in the second test by improving the cutting techniques to give more uniform thicknesses of peel and flesh tissues. In both experiments, the incorporation of $C^{14}$ (counts/minute/gram of fresh weight) in the flesh and core of apples A through H was inversely related to the apple diameter.

The average radioactivity per gram of flesh tissue was slightly greater than for the core, and considerably higher than for the peel.

Controlled atmosphere and regular-stored McIntosh and Jonathan apples were exposed to 25 percent labeled carbon dioxide (433.3 microcuries of $C^{14}$)

Table 17

The Quantity of $C^{14}$ Recovered in the Core, Flesh and Peel Tissue of CA Jonathan Apples Exposed for 14 Days to 50 Percent $CO_2$ Labeled with $C^{14}O_2$, with 3 Percent $O_2$

| Fruit Sample | Fruit Diameter (inches) | Core | | Flesh | | Peel | |
|---|---|---|---|---|---|---|---|
| | | Total cpm | cpm/gram | Total cpm | cpm/gram | Total cpm | cpm/gram |
| A | 3 | 122, 000 | 6, 100 | 701, 500 | 5, 810 | 72, 000 | 4, 250 |
| B | 2-3/4 | 59, 500 | 2, 975 | 491, 000 | 5, 660 | 58, 000 | 4, 540 |
| C | 2-1/2 | 60, 300 | 3, 350 | 580, 500 | 4, 899 | 26, 000 | 1, 735 |
| D | 2-1/4 | 45, 500 | 2, 400 | 208, 000 | 2, 777 | 13, 500 | 1, 230 |
| E | 3 | 57, 250 | 2, 862 | 372, 500 | 3, 010 | 15, 800 | 1, 112 |
| F | 2-3/4 | 46, 800 | 2, 600 | 360, 000 | 2, 972 | 13, 890 | 992 |
| G | 2-1/2 | 50, 500 | 2, 518 | 271, 000 | 2, 710 | 12, 300 | 1, 118 |
| H | 2-1/4 | 47, 490 | 2, 499 | 249, 900 | 2, 762 | 7, 630 | 763 |
| Average (E - H) | | | 2, 619. 7 | | 2, 863. 5 | | 996. 2 |

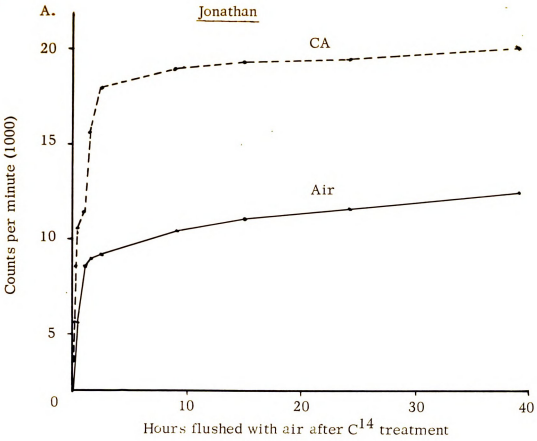
and 10 percent oxygen at 75° F for 17 days. The subsequent cumulative evolution of carbon$^{14}$ dioxide from the intact fruit is illustrated in Figure 22. In both varieties and from CA (150. 4 cpm) and regular storage 43. 3 cpm) $C^{14}O_2$ was released rapidly for the first 2 1/2 hours following removal from the atmosphere containing $C^{14}O_2$. Thereafter, the rates of evolution were approximately 0. 5 and 1. 0 counts per minute for CA and regular stored fruit, respectively. After 24 hours the rates of evolution of CA and regular stored fruit were approximately the same (0. 5 cpm). For both varieties the total $C^{14}O_2$ evolved in 40 hours by CA fruit was approximately twice that evolved by fruit from regular storage.

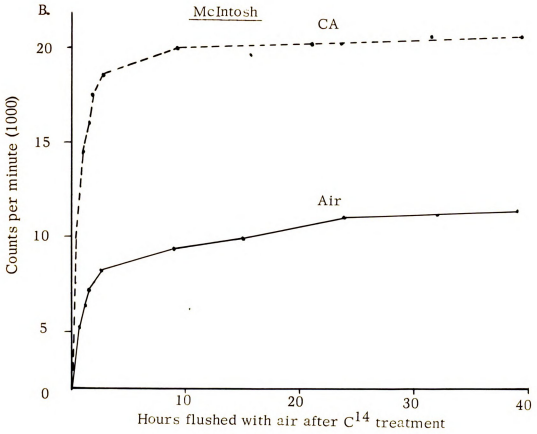
The experiment was repeated by exposure of controlled atmosphere and regular stored Jonathan apples to 25 percent carbon dioxide labeled with 260 microcuries of $C^{14}$, and 10 percent oxygen at 75° F for 17 days. Measurement of $C^{14}O_2$ evolved at 4, 24, and 56 hours following exposure gave cumulative totals for CA fruit of 12, 000, 15, 200 and 16, 400 cpm, respectively, and for fruit from regular storage of 6, 010, 9, 500 and 11, 000 cpm, respectively. The $C^{14}O_2$ evolution pattern was similar to that of the previous experiment.

The cumulative release of $C^{14}O_2$ by Jonathan apples which had been stored at 13 percent carbon dioxide and 3 percent oxygen for 5 months at 32° F and subsequently exposed to 25 percent carbon dioxide labeled atmosphere

Figure 22

Cumulative evolution of carbon$^{14}$ dioxide by storage apples following 17 days of exposure to 25 percent carbon dioxide containing 433.3 microcuries of $C^{14}$ at 75° F. Chart A is for Jonathan apples previously stored in 2 1/2 percent carbon dioxide with 3 percent oxygen at 32° F and in regular storage at 32° F for four months. Chart B is for McIntosh apples previously stored in 5 percent carbon dioxide and 3 percent oxygen and in regular storage at 32 to 33° F for four months.


A.
Jonathan
CA
Air
Counts per minute (1000)
0
5
10
15
20
10
20
30
40
Hours flushed with air after C14 treatment


B.
McIntosh
CA
Air
Counts per minute (1000)
0
5
10
15
20
10
20
30
40
Hours flushed with air after C14 treatment

with 10 percent oxygen at 32 and 75° F for seven days is depicted in Figure 23. The $C^{14}O_2$ evolved in the first ten hours after exposure to labeled carbon dioxide atmosphere was greatest from fruit which had been exposed at 32° F. By 24 hours, similar amounts had been evolved from fruit at both temperatures, and the amounts remained similar (approximately 0.7 cpm) throughout the test period.

The cumulative release of carbon$^{14}$ dioxide from Jonathan apples stored for four months in normal air, 5 percent carbon dioxide and 3 percent oxygen and 13 percent carbon dioxide with 3 percent oxygen with subsequent exposure to 25 percent carbon dioxide (labeled with 457 microcuries of $C^{14}$) with 10 percent oxygen is presented in Figure 24. The greatest amount of $C^{14}O_2$ was released by fruit from all three storage treatments during the first ten hours. The fruit stored in 13 percent carbon dioxide, however, evolved a greater amount (60 cpm) during this time than fruit from 5 percent carbon dioxide and 3 percent oxygen (23.3 cpm) or normal air (16.6 cpm). Fruit previously stored in 5 percent carbon dioxide released a greater amount of $C^{14}O_2$ than that stored in normal air. After ten hours, fruit from 13 percent carbon dioxide and 3 percent oxygen released $C^{14}O_2$ at a steady rate until the experiment was terminated. Between 10 and 24 hours fruit from 5 percent carbon dioxide with 3 percent oxygen (4.8 cpm) and normal air (5.9 cpm), however, continued to evolve $C^{14}O_2$ at a faster rate than fruit from 13 percent carbon dioxide and 3 percent oxygen (1.2 cpm)

Figure 23

Cumulative evolution of $C^{14}O_2$ by Jonathan apples following exposure to 25 percent carbon$^{14}$ dioxide with 10 percent oxygen at 32° F and 75° F for seven days. These apples had been stored prior to $C^{14}$ treatment in 13 percent carbon dioxide with 3 percent oxygen at 32° F for five months.

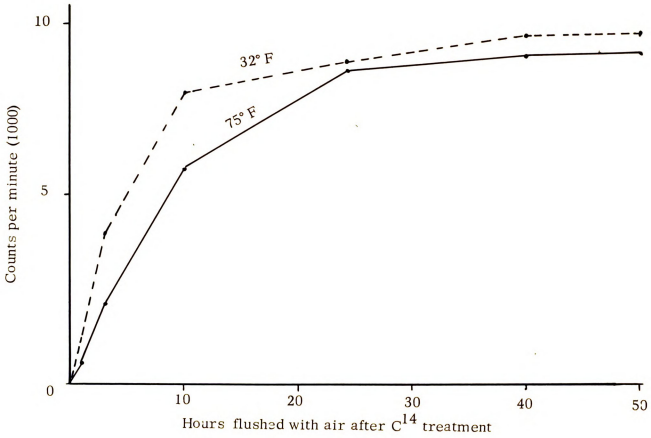
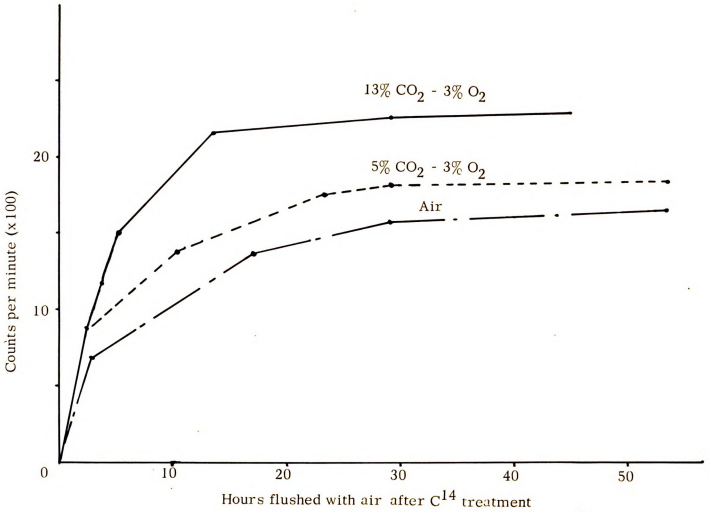

10
5
0
32° F
75° F
Counts per minute (1000)
10
20
30
40
50
Hours flushed with air after $C^{14}$ treatment

Figure 24

Cumulative evolution of $C^{14}O_2$ from Jonathan apples stored for four months in 13 percent carbon dioxide with 3 percent oxygen, 5 percent carbon dioxide with 3 percent oxygen and normal air at 32°F. This fruit was then exposed to 25 percent carbon dioxide containing 457 microcuries of $C^{14}$, with 10 percent oxygen for seven days and the evolution of $C^{14}O_2$ measured when flushed with normal air.


13% $CO_2$ - 3% $O_2$
5% $CO_2$ - 3% $O_2$
Air
Counts per minute (x 100)
20
10
0
10
20
30
40
50
Hours flushed with air after $C^{14}$ treatment

but at a slower rate than the first ten hours. After 24 hours, fruit from the three storage treatments evolved approximately the same amount of carbon$^{14}$ dioxide until the experiment was terminated. The total $C^{14}O_2$ evolved by the fruit stored in 13 percent carbon dioxide with 3 percent oxygen was 4000 and 6000 cpms greater than by fruit stored in 5 percent carbon dioxide with 3 percent oxygen and normal air, respectively; evolution by the fruit in 5 percent carbon dioxide with 3 percent oxygen was 2000 cpms greater than from storage in normal air.

The distribution of radioactivity in the internal atmosphere of a median Jonathan apple slice (3/8 inch thick) is diagrammed in Figure 25. The apples had been stored in 13 percent carbon dioxide with 3 percent oxygen at 32° F for four months prior to exposure to 25 percent labeled carbon dioxide (166 microcuries of $C^{14}$), with 10 percent oxygen. The radioactivity in each 1/4 inch square of cross-section fruit surface was determined. The areas of activity greater than 90 cpm/1/4 inch square are outlined in diagram A, one occurred in the cortical tissue and the smaller area extended into the pith tissue. There was a wide variation in the radioactivity of the measured blocks; the greatest gradient between adjacent blocks was 89 cpm.

As shown in diagram B of Figure 25 the greatest radioactivity concentration developed in the seed cavity, pith tissue and some of the cortical tissue surrounding the pith tissue. A sharper line of demarcation occurred between the areas of high and low activity.

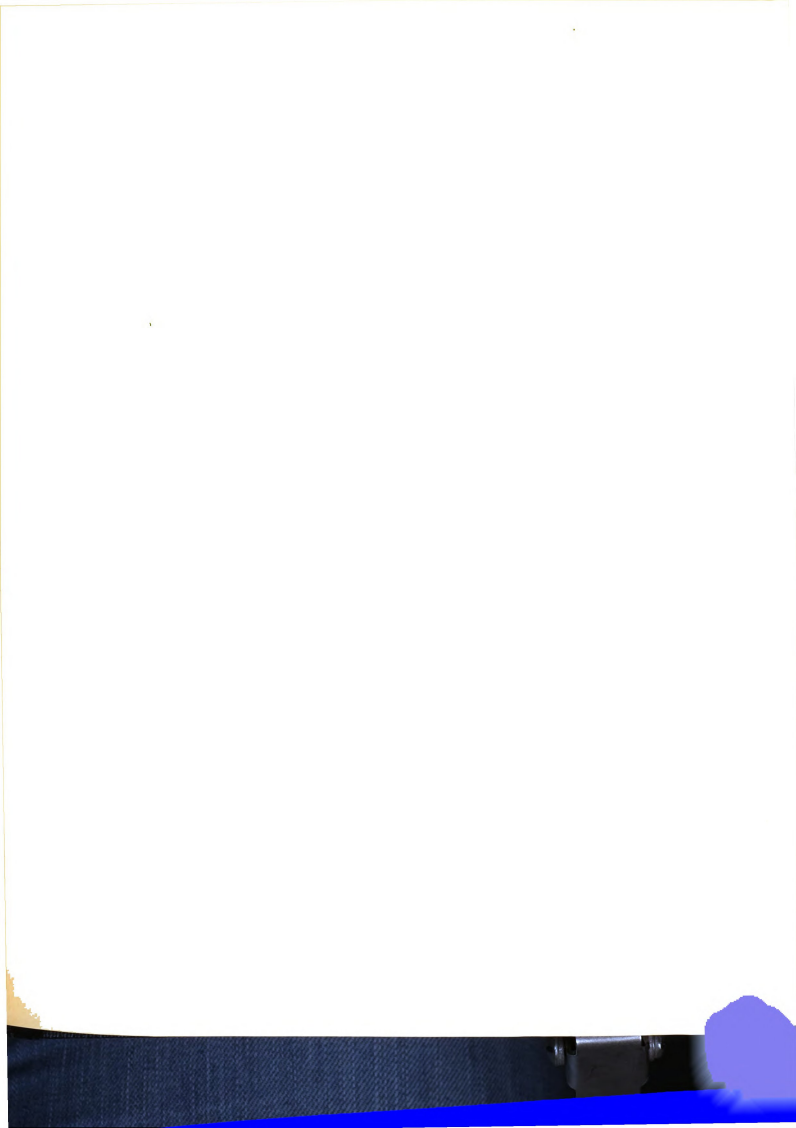
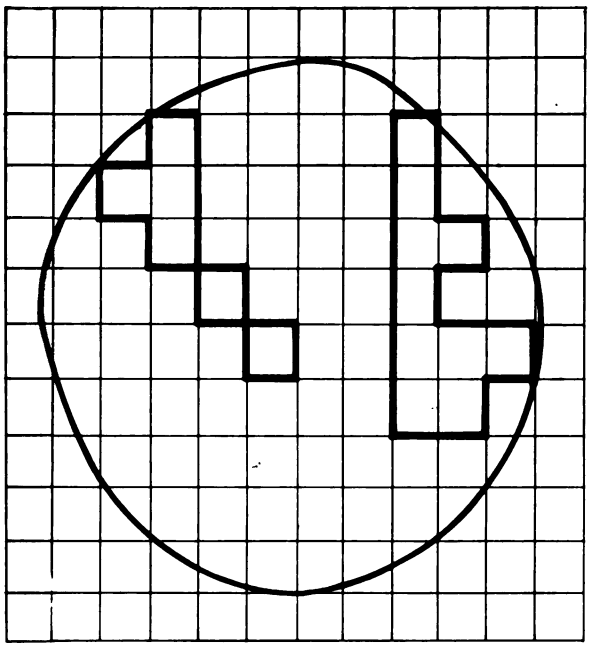
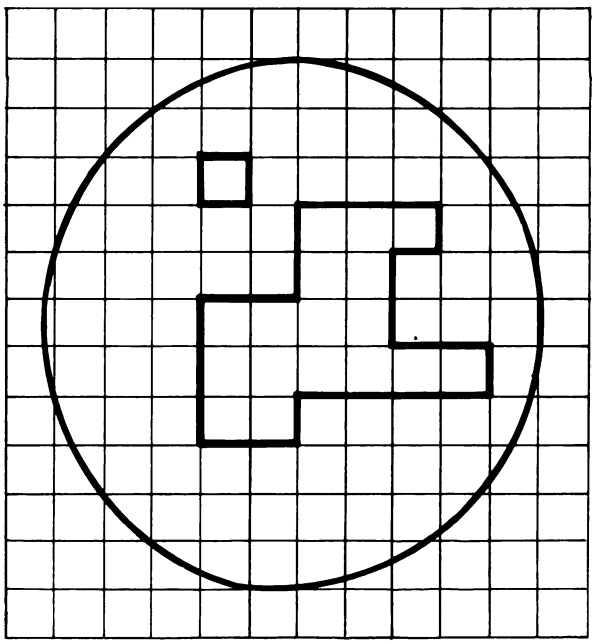


Figure 25

Diagram showing the distribution of $C^{14}O_2$ from the internal atmosphere of a 3/8 inch thick median transverse Jonathan apple slice. The internal atmosphere was withdrawn from the apple slice onto a treated blotting paper. The paper was dried and cut into 1/4 inch squares for radioactive measurement.



A



B

The distribution of radioactivity in an apple slice (3/8 inch thick) is portrayed in Figure 26. Although the resolution is poor, it may be seen that the greatest radioactivity occurred in the primary vascular bundles and the cambial line. The radioactivity pattern in the cortical tissues shows small pockets that contained no $C^{14}$.

The distribution of the internal atmosphere on the treated filter paper (Figure 27) shows that the greatest radioactive concentration in the seed cavity, cambial line, with lesser amount in the pith and cortical tissue adjacent to the pith tissue. Some radioactivity outside the outline of the fruit indicated that a small amount of radioactive material diffused laterally in the filter paper (see upper left hand corner of Figure 27).

By autoradiography, slight radioactivity was detected in the pith and cortical tissue when intact fruit had been exposed to labeled carbon dioxide for four hours. The greatest concentration occurred in the seed cavities with a lesser amount in the pith and cortical tissues. Fruit exposed to $C^{14}O_2$ for one hour showed approximately equal radioactivity in the seed cavity, pith and cortical tissue (10-20 cpm).

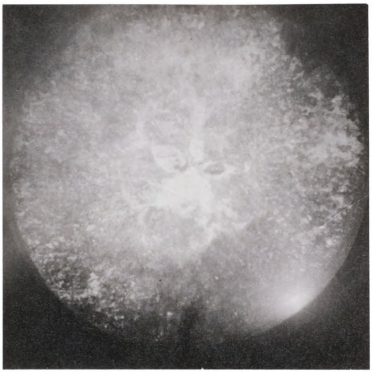
A second set of filter paper was treated with 10 percent phosphoric acid to release the $C^{14}O_2$ to see if other materials containing $C^{14}$ were withdrawn from the apple slice. Slight radioactivity (5-25 cpm) was found evenly distributed on the filter paper.

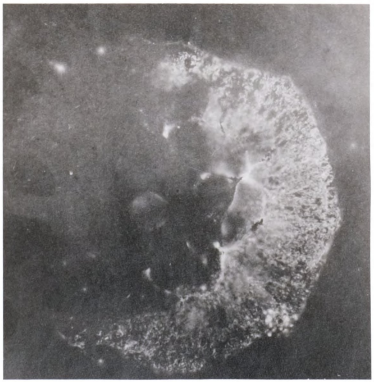
Figure 26

An autoradiogram of a 3/8 inch thick Jonathan apple slice taken from an intact apple that had been exposed to controlled atmosphere containing $C^{14}0_2$ (light areas radioactive). The shaded area on the right of the photograph was due to fogging of the film during exposure to the X-ray film.

Figure 27

An autoradiogram showing the $C^{14}$ distribution (light areas) from the internal atmosphere of a 3/8 inch thick median Jonathan apple slice. The internal atmosphere was withdrawn from the apple slice onto BaOH treated filter paper. The filter paper was exposed to X-ray film for 72 days.





The total radioactivity of slices from which the atmosphere had not been withdrawn was determined by assay of the tissue. The total radioactivity in the complete slice for one hour of exposure was 3, 000 cpm, 10, 500 for four hours, 22, 000 for 24 hours, 30, 500 for 168 hours, and 880, 000 cpm for 384 hours.

# DISCUSSION

The development of air pockets or voids in fruit tissues was the only important disorder noted in these experiments which could be attributed to controlled atmosphere storage. Other disorders, namely, core browning and breakdown, were not limited to fruit stored in controlled atmospheres, but were aggravated by the modified levels of carbon dioxide and oxygen employed. Although surface injuries appeared only in controlled atmosphere fruit, they were of such limited quantity that the causal factors could not be ascertained. Quite often voids and breakdown were extensive so as to adversely affect the marketability of the fruit. Core browning, although frequently prevalent, was limited and was not considered to be detrimental to the fruit quality.

A high level of carbon dioxide (13 percent) invariably increased the amounts of fruit with voids and core browning over the low levels (5 percent) as used in these tests. These concentrations of carbon dioxide were always employed in conjunction with 3 percent oxygen. Plagge (1942), Smock (1949) and Dewey et al. (1957) found that the greatest amounts of voids developed in Jonathan apples when stored in relatively high levels of carbon dioxide. Dewey et al. (1957) also found dry pith areas in the flesh near the core, similar to the advanced stages of core browning, developed in the higher concentrations of carbon dioxide.

Breakdown in controlled atmosphere storage has also been attributed to high carbon dioxide (Carne and Martin, 1935, 1938; Huelin and Tindale, 1947; Ballinger, 1955). This was found to be true for the 1957-58 tests, but in 1958-59 no significant differences appeared in the amount of breakdown from the storage treatments. The quantities noted in fruit from regular storage was 4.0 percent, in 13 percent carbon dioxide with 3 percent oxygen, 1.2 percent, and in 5 percent carbon dioxide with 3 percent oxygen, 1.4 percent.

Low temperatures (32° F) in controlled atmosphere storage were more favorable for the development of voids, core browning and total breakdown than the higher controlled atmosphere temperature (38° F) in the one season in which temperatures were compared. This effect of low temperature on the formation of voids did not agree with the findings of Dewey et al. (1957), but did agree with Trout et al. (1940) and Huelin and Tindale (1940).

Fruit of advanced maturity were considerably more susceptible to the development of voids and breakdown than fruit picked at earlier stages of maturation. Mandeno and Padfield (1953), Trout et al. (1940) and Huelin and Tindale (1947) also found that the more mature fruit was susceptible to CA disorders and breakdown in CA storage. However, the effects of fruit maturity on the development of core browning was not as clear cut. In 1957, factors other than maturity masked any distinct effect that maturity may have contributed. The most mature fruit of the 1958 study (harvested on

October 14) yielded the greatest amount of core browning in CA storage. The degree of susceptibility to core browning appeared to be due to a predisposition of the fruit tissue to core browning during the growing season. For example, some of the fruit stored in regular storage in 1957 developed core browning, whereas no core browning was found in fruit from regular storage of the 1958 storage season. Also, the fruit harvested for the 1957 storage tests was more susceptible to core browning than fruit picked for comparable storage tests in 1958.

The effect, if any, of the various prestorage treatments on the development of disorders appeared to be concealed by some factor or factors other than the treatment itself. Where apparently opposite treatments had been applied, for example, thinning and excess crop, no differences in the occurrence of voids, core browning and breakdown were observed between similar storage conditions. The defoliation treatment when applied in 1957 greatly increased the incidence of voids in CA storage, but in 1958, when a more thorough experiment was conducted, defoliation had an opposite effect on the development of voids. Defoliation in 1957 increased the incidence of core browning in regular storage, but decreased it in CA storage; also, breakdown was increased slightly in regular and CA storage over the non-treated fruit held in comparable storage treatments. In 1958, defoliation had no significant effect on the development of core browning or breakdown.

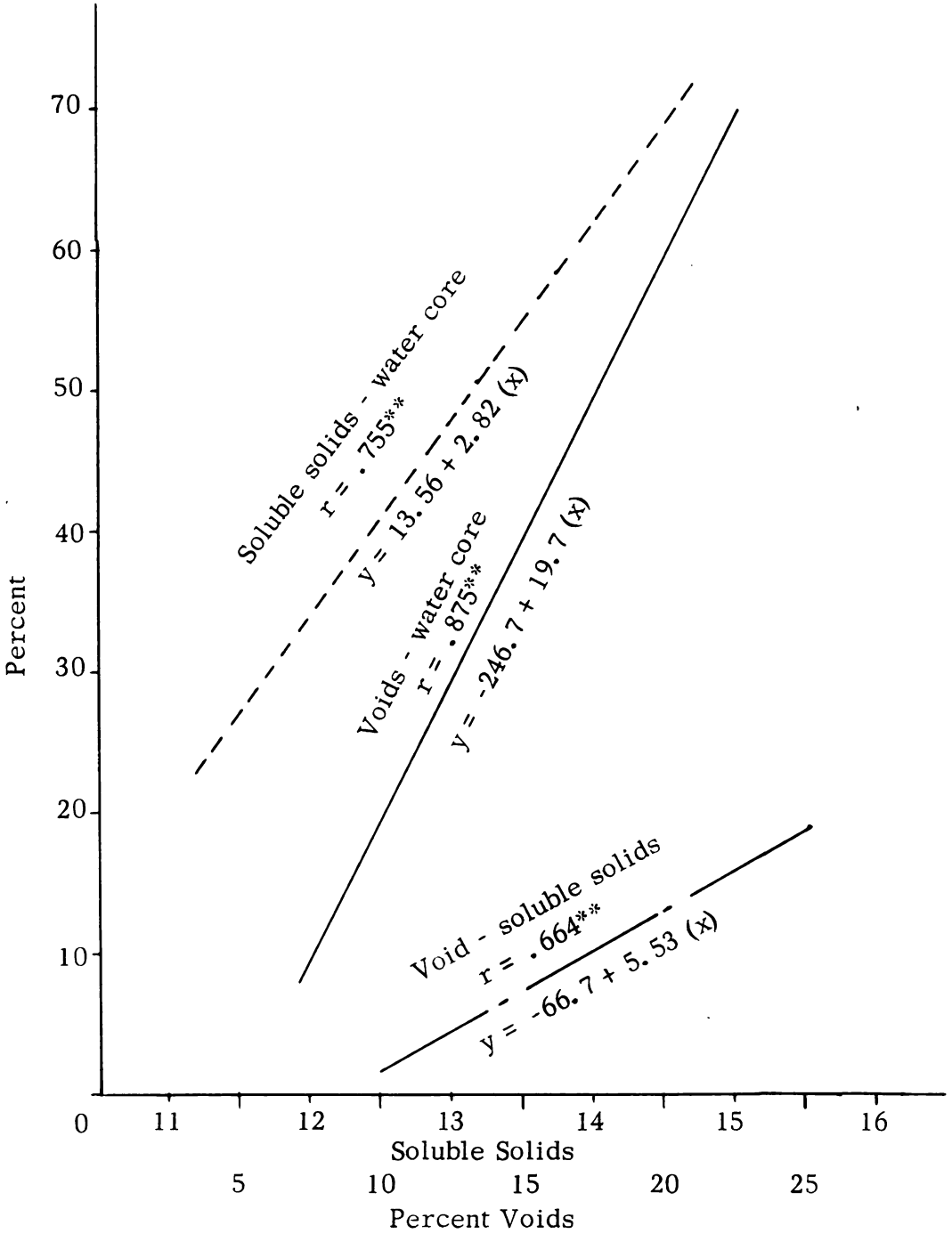
In 1957, fruits having a high percentage of water core at harvest had a high percentage of voids upon removal from controlled atmosphere storage. Jonathan apples from the non-defoliated branches in 1958 developed an amount of water core significantly greater than the amount in fruit from the defoliated limbs. After seven months of CA storage at 32° F, a significantly greater amount of voids developed in fruit from the non-defoliated limbs than from the defoliated limbs. From these data, a highly significant positive correlation occurred between the percent water core developed at harvest and the percent voids appearing in fruit held in 13 percent carbon dioxide with 3 percent oxygen at 32° F for seven months (Figure 28). This relationship did not hold true for the fruit held in 5 percen carbon dioxide with 3 percent oxygen since few voids were dormed in this atmosphere.

Fruit at harvest from the non-defoliated limbs had a significantly higher soluble solids content that fruit from the defoliated limbs. A positive correlation was found between the percent soluble solids and the percent water core in the fruit from the non-defoliated limbs when measured at harvest (Figure 28). In turn, a significant positive correlation occurred between the percent solubls solids and the percent voids developed in the fruit stored in 13 percent carbon dioxide with 3 percent oxygen at 32° F for seven months (Figure 28).

Although significantly correlated with the occurrence of water core, the

Figure 28

Correlation of percent voids with soluble solids of fruit from non-defoliated branches after seven months storage in 13 percent carbon dioxide - 3 percent oxygen at 32° F and with percent water core at harvest - 1958-59.


Percent
70
60
50
40
30
20
10
0
Soluble solids - water core
r = .755**
y = 13.56 + 2.82 (x)
Voids - water core
r = .875**
y = -246.7 + 19.7 (x)
Void - soluble solids
r = .664**
y = -66.7 + 5.53 (x)
11
12
13
14
15
16
Soluble Solids
5
10
15
20
25
Percent Voids

development of voids was not necessarily caused by water core. Voids developed in some fruits which did not show water core, and when both disorders appeared together in the same fruit, the same tissues were not always involved. The conditions of the cells responsible for susceptibility to water core may, however, cause them to be susceptible to controlled atmosphere injury. Other factors not determined here ultimately seemed to determine whether or not injury developed.

The injury characterized by air pockets or voids occurred most often in the pith tissues. Microscopic examination of these tissues revealed that the tissues had collapsed to form the air pocket and the remaining affected cell walls had been ruptured or partially disintegrated. The autoradiograms of the $C^{14}O_2$ activity of the internal atmospheres of the slice from intact fruits exposed to an atmosphere containing $C^{14}O_2$, in most cases, showed the larger amounts of $C^{14}O_2$ in the seed cavity, pith tissues, cambial line, vascular bundles and the cortical tissues immediately surrounding the pith tissues. With a carbon dioxide buildup in the pith tissues, it is possible that the metabolic processes of the tissue are disrupted so as to cause an accumulation of toxic materials in the cells. Hulme (1956) noted an accumulation of succinic acid in tissues which had been injured by higher than normal levels of carbon dioxide. Allentoff et al. (1954) found that intact McIntosh apples exposed to an external source of $C^{14}O_2$ incorporated $C^{14}$ in the malic acid, aspartic

acid and glumatic acid. These authors also found that McIntosh apples in storage produced malic acid by fixation of carbon dioxide and the rate of fixation increased with a rising concentration of carbon dioxide in the external atmosphere. It would, therefore, appear that there is a mechanism affected by a high external source of carbon dioxide which eventually could lead to the buildup of injurious amounts of a toxic material.

The appearance of core browning in controlled atmosphere storage was not associated with the formation of voids or breakdown. Since it occurred in an area showing accumulation of $C^{14}O_2$, and was more severe in CA than in regular storage, the accumulation of carbon dioxide may be the causal factor. As true for other disorders, the susceptibility of the fruit varied widely, being highly susceptible in 1957-58 so as to develop as a result of the normal accumulation of carbon dioxide even in regular storage.

The three types of breakdown appeared to originate in the cortical tissues with internal breakdown and brown heart spreading to include the pith tissues. The points of origin of these disorders did not coincide with the areas found to have high $C^{14}O_2$ activity. Over-maturity of the fruit upon placement in CA storage seemed to be the primary factor responsible for breakdown. This was found to be true also in the studies of Trout et al. (1940) and Huelin and Tindale (1947).

Several external injuries appeared as a result of storage of fruit in con-

trolled atmosphere conditions but none appeared in amounts greater than 0.1 percent. Although different in appearance, these injuries were similar in size and shape to those on Jonathan apples in regular storage caused by soft scald. Trout et al. (1940) has reported soft scald on fruit stored in controlled atmospheres. The relatively small amounts of surface injuries that developed in the tests reported here did not permit direct correlations with soft scald developed in regular storage.

Controlled atmospheres apparently affected the pith and cortical cells so as to change the diffusion rate of carbon dioxide. When controlled atmosphere and regular stored Jonathan and McIntosh apples were exposed to an atmosphere containing $C^{14}O_2$, the pattern of release was similar; however, fruit from controlled atmospheres evolved $C^{14}O_2$ at a much greater rate during the first ten hours and released a greater total amount. Possibly the cell contents are changed under controlled atmosphere conditions so as to allow a greater adsorption of $C^{14}O_2$; or possibly during controlled atmosphere storage the permeability of the tissues to gases is altered. Either or both of these changes would account for the difference in the diffusion of carbon dioxide from these tissues.

Evidence that these changes in cell properties are affected by carbon dioxide content of the atmosphere was the differences in $C^{14}O_2$ evolution from fruit previously stored in the different atmospheres (Figure 24). Apples pre-

viously stored in 13 percent carbon dioxide with 3 percent oxygen released a greater total amount of $C^{14}O_2$ and at a higher initial rate than fruit stored in 5 percent carbon dioxide with 3 percent oxygen and these in turn released a greater total amount of $C^{14}O_2$ and at a greater initial rate than fruit which had been stored in normal air.

The higher rate of evolution of $C^{14}O_2$ from fruit released at 32° F than fruit treated at 75° F (Figure 23) can be attributed to the greater solubility of carbon dioxide in solutions at low temperature. For example, the solubility of carbon dioxide per 100 ml of water at 32° F is 0.348 grams and 0.145 grams at 77° F.

## SUMMARY AND CONCLUSIONS

The purpose of these experiments was to investigate the effects of fruit maturity and various prestorage factors on the development of controlled atmosphere storage disorders in Jonathan apples.

Core browning, a form of brown discoloration in some or all of the pith tissues of the fruit, was aggravated by CA storage, but also appeared in fruit in regular storage during one of the two years in which these experiments were conducted. Cells injured by core browning showed a vacuole-like sac containing many particles surrounded by a brown fluid.

High concentrations of carbon dioxide (13 percent) and low temperatures (32° F) increased the incidence of core browning in CA fruit over 5 percent carbon dioxide and a storage temperature of 38° F. Advanced fruit maturity, associated with delaying the time of harvest of fruit for storage, tended to increase the amount of fruit showing core browning in CA storage. Removal of the leaves from branches bearing the test fruit two months prior to fruit harvest did not have a significant effect on the development of core browning in storage. The time of appearance of core browning in controlled atmosphere and regular storage differed in the two years of this study. In 1957-58, core browning appeared after 156 days of storage, whereas, in 1958-59, it appeared after 76 days of storage.

Air pockets or voids in the pith tissues and occasionally in the cortical

tissue of the fruit appeared only in fruit stored in controlled atmospheres. It was favored by high concentrations of carbon dioxide (13 percent) and low temperatures (32° F), and the larger and more mature fruits were more susceptible to this injury than small apples or apples harvested early in the picking season. Prestorage treatments of defoliation, delayed storage, thinning, ringing, excess crop, and the plastic bag fruit covering showed no consistent effect on the development of voids in controlled atmosphere storage. Voids first appeared between 125 days and 176 days after placement in CA storage.

Three types of fruit breakdown developed in these tests. Internal breakdown appeared in regular and controlled atmosphere storage, soggy breakdown occurred only in regular storage, whereas brown heart developed only in controlled atmosphere conditions.

Late-harvested fruit stored in 13 percent carbon dioxide with 3 percent oxygen at 32° F showed the greatest amount of breakdown. The early harvested fruits were the least susceptible to breakdown in both controlled atmosphere and regular storage. Prestorage treatments in 1957 had no consistent effects on the development of breakdown. Breakdown appeared initially after 175 days in controlled atmosphere and regular storage at 32° F; however, fruit held at 38° F in CA storage and fruit stored in regular and controlled atmosphere storage in 1958 developed breakdown after 210 days.

Several external injuries appeared on fruit held in CA storage, but none developed in amounts greater than 0.1 percent.

There was a positive linear correlation of water core in fruit at harvest, with the soluble solids contents of the fruit juice both before and after storage, and with the development of voids in high concentrations of carbon dioxide (13 percent) during storage.

The best storage conditions for avoiding severe core browning, for minimum development of voids and breakdown, and for retention of good eating quality was the controlled atmosphere containing 5 percent carbon dioxide and 3 percent oxygen at a temperature of 32° F. Fruit of advanced maturity or showing water core at harvest should be avoided when selecting Jonathan apples for controlled atmosphere storage.

Techniques were developed for the utilization of labeled carbon to follow the movement and distribution of carbon dioxide in fruit tissues. More $C^{14}$ accumulated in the core and flesh of the larger fruit, on a per gram basis, than in the smaller fruit. The flesh and the core contained a higher concentration of radioactivity than the peel of individual fruits.

Controlled atmosphere Jonathan and McIntosh apples, following exposure to $C^{14}O_2$, evolved a greater amount of $C^{14}O_2$ and at a higher initial rate than fruit from regular storage. Fruit previously stored in 13 percent carbon dioxide also evolved a greater total amount of $C^{14}O_2$ than fruit stored in 5 per-

cent carbon dioxide. Fruit stored in 5 percent carbon dioxide released a greater total amount of $C^{14}O_2$ than fruit from normal air. The greatest radioactivity occurred in the seed cavities, cambial line, vascular bundles, pith tissues and the cortical tissues immediately surrounding the pith tissues of the fruit. These studies indicate that the properties of the fruit cells in respect to accumulation and/or permeability to carbon dioxide are altered as a result of storage in controlled atmospheres. Further study is needed to define these changes.

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# APPENDIX TABLES

## APPENDIX TABLE I

Experiment 1: The Effect of Time of Harvest on Jonathan Apples During Storage in Controlled Atmospheres and Regular Air - 1957-58

| Time of Harvest | Atmospheres | | Temperature (°F) | Water-core[a] (%) | Jonathan Spot (%) | Soft Scald (%) | Breakdown[b] (%) | Core Browning (%) | Voids (%) |
|---|---|---|---|---|---|---|---|---|---|
| | %$CO_2$ | %$O_2$ | | | | | | | |
| | 5 | 3 | 32 | | 0.0 | 0.0 | 0.0 | 80.0 | 3.0 |
| | 5 | 3 | 38 | | 0.0 | 0.0 | 0.0 | 43.0 | 0.0 |
| September 20 | 13 | 3 | 32 | 0.0 | 0.0 | 0.0 | 0.0 | 57.0 | 33.0 |
| | 13 | 3 | 38 | | 0.0 | 0.0 | 0.0 | 57.0 | 0.0 |
| | Air | | 32 | | 71.0 | 0.0 | 0.0 | 100.0 | 0.0 |
| | 5 | 3 | 32 | | 0.0 | 0.0 | 0.0 | 73.0 | 0.0 |
| | 5 | 3 | 38 | | 0.0 | 0.0 | 3.0 | 20.0 | 0.0 |
| September 25 | 13 | 3 | 32 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 10.0 |
| | 13 | 3 | 38 | | 0.0 | 0.0 | 0.0 | 57.0 | 0.0 |
| | Air | | 32 | | 67.0 | 0.0 | 0.0 | 80.0 | 0.0 |
| | 5 | 3 | 32 | | 0.0 | 0.0 | 16.0 | 37.0 | 30.0 |
| | 5 | 3 | 38 | | 0.0 | 0.0 | 3.0 | 20.0 | 6.0 |
| October 1[c] | 13 | 3 | 32 | 44.0 | 0.0 | 0.0 | 100.0 | 83.0 | 100.0 |
| | 13 | 3 | 38 | | 0.0 | 0.0 | 6.0 | 10.0 | 27.0 |
| | Air | | 32 | | 93.0 | 6.0 | 0.0 | 57.0 | 0.0 |
| | 5 | 3 | 32 | | 0.0 | 0.0 | 16.0 | 74.0 | 40.0 |
| | 5 | 3 | 38 | | 0.0 | 0.0 | 0.0 | 16.0 | 0.0 |
| October 5 | 13 | 3 | 32 | 60.0 | 0.0 | 0.0 | 100.0 | 90.0 | 97.0 |
| | 13 | 3 | 38 | | 0.0 | 0.0 | 3.0 | 40.0 | 10.0 |
| | Air | | 32 | | 100.0 | 27.0 | 13.0 | 75.0 | 0.0 |

[a] Average at time of harvest

[b] Average of internal breakdown, brown heart and soggy breakdown

[c] Considered optimum date for commercial harvest

APPENDIX TABLE II

Experiment 2: The Effect of Time of Harvest on Jonathan Apples in Controlled Atmosphere and Regular Storage - 1958-59

| Time of Harvest | Atmospheres $\%CO_2$ | Atmospheres $\%O_2$ | Temperature (°F) | Water-core[a] (%) | Jonathan Spot (%) | Soft Scald (%) | Breakdown[b] (%) | Core Browning (%) | Voids (%) |
|---|---|---|---|---|---|---|---|---|---|
| | 5 | 3 | 32 | | 0.0 | 0.0 | 0.0 | 18.3 | 0.0 |
| September 20 | 13 | 3 | 32 | 0.0 | 0.0 | 0.0 | 0.0 | 33.3 | 0.0 |
| | Air | | 32 | | 21.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 5 | 3 | 32 | | 0.0 | 0.0 | 0.0 | 21.0 | 0.0 |
| September 30[c] | 13 | 3 | 32 | 0.0 | 0.0 | .5 | 0.0 | 55.0 | 0.0 |
| | Air | | 32 | | 13.0 | 13.8 | 0.0 | 0.0 | 0.0 |
| | 5 | 3 | 32 | | 0.0 | 0.0 | 0.0 | 56.0 | 0.1 |
| October 14 | 13 | 3 | 32 | 44.0 | 0.0 | 0.0 | 13.0 | 70.0 | 4.2 |
| | Air | | 32 | | 40.0 | 16.6 | 13.2 | 0.0 | 0.0 |

[a] Average at harvest

[b] Average of internal breakdown, brown heart, and soggy breakdown

[c] Considered optimum date for commercial harvest

## APPENDIX TABLE III

Experiments 3-8: The Effect of Prestorage Treatments on Jonathan Apples in Controlled Atmosphere and Regular Storage - 1957-58

| Experiment | Treatment | Atmospheres % $CO_2$ | Atmospheres % $O_2$ | Temperature (°F) | Water Core[a] (%) | Jonathan Spot (%) | Soft Scald (%) | Breakdown[b] (%) | Core Browning (%) | Voids (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 10 days delay at 55°F in air | 5 | 3 | 32 | | 0 | 0 | 16 | 40 | 40 |
| | | 5 | 3 | 38 | | 0 | 0 | 0 | 0 | 0 |
| | | 13 | 3 | 32 | 30 | 0 | 0 | 100 | 80 | 80 |
| | | 13 | 3 | 38 | | 0 | 0 | 3 | 10 | 10 |
| | | Air | | 32 | | 60 | 40 | 13 | 76.5 | 0 |
| 4 | Ringing | 5 | 3 | 32 | | 0 | 0 | 0 | 90 | 0 |
| | | 5 | 3 | 38 | 40 | 0 | 0 | 30 | 0 | 37 |
| | | 13 | 3 | 32 | | 0 | 0 | 73 | 47 | 47 |
| | | Air | | 32 | | 47 | 3 | 16 | 41.5 | 0 |
| 5 | Defoliation | 5 | 3 | 32 | 47 | 0 | 0 | 20 | 27 | 87 |
| | | Air | | 32 | | 50 | 12 | 10 | 100 | 0 |
| 6 | Thinning | 5 | 3 | 32 | | 0 | 0 | 0 | 22 | 0 |
| | | 13 | 3 | 38 | 10 | 0 | 0 | 4 | 6 | 2 |
| | | Air | | 32 | | 70 | 25 | 3 | 83 | 0 |
| 7 | Excess crop | 5 | 3 | 32 | | 0 | 0 | 3 | 40 | 0 |
| | | 13 | 3 | 38 | 0 | 0 | 0 | 3 | 2 | 0 |
| | | Air | | 32 | | 77 | 30 | 3 | 100 | 0 |
| 8 | Plastic bags[c] | 13 | 3 | 38 | 3 | 0 | 0 | 75 | 0 | 15 |
| | | Air | | 32 | | 80 | 16 | 16 | 35 | 0 |

[a] Average at time of harvest

[b] Average of internal breakdown, brown heart and soggy breakdown

[c] Average of black and white plastic bags

Check fruit - see Appendix Table I - fruit harvested on October 1

## APPENDIX TABLE IV

Percentage of Fruit with Core Browning from Defoliated and Non-defoliated Trees of Five Growers. Fruit Stored in Controlled Atmosphere and Regular Storage at 32° F for Seven Months (1958-59)

| | Percent Core Browning | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Growers | | | | | | | | | | |
| | 1 | | 2 | | 3 | | 4 | | 5 | | |
| | Field Treatments (D = defoliated; ND = non-defoliated) | | | | | | | | | | |
| | ND | D | ND | D | ND | D | ND | D | ND | D | Ave. |
| 5% $CO_2$ - 3% $O_2$ | 10.8[a] | 2.5 | 5.8 | 5.8 | 10.0 | 14.1 | 24.2 | 35.0 | 10.3 | 10.8 | 13.2 |
| 13% $CO_2$ - 3% $O_2$ | 13.8 | 10.2 | 12.5 | 31.6 | 25.0 | 25.0 | 60.0 | 25.0 | 34.1 | 27.3 | 26.1 |
| Air | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Field treatment average | 8.2 | 4.2 | 6.1 | 12.5 | 11.7 | 13.5 | 28.2 | 20.0 | 15.8 | 12.7 | |
| Grower average | 6.1 | | 9.3 | | 12.3 | | 24.1 | | 14.6 | | |

ANALYSIS OF VARIANCE TABLE

| Source | Degrees of Freedom | Variance |
|---|---|---|
| Replications (trees within each grower) | 3 | |
| Growers | 4 | 1632.7 |
| Error (a) | 12 | 650.3 |
| Field treatments (defoliation) | 1 | 382.8 |
| Grower x field treatment | 4 | 50.4 |
| Error (b) | 15 | 153.8 |
| Storage atmospheres | 2 | 9049.9** |
| Grower x storage atmospheres | 8 | 236.3** |
| Field treatments x storage atmospheres | 2 | 267.3** |
| Grower x field treatment x storage atmospheres | 8 | 376.9** |
| Error (c) | 60 | 41.5 |

[a] Each entry average value of four trees

## APPENDIX TABLE V

Percentage of Fruit with Voids from Defoliated and Non-defoliated Trees of Five Growers. Fruits Stored in Controlled Atmosphere and Regular Storage at 32° F for Seven Months (1958-59)

| | Percent Voids | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Grower | | | | | | | | | | |
| | 1 | | 2 | | 3 | | 4 | | 5 | | |
| | Field Treatment (D = defoliated; ND = non-defoliated) | | | | | | | | | | |
| | ND | D | ND | D | ND | D | ND | D | ND | D | Ave. |
| 5% $CO_2$ - 3% $O_2$ | 0.0[a] | 0.0 | 0.0 | 0.0 | 0.0 | 0.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 |
| 13% $CO_2$ - 3% $O_2$ | 0.0 | 0.0 | 4.1 | 0.0 | 10.8 | 0.1 | 16.6 | 0.5 | 2.0 | 0.0 | 3.4 |
| Air | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Field treatment average | 0.0 | 0.0 | 1.4 | 0.0 | 3.6 | 0.3 | 5.5 | 0.2 | 0.7 | 0.0 | |
| Grower average | 0.0 | | 0.7 | | 1.9 | | 2.8 | | 0.3 | | |

[a] Each entry average value of four trees.

ANALYSIS OF VARIANCE TABLE

| Source | Degrees of Freedom | Variance |
|---|---|---|
| Replication (trees within each grower) | 3 | |
| Grower | 4 | 83.99** |
| Error (a) | 12 | 8.85 |
| Field treatments (defoliation) | 1 | 404.25** |
| Grower x field treatments | 4 | 34.20* |
| Error (b) | 15 | 9.15 |
| Storage atmospheres | 2 | 596.39** |
| Grower x storage atmosphere | 8 | 77.37** |
| Field treatment x storage treatment | 2 | 464.64** |
| Grower x field treatment x storage treatment | 8 | 43.57** |
| Error (c) | 60 | 11.06 |

## APPENDIX TABLE VI

Percentage of Fruit with Breakdown from Defoliated and Non-defoliated Trees of Five Growers. Fruit Stored in Controlled Atmosphere and Regular Storage at 32° F (1958-59)

| | Percent Breakdown | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Grower | | | | | | | | | | |
| | 1 | | 2 | | 3 | | 4 | | 5 | | |
| | Field Treatment (D = defoliated; ND = non-defoliated) | | | | | | | | | | |
| | ND | D | ND | D | ND | D | ND | D | ND | D | Ave. |
| 5% $CO_2$ - 3% $O_2$ | 0.0[a/] | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 6.6 | 1.7 | 2.5 | 1.4 |
| 13% $CO_2$ - 3% $O_2$ | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10.8 | .8 | 0.0 | 0.8 | 1.2 |
| Air | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.1 | 21.0 | 1.2 | 11.0 | 6.5 | 4.0 |
| Field treatment average | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 11.7 | 2.9 | 4.2 | 3.3 | |
| Grower average | 0.0 | | 0.0 | | 0.0 | | 7.3 | | 3.7 | | |

ANALYSIS OF VARIANCE TABLE

| Source | Degrees of Freedom | Variance |
|---|---|---|
| Replication (trees within each grower) | 3 | |
| Growers | 4 | 476.52 |
| Error (a) | 12 | 645.53 |
| Field treatment (defoliation) | 1 | 202.90 |
| Grower x field treatment | 4 | 397.14 |
| Error (b) | 15 | 291.61 |
| Storage atmospheres | 2 | 2443.45 |
| Grower x storage atmospheres | 8 | 623.29 |
| Field treatment x storage atmospheres | 2 | 232.83 |
| Grower x field treatment x storage atmospheres | 8 | 378.73 |
| Error (c) | 60 | 9894.68 |

[a/] Each entry average value of four trees.

## APPENDIX TABLE VII

Percentage of Jonathan Apples from Defoliated and Non-defoliated Trees with Water Core at Harvest (1958-59)

| | Grower | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Ave. |
| | (%) | (%) | (%) | (%) | (%) | (%) |
| Non-defoliated | 2.0[a] | 20.2 | 52.8 | 69.1 | 29.5 | 34.9 |
| Defoliated | 0.0 | 0.0 | 14.5 | 7.1 | 2.6 | 4.9 |
| Grower average | 1.3 | 10.2 | 33.7 | 38.1 | 16.1 | |

ANALYSIS OF VARIANCE TABLE

| Source | Degrees of Freedom | Variance |
|---|---|---|
| Replication | 3 | 430.7 |
| Growers | 4 | 1974.4** |
| Error (a) | 12 | 295.7 |
| Field treatment (defoliation) | 1 | 8880.4** |
| Grower x field treatment | 4 | 993.2** |
| Error (b) | 15 | 129.3 |

[a] Each entry average value of four trees.

## APPENDIX TABLE VIII

Mean Flesh Firmness According to Pressure Tests of Jonathan Apples at Various Times of Harvest and Firmness Loss after 210 Days in Controlled Atmosphere and Regular Storage

| Time of Harvest | Year | At Harvest | 30° F | | | 38° F | | |
|---|---|---|---|---|---|---|---|---|
| | | | Air | 5% $CO_2$ 3% $O_2$ | 13% $CO_2$ 3% $O_2$ | 5% $CO_2$ 3% $O_2$ | 13% $CO_2$ 3% $O_2$ | Ave. |
| | | (lbs) | (Pounds loss during storage) | | | | | |
| September 20 | 1957 | 16.9 | 3.8 | 0.7 | 1.3 | 3.7 | 2.7 | 2.2 |
| September 25 | 1957 | 16.5 | 3.4 | 1.4 | 1.7 | 3.8 | 1.9 | 2.4 |
| October 1 | 1957 | 16.5 | 3.3 | 2.1 | 2.3 | 3.4 | 2.8 | 2.8 |
| October 5 | 1957 | 16.7 | 4.0 | 0.5 | 2.2 | 4.3 | 2.4 | 2.7 |
| Average | | 16.5 | 3.6 | 1.2 | 1.9 | 3.8 | 2.5 | |
| September 20 | 1958 | 17.1 | 5.5 | 3.0 | 4.5 | - | - | 4.3 |
| September 30 | 1958 | 16.5 | 4.2 | 2.9 | 3.5 | - | - | 3.5 |
| October 14 | 1958 | 17.9 | 6.3 | 5.1 | 6.0 | - | - | 5.8 |
| Average | | 17.2 | 5.3 | 3.6 | 4.6 | | | |

APPENDIX TABLE IX

Flesh Firmness According to Pressure Tests Given Various Prestorage Treatments at Harvest and After 210 Days in Controlled Atmosphere and Regular Storage (1957-58)

| Treatment | At Harvest | 32° F | | | 38° F | |
|---|---|---|---|---|---|---|
| | | Air | 5% $CO_2$ 3% $O_2$ | 13% $CO_2$ 3% $O_2$ | 5% $CO_2$ 3% $O_2$ | 13% $CO_2$ 3% $O_2$ |
| | (lbs) | (Pounds loss during storage) | | | | |
| 10 days delay at 55° F in air | 14.8[a] | 2.2 | 1.8 | 1.2 | 2.4 | 2.4 |
| Ringing | 16.5 | 5.3 | 2.7 | 2.8 | - | - |
| Defoliation | 16.5 | 3.4 | 2.8 | - | - | - |
| Thinning | 16.7 | 5.0 | 2.6 | - | - | 1.8 |
| Excess crop | 17.2 | 4.1 | 3.7 | - | - | 2.8 |
| Plastic bags | 15.9 | 3.6 | - | - | - | 2.7 |
| Check | 16.5 | 3.3 | 2.1 | 2.3 | 3.4 | 2.8 |

[a] After 10 days delay at 55° F in air; this fruit had a pressure of 16.8 pounds at harvest.

APPENDIX TABLE X

Fruit Firmness (Pounds) According to Pressure Tests of Jonathan Apples at Harvest. Fruit Harvested from Defoliated and Non-defoliated Areas of Four Trees from Five Growers (1958)

| Field Treatment | Growers | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Ave. |
| | (%) | (%) | (%) | (%) | (%) | (%) |
| Non-defoliated | 17.1[a/] | 18.0 | 17.7 | 18.2 | 17.1 | 17.6 |
| Defoliated | 17.1 | 17.9 | 17.5 | 17.9 | 17.1 | 17.4 |
| Grower average | 17.1 | 17.9 | 17.6 | 18.0 | 17.1 | |

ANALYSIS OF VARIANCE TABLE

| Source | Degrees of Freedom | Variance |
|---|---|---|
| Replication | 3 | 0.3 |
| Growers | 4 | 2.05 |
| Error (a) | 12 | 0.78 |
| Field treatment (defoliation) | 1 | 0.2 |
| Grower x field treatment | 4 | 0.16 |
| Error (b) | 15 | 0.26 |

[a/] Each entry average value of four trees.

## APPENDIX TABLE XI

Fruit Firmness (Pounds) According to Pressure Tests of Jonathan Apples after 210 Days in Controlled Atmosphere and Regular Storage. Fruit Harvested from Defoliated and Non-defoliated Areas of Four Trees from Five Orchards (1958-59)

| | Growers | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | |
| | Field Treatment (D = defoliated; ND = non-defoliated) | | | | | | | | | | |
| | ND | D | ND | D | ND | D | ND | D | ND | D | Ave. |
| 5% $CO_2$ - 3% $O_2$ | 13.0[a/] | 12.0 | 12.9 | 13.4 | 12.6 | 12.5 | 13.5 | 13.4 | 13.0 | 12.7 | 12.9 |
| 13% $CO_2$ - 3% $O_2$ | 14.6 | 14.6 | 15.6 | 15.8 | 15.3 | 15.6 | 14.7 | 14.4 | 14.0 | 14.6 | 14.92 |
| Air | 11.6 | 12.5 | 12.9 | 13.4 | 12.4 | 13.6 | 12.8 | 11.4 | 12.1 | 11.0 | 12.37 |
| Field treatment average | 13.1 | 13.0 | 13.8 | 14.2 | 13.5 | 13.9 | 13.7 | 13.1 | 13.0 | 12.8 | |
| Grower average | 13.05 | | 14.0 | | 13.7 | | 13.4 | | 12.9 | | |

ANALYSIS OF VARIANCE TABLE

| Source | Degrees of Freedom | Variance |
|---|---|---|
| Replication (trees within each grower) | 3 | 0.02 |
| Grower | 4 | 3.83* |
| Error (a) | 12 | 1.03 |
| Field treatment | 1 | 0.26 |
| Grower x field treatment | 4 | 0.91** |
| Error (b) | 15 | 0.1 |
| Storage atmospheres | 2 | 72.73** |
| Grower x storage atmospheres | 8 | 2.28** |
| Field treatment x storage atmospheres | 2 | 0.84 |
| Growers x field treatment x storage atmospheres | 8 | 1.19* |
| Error (c) | 60 | 0.45 |

[a/] Each entry average value of four trees.

APPENDIX TABLE XII

Percentage Soluble Solids of Juice from Jonathan Apples at Various Times of Harvest and Percentage Loss After 210 Days of Controlled Atmosphere and Regular Storage (1958-59)

| Time of Harvest | At Harvest | Change During Storage at 32° F | | | |
|---|---|---|---|---|---|
| | | Air | 5% $CO_2$ 3% $O_2$ | 13% $CO_2$ 3% $O_2$ | Average |
| September 20 | 12.0 | +1.0 | +0.7 | +0.5 | +0.7 |
| September 30 | 13.5 | -0.5 | -0.5 | 0.0 | -0.3 |
| October 14 | 13.5 | -0.3 | 0.0 | +0.2 | -0.1 |

Data for time of harvest studies 1957 not available.

## APPENDIX TABLE XIII

Percentage Soluble Solids of Juice from Jonathan Apples from Various Prestorage Treatments at Harvest and Percentage Loss after 210 Days in Controlled Atmosphere and Regular Storage (1957-58)

| Treatment | At Harvest (%) | Change During Storage At 32° F | | | 38° F | |
|---|---|---|---|---|---|---|
| | | Air | 5% $CO_2$ 3% $O_2$ | 13% $CO_2$ 3% $O_2$ | 5% $CO_2$ 3% $O_2$ | 13% $CO_2$ 3% $O_2$ |
| 10 days delay at 55° F in air | 13.0 | -0.3 | -0.8 | 0.0 | +0.2 | -0.3 |
| Ringing | 13.5 | -1.0 | -0.7 | -0.5 | +0.3 | - |
| Defoliation | 13.0 | -0.8 | -0.2 | - | - | - |
| Thinning | 13.0 | -0.5 | -0.2 | - | - | 0.0 |
| Excess crop | 12.5 | -0.6 | 0.0 | - | - | 0.0 |
| Plastic bags | 13.7 | +0.2 | - | - | - | -0.2 |
| Check | 13.5 | -0.5 | -0.4 | 0.0 | 0.0 | 0.2 |

## APPENDIX TABLE XIV

Percentage Soluble Solids of Juice from Jonathan Apples at Harvest. Fruit Harvested from Defoliated and Non-defoliated Areas of Four Trees from Five Growers (1958-59)

| Field Treatments | Growers 1 | 2 | 3 | 4 | 5 | Ave. |
|---|---|---|---|---|---|---|
| | (%) | (%) | (%) | (%) | (%) | (%) |
| Non-defoliated | 12.6[a/] | 13.6 | 13.5 | 14.0 | 13.5 | 13.45 |
| Defoliated | 12.2 | 12.7 | 12.1 | 12.9 | 12.4 | 12.45 |
| Grower average | 12.4 | 13.5 | 12.8 | 13.5 | 12.9 | |

ANALYSIS OF VARIANCE TABLE

| Source | Degrees of Freedom | Variance |
|---|---|---|
| Replication | 3 | 0.06 |
| Growers | 4 | 0.87 |
| Error (a) | 12 | 0.45 |
| Field treatment | 1 | 10.50** |
| Field treatment x growers | 4 | 0.37 |
| Error (b) | 15 | 0.39 |

[a/] Each entry average value of four trees.

## APPENDIX TABLE XV

Percentage Soluble Solids of Jonathan Apples after 210 Days in Controlled Atmosphere and Regular Storage. Fruit Harvested from Defoliated and Non-defoliated Areas of Four Trees from Five Growers (1958-59)

| | Growers | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | |
| | Field Treatment (D = defoliated; ND = non-defoliated) | | | | | | | | | | |
| | ND | D | ND | D | ND | D | ND | D | ND | D | Ave. |
| 5% $CO_2$ - 3% $O_2$ | 13.0[a] | 12.0 | 12.9 | 12.3 | 12.6 | 12.5 | 13.6 | 13.4 | 13.0 | 12.8 | 12.81 |
| 13% $CO_2$ - 3% $O_2$ | 12.9 | 12.5 | 12.6 | 13.2 | 14.0 | 13.9 | 14.3 | 14.0 | 13.9 | 13.2 | 13.95 |
| Air | 12.4 | 12.1 | 12.5 | 12.4 | 12.9 | 12.6 | 13.2 | 12.5 | 12.9 | 13.0 | 12.75 |
| Field treatment average | 12.8 | 12.2 | 12.7 | 12.9 | 13.2 | 13.0 | 13.7 | 13.3 | 13.2 | 13.0 | |
| Grower average | 12.5 | | 12.8 | | 13.1 | | 13.5 | | 13.1 | | |

ANALYSIS OF VARIANCE TABLE

| Source | Degrees of Freedom | Variance |
|---|---|---|
| Replication (trees with each grower) | 3 | 1.37 |
| Grower | 4 | 3.88 |
| Error (a) | 12 | 1.43 |
| Field treatment (defoliation) | 1 | 2.2** |
| Grower x field treatment | 4 | 0.25 |
| Error (b) | 15 | 0.53 |
| Storage atmospheres | 2 | 7.2** |
| Growers x storage atmospheres | 8 | 0.55 |
| Field treatment x storage atmospheres | 2 | 0.1 |
| Growers x field treatment x storage atmospheres | 8 | 0.35 |
| Error (c) | 60 | 0.32 |

[a] Each entry average value of four trees.

APPENDIX TABLE XVI

Total Carbon Dioxide (Grams) per Container at Standard Temperature and Pressure

| Total Volume of Atm/Container (Liter) | % $CO_2$/Container | Total $CO_2$ (Grams) |
|---|---|---|
| 4 | 50 | 3.954 |
| 4 | 25 | 1.977 |
| 4 | 13 | 1.028 |
| 4 | 5 | 0.395 |
| 1.2 | 25 | 0.593 |
| 1.2 | 13 | 0.315 |
| 1.2 | 5 | 0.119 |
| 12.6 | 25 | 6.426 |
| 12.6 | 13 | 3.153 |
| 12.6 | 5 | 1.246 |
| 19.6 | 25 | 9.687 |
| 19.6 | 13 | 4.942 |
| 19.6 | 5 | 1.937 |

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