FRUIT FACTORS AFFECTING GAS EXCHANGE AND THE DEVELOPMENT OF BROWN HEART IN JONATHAN APPLES STORED IN CONTROLLED ATMOSPHERES

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THESIS

This is to certify that the

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FRUIT FACTORS AFFECTING GAS EXCHANGE AND THE DEVELOPMENT OF BROWN HEART IN JONATHAN APPLES STORED IN CONTROLLED ATMOSPHERES

By

Dadi N. Kerawala

AN ABSTRACT OF A THESIS

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ABSTRACT

FRUIT FACTORS AFFECTING GAS EXCHANGE AND THE DEVELOPMENT OF BROWN HEART IN JONATHAN APPLES STORED IN CONTROLLED ATMOSPHERES

by Dadi N. Kerawala

Brown heart, a physiological disorder of apples that occurs during long-term storage at low temperatures in controlled atmospheres, is characterized by browning or necrosis of the core tissues. In severe cases voids develop and the browning may extend into the cortical tissues. Factors or conditions studied for their possible effects on the development and incidence of this disorder in Jonathan apples during controlled atmosphere storage included: fruit source by orchards and trees, time of fruit harvest, fruit load on trees, fruit size (weight, volume, and volume-weight ratios), composition of internal atmospheres and rates of gaseous exchange, epidermal punctures and waxing, and porosity measured from rate of gas flow through fruits. The storage studies were made at 40°F with atmospheres of approximately 18% CO_2 and 3% O_2, so as to favor the development of CO_2 injury.

The incidence and severity of CO_2 injury varied significantly for orchards and trees. Susceptibility increased with ripening at harvest, light crop loads on a tree and increased fruit size.

The incidence and severity of CO_2 injury differed significantly by apple variety, and incidence was positively correlated with the physical characteristics of fruit weight, volume, and volume-weight ratio. The volume-weight ratios differed by variety, but increased with increases in fruit weight in Rome Beauty, Delicious and Jonathan varieties, thus

indicating that cellular structure of fruits differed both by varieties and fruit size. In Jonathan fruit the correlation coefficients (r values, non-linear terms) for incidence of CO_2 injury and fruit weight, volume, and volume-weight ratio were 0.78, 0.79, and 0.51, respectively; and 0.60, 0.60, and 0.26 for internal breakdown incidence. CO_2 injury increased markedly from 9% in fruits of 121-125 grams in weight to 45% in 126 to 130 gram fruits, to 100% in fruits weighing 160 grams and more.

Fruits with brown heart and internal breakdown had significantly higher rates of O_2 diffusion than fruits of comparable size but free of these disorders. The rate of O_2 diffusion was significantly higher in large than in small fruits; also, CO_2 injury was increased by epidermal punctures which enhanced gas exchange in fruits.

Fruit porosity increased with increases in fruit size as measured by weight, volume and volume-weight ratio, the correlation coefficients being 0.71, 0.76, and 0.63, respectively. Porosity also increased with maturation. High porosity fruits had slightly higher rates of O_2 uptake at 30^oC than low porosity fruits, while the reverse occurred at 20^oC indicating gas exchange was not limiting for respiration. The similar correlations of fruit size to CO_2 injury and to porosity indicate that factors facilitating gas exchange favor increases in CO_2 injury and other internal disorders of stored apples.

The results of these researches suggest that a supply of oxygen to the tissues is essential for the development of internal disorders.

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DEDICATION

TO MY MASTER, AVATAR MEHER BABA, Without Whose Encouragement this work would never have begun, Whose inspiration throughout has brought it to this conclusion.

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INTRODUCTION

The controlled atmosphere (CA) storage of apples involves the utilization of low temperatures in conjunction with below normal levels of O_2 and above normal levels of CO_2 in the storage room atmosphere. compared to air. All three factors when properly applied, favorably affect fruit metabolism so that nearly year-a-round storage of apples is practical. Even so, difficulties may be encountered. The use of excessive levels of CO_2 in the storage atmospheres may promote the development of off-flavors and physiological disorders that are commonly referred to as CO_2 injury in a wide range of fruits, vegetables, bulbs and tubers (Thornton, 1931; Smith, 1963). In apples CO_2 injury may manifest itself as brown heart, characterized by tissue necrosis and browning of the tissues in core area. The disorder was first reported in relation to high CO_2 levels in 1920 (Anon., 1925).

Carne (1948) considers storage temperatures, variety and fruit maturity to be associated in CO_2 injury. Others (Nylen and Johansson, 1964) have studied CO_2 injury in apples from a nutritional and environmental standpoint, still others (Hulme, 1956; Thomas, 1925; 1929; Bogdanski, 1960) have hypothesized that the injury is caused by basic alterations in the intermediary metabolism of fruits when stored under critically high levels of CO_2. Thornton (1931, 1933, 1933a), Smock and Van Doren (1941), Rasmussen (1961) and Eaves _et al_. (1964) have directed research towards determining the role of both CO_2 and O_2 gases in the storage atmospheres in an attempt to obtain maximum duration of storage with little or no CO_2 injury. Consequently, considerable concern has developed about the regulation of both CO_2 and O_2 levels and current recommendations are well summarized by Hardenburg (1965).

With the technological advances made in recent years for the operation of CA rooms, the problem of CO_2 injury to apples and pears have, except for accidental occurrences, been largely eliminated. Dewey (1962) reports continued success in commercial storage of Jonathan apples, if recommended levels of CO_2 and O_2 are employed.

Nevertheless, occurrences of CO_2 injury including brown heart, and of internal breakdown have not been completely eliminated in commercial CA storage. The amount and severity of these disorders are known to vary from year to year and from one lot of apples to another under the same storage conditions. Even though most difficulties have been attributed to the storage conditions, the inherent characteristics of the fruit seem equally important.

This research was initiated to study the possible factors influencing the development of CO_2 injury to Jonathan apples under CA storage. This was approached by employing above normal levels of CO_2 (above 15%) and higher than normal temperature of 40°F in the CA storage, since these conditions tend to promote both brown heart (CO_2 injury) and internal breakdown disorders. Fruit factors of size, maturity at harvest, cropping levels, fruit finish, and others which are know, or thought, to influence these CA room disorders were investigated primarily from the standpoint of their possibly affecting the fruit structure and thus gas exchange rates. These and other factors were considered in relation to the likely influence of environmental and cultural factors, physical properties of apple fruits, pre-storage fruit treatment practices and fruit porosity.

REVIEW OF LITERATURE

Although elevated concentrations of CO_2, within certain limits, have a beneficial effect in prolonging the storage life of apples excessive concentrations can cause a physiological disorder known as brown heart. This is called CO_2 injury, with tissue necrosis and browning in the core region being the principal symptoms.

Unfortunately, the nomenclature or terminology for CO_2-induced storage disorders is not standardized and often creates confusion. As stated by Roberts et al (1965) "The terms low temperature breakdown, senescence breakdown, brown heart, CO_2 injury are all used to describe brown or dead cortical tissue of pome fruits. The descriptive nature of these terms implies that the cause of the disorder has been established but this is usually not so. We have often found several types of lesions in one fruit...We do not believe that it is feasible to differentiate consistently on symptoms alone. Critical metabolic studies appear to be necessary for identification of the cause of breakdown and may also produce a better diagnostic tool."

Roberts et al (1965), therefore suggested two types of breakdown: one, induced by high CO_2 and low O_2, the other, induced by low CO_2 and high O_2; the former type being due to the accumulation of toxic compounds, the latter to the death of tissue upon senescence. Some of the CO_2 disorders reported in the literature are: core flush (Kidd and West, 1936; Fidler and North, 1963); core browning (Eaves et al, 1964; Dewey, 1962); brown heart (Carne and Martin, 1938; Carne, 1948; Kidd and West, 1923, Smock and Van Doren, 1941; Mandeno and Padfield, 1953). Besides these, Smith (1963) lists low temperature injury and superficial scald of the skin tissues as being induced by high CO_2 levels in the storage atmospheres.

Environmental and Cultural Factors Associated with CO_2 Injury: Historically, Carne and Martin (1935) recorded brown heart in apples as early as 1911 in a shipment of apples from Australia to England. It was, however, not until 1918 that Kidd and West (1923) were able to reproduce it experimentally and to establish its relationship to excess CO_2 in the storage atmosphere. Carne (1950) mentioned the serious damage that occurred in the Australian shipments during the twenties and reported the following interesting incident "... while shipping companies in subsequent years slowly made some concessions to the findings of the DSIR* survey (team)...a rapid improvement was delayed by a court decision that brown heart was due to inherent vice in the fruit. In the meantime the disorder continued to turn up badly in at least one shipment from Tasmania every year with an occasional one from New Zealand. Incidentally, the legal decision that the fruit and not ship's carriage was responsible for brown heart was only reversed in 1940..."

Carne (1950) further observed that the estimates of actual loss were not possible, the number of fruits affected in a box ranged from a few to all depending upon fruit origin; apple fruits from Tasmania came to be recognized in the U.K. market as more susceptible to brown heart than fruits from the Australian mainland. His important conclusions were that for most varieties of apples the critical level for injury was about 10% but for Tasmanian Sturmer the safe limit was 3-4% CO_2. He reported minor occurrences of brown heart at concentrations below 4% in two shipments of Tasmanian Sturmer in 1949 and added "this coincided with an abnormally light crop in Tasmania

* Department of Scientific and Industrial Research, Great Britain.

for all varieties, probably the lowest per tree ever experienced in that State." Martin and Carne (1950) described Sturmer apples as very susceptible to CO_2 injury, Jonathan as relatively resistant to injury up to 12% CO_2 levels, and fruits of French Crab as intermediate in their response. Smith (1963) also pointed out that apple varieties varied greatly in their tolerance to CO_2, fruits of Bramley's Seedling tolerated 8-9% while for many varieties the safe limit was only 5% though some show injury at very low levels of CO_2.

Though considerable literature is available, little is known as to why varieties show such marked differences in CO_2 injury; Bunemann (1963), for instance, found 10% concentrations of CO_2 injurious to Jonathan, but not for the Golden Delicious, and attributed this to differences associated with the origin of the fruits. Smock and Van Doren (1941) found Rhode Island Greening, Jonathan, Baldwin and Wealthy quite susceptible to internal or core browning when stored under CO_2 atmospheres.

There are several environmental factors that influence the incidence of CO_2 injury in CA storages. Fruits of a variety grown under different environmental conditions, are reported to respond differently to CA storage atmospheres (Smock, 1944); Tasmanian Sturmer have been observed by Carne (1950) to be more susceptible to CO_2 injury than those from the Australian mainland. Plagge (1942) reported Iowa-grown Jonathan apples were able to tolerate up to 5% CO_2 at 40°F, while the New York-grown Jonathans developed severe injury under similar conditions of storage (Smock and Van Doren, 1941).

Dewey (1962) observed CO_2 injury in Michigan Jonathan occurred at 7% CO_2 or above and that the incidence was higher in overmature fruits and fruits with water-core at harvest. Chace (1959) also found the incidence of CO_2 injury (brown heart) was associated with advanced fruit maturity. Other workers including Carne et al (1938), Smock and Blanpied (1963), Trout et al (1940) also have reported increased susceptibility of fruits harvested late in the season. Phillips (1939) on the other hand, found McIntosh apples stored better and were less susceptible to the CO_2 injury if the climateric peak in respiration was allowed to pass on the tree. Hansen and Mellenthin (1962) observed that brown core in pears did not have a direct relationship to a specific CO_2 concentration, but varied according to the degree of susceptibility of the fruit to injury; the factors associated being over-maturity of fruit at harvest, fruit senescence due to over-storage, season and area where fruit was grown, tree vigor, delayed storage and cooling rates.

Australian workers (Anon., 1956) concluded that the influence of nitrogen on storage disorders was mainly through fruit size with little direct effect, and suggested that the ill effects of high nitrogen on yield and keeping quality may be less serious than the effects of nitrogen starvation. Workers in America and in Canada, however, believed high nitrogen applications produced softer fruits which impaired both table and keeping quality (Bunemann et al, 1959; Fisher and Porritt, 1951). Eaves et al (1964), on the other hand, found McIntosh apples from trees with low nitrogen fertilization more firm at harvest, but softened more rapidly in storage than fruits from trees with high nitrogen fertilization. Further, they observed that the CO_2 injury was severe in low nitrogen fruits stored at 5% CO_2 and O_2 levels above 2.5%. They found the index for core browning higher for air stored McIntosh apples from high nitrogen trees than from low nitrogen trees. This conflicting result is probably due to the fact that the authors had not attempted to differentiate between the two common disorders of core browning or brown heart caused directly by high CO_2 concentrations, and the internal breakdown disorder or senescent breakdown known to be associated with high oxygen levels in storage atmospheres. Nylen and Johansson (1964) correlated K and low P contents in the apple juice with a high incidence of brown heart and suggested that though the incidence varied greatly with fruit origin and season, the incidence was most pronounced in fruits from the light soils.

Storage Conditions Influencing the Incidence of CO_2 Injury: CO_2 injury is known to be influenced by the oxygen and carbon dioxide composition of the storage atmospheres, storage temperatures and the duration of storage. Research workers seem agreed that the incidence of CO_2 injury increases with increased concentrations of CO_2 in the storage atmospheres (Thornton, 1931; Stevenson et al, 1963; Carne et al, 1938; Chace, 1959; Johansson, 1964). A high correlation between brown heart and high CO_2 levels in the storage atmospheres has been reported (Anon., 1925). Wright (1953) considered the occurrence of brown heart in apples stored in sealed containers or heavily coated with waxes was due to the interference with normal air exchange.

Considerable evidence is available in the literature regarding the adverse effects of increasing concentrations of CO_2 in conjunction with fixed levels of O_2, yet experimental evidence on the possible involvement of different O_2 levels on the incidence of CO_2-induced disorders of core or internal browning are rather limited. Rasmussen (1961) demonstrated the important influence of O_2 concentration on the occurrence of internal browning in Cox's Orange Pippen apples stored under increasing concentrations up to 6% CO_2. He found the percentage internal browning to be consistently higher with 9% O_2 in the storage atmospheres than with 3% O_2. The findings of Eaves <u>et al</u> (1964) on the storage of McIntosh apples in atmospheres of 5% CO_2 and varying amount of O_2 (2.5 to 20%) also indicated that the incidence of core browning was particularly serious at O_2 concentrations above 2.5%. Fidler and North (1963) studied the incidence of core flush in several English apple varieties stored under 5 to 6% O_2. They considered CO_2 as the causal agent but were not able to explain the beneficial effects of low O_2 concentrations which reduced the incidence of core flush. Smock and Blanpied (1963) studied the incidence of internal CO_2 injury to McIntosh apples stored in 2% CO_2 1 month, then 5% CO_2 with 3% O_2 at 35^{o}F, and found the effects of a rapid O_2 reduction at the beginning of CA storage resulted in no benefits in some experiments though in others it gave slightly firmer fruit and less scald. In another study, Blanpied and Smock (1961) found that the O_2 level in the storage had no effect on the internal CO_2 injury, but was inversely related to the incidence of external CO_2 injury. Hansen and Mellenthin (1962) also reported a variable susceptibility to brown core for several pear varieties; the damage was influenced by the CO_2:O_2 ratio in the storage atmospheres and the amount of injury at a specific CO_2 concentration was greatly increased at reduced O_2 levels,

Similarly, the influence of storage temperature on the incidence of CO_2 injury are not clear cut. Thus, Blanpied and Smock (1961) could give no logical explanation for the lack of agreement between two years on the effect of temperature on the incidence of internal CO_2 injury to CA-stored McIntosh apples and attributed this to seasonal variations in fruits.

Subsequent studies of Smock and Blanpied (1963) did however indicate that off-flavors and excessive CO_2 damage occurred to McIntosh apples when stored at 32°F or 35°F, but not as much when stored at 38°F. Carne et al (1938) reported a higher incidence of brown heart in apples stored at 38° and 44°F than at 32° - 34°F. Mandeno and Padfield (1953) also found brown heart to occur at temperatures exceeding 40°F, but not at 38°F, for both Jonathan and Sturmer varieties. Kidd et al (1923, 1927), however, reported that apples were more susceptible to brown heart at lower than higher temperatures; they attributed this to excessive concentrations of CO_2 in the tissue fluids at lower temperatures and suggested that "for most effective gas storage at lower temperatures (the atmosphere) should contain less O_2, and at higher temperatures more O_2, than the equivalent of the safety maximum concentrations of CO_2." Smock and Van Doren (1941) also considered temperatures lower than 40°F could become a factor in the development of CO_2 injury. Dewey et al (1957) showed brown heart incidence was related to high temperatures and high CO_2 levels during storage; however, studies of Chace (1959) revealed that the injury was more serious under low temperatures and high CO_2 levels.

Duration of storage is also a factor in the development of CO_2 injury, but it is affected by the temperature of storage, and the concentration of CO_2. Carne (1948) considered high concentrations of CO_2 in the storage atmospheres induced injury earlier than low concentrations. Mandeno and Padfield (1953) studying the brown heart disorder in several apple varieties stored in 8% CO_2 at 41°F, observed the injury to occur only after 21-22 weeks storage. Roberts et al (1964) found the incidence of brown heart in Williams Bon Chretien pears increased linearly from about

9% after 56 days to about 51% after 117 days of storage in polyethelene bags.

Several workers have shown that the injury was caused by basic alterations in the intermediary metabolism of fruits stored under critically high levels of CO_2. Hulme (1956) considered the injury was brought about by the inhibition of succinic oxidase system by high levels of CO_2 since toxic levels of succinate were found in apple tissues affected with brown heart. Thomas (1929) observed that changes in the respiration of apple cells under high CO_2 levels resulted in the production of ethyl alcohol and acetaldehyde which then caused brown heart. Bogdanski (1960) on the other hand, suggested that apples stored under high CO_2 atmospheres, the O_2 concentration in the fruit's internal atmosphere closely approximated the external atmosphere and that this caused the oxidation of the ascorbic acid present in the so-called "low-zone" areas, namely, the core region shown to be lowest in ascorbic acid (Bogdanski, 1960a). According to him complete oxidation of ascorbic acid preceded the development of the brown heart. Smock and Neubert (1950) have reported results of studies which showed greater or more rapid losses of ascorbic acid in CA-stored apples than those stored in air. Thornton (1933a), who examined at great length the influence of CO_2 on the acidity of the plant and fruit tissues, observed that in the absence of O_2 there was a decrease in the pH of the tissue, while in the presence of O_2 there was decided increase in the pH and this alkaline reaction was harmful to the tissues. He also concluded that (a) CO_2 injury was related directly to the firmness of fruit tissues; (b) toxicity of CO_2 increased with increase in temperature; and (c) in rapidly respiring materials, the O_2

content of the atmosphere may be the controlling factor in CO_2 injury.

Fruit Factors Related to Storage Disorders: Carne (1948) discussed at length the various factors influencing the susceptibility of apples to non-parasitic disorders; among those listed were:

a) Varietal characteristics of: specific gravity - fruits of 'heavy' varieties were found to store better than 'light' varieties; season maturity - early maturing varieties had shorter storage life than late maturing varieties; skin waxing - varieties with pronounced waxy bloom generally stored better (without becoming mealy and flavorless) than those with little bloom such as Jonathan and Delicious.

b) Size of fruit and crop - in general large fruits and fruits of light crop were observed to be more susceptible to physiogenic disorders than small fruits or fruits of heavy crops. Also, length of optimum picking time was much shorter and fruit maturity more difficult to determine in light than in heavy crop fruits. He also found light crop apples had higher penetrometer tests than heavy crop fruits.

c) Over-maturity at harvest; and

d) Climatic conditions - particularly low temperatures during 3 weeks or so preceding the normal harvest time increased the susceptibility to low temperature breakdown whereas high temperature decreased the susceptibility.

Martin (1953, 1954, 1954a) reported a high correlation between susceptibility to disorders of pit or breakdown and fruit size. He further observed that the correlation of the disorder breakdown with fruit diameter was extremely high and "the mean fruit diameter per tree is by far the best index of phsyiological behavior of the fruit from it, being more reliable than any measure of crop in terms of numbers of any of the common chemical or physical changes associated with ripening." And concluded "...in the final analysis, seasonal variation in the level of disorders was mainly related to differences in mean fruit size and when the size factor was held constant between seasons the differences remaining were relatively small."

Smock and Van Doren (1941) reported all Jonathan apples over 2 1/2 inches in diameter were severely affected with flesh browning when stored at 5% CO_2 and 2% O_2 at 40°F. In another study, they found Jonathan apples developed 25% browning when stored at 5% CO_2 and 15% O_2 at 45°F, while the injury increased to 90% with 10% CO_2 and 10% O_2. Chace (1959) also found brown heart to increase from 4% for 2 1/4 inch diameter fruits to 17% for 3 inch diameter fruits of Jonathan variety at the end of 7 months storage in 13% CO_2 and 3% O_2 at 32°F. His most interesting finding was related to the quantity of ^{14}C recovered from $^{14}CO_2$ in the atmosphere in core, flesh and peel of Jonathan fruits of 4 different diameters; again the 3 inch diameter fruits consistently had higher cpm/gram tissue as compared to fruits of lesser diameters. Allentoff (1954) not only found a linear relationship between the rate of CO_2 fixation and the concentration of external CO_2 but also the rate of fixation at harvest to be concurrent with the respiratory climateric.

Several workers have studied the relationship of fruit size to cell number and cell size. Smith (1940) showed a correlation between fruit size and cell size. Larger apples had larger cells, and though they also had a larger number of cells than smaller apples, he concluded that the better keeping varieties had a longer growing period, smaller cell numbers and lower respiration rates, while poorer keeping varieties were early maturing and higher in cell numbers and respiration rates. By further study Smith (1950) showed that mean individual fruit weight at harvest for a given tree or set of trees varied from year to year, being higher in light crop years than in heavy crop years, and that while mean cell volume also varied from year to year, no strict relation to fruit size was observed. From this he concluded that fruit weight may be determined one year primarily by amount of cell multiplication while in another by amount of cell enlargement. Denne (1960), however, showed that variations in fruit size within a tree and between trees were related to both cell size and cell number; and that late thinning appeared to increase fruit size by increases in cell number. Martin et al (1964) also confirmed the effects of thinning on the increase in cell numbers. Letham (1961) showed significant correlation between mean cell volume and internal breakdown in each of the three years studied. Sharples (1967) however failed to observe a similar relationship, though he observed a significant correlation between fruit weight and cell number, while respiration per cell was correlated with cell volume. Even though Bain and Robertson (1951) found variations in fruit size mostly due to variations in cell number and only to a small extent to mean cell size; they observed that cell enlargement continued throughout the life of the fruits on the tree. A most significant finding of theirs was that the air space as percent of fruit volume in

Granny Smith apples increased rapidly with size of fruit and related this with the decrease in the specific gravity of the fruit with increase in weight. They further observed that air space as percent of fruit volume "in the large fruits approaches an asymptote at about 27% of volume." Reeve (1953) estimated that the intercellular spaces or air space averaged 20-22% for Delicious as compared to 23-24% for Rome Beauty and contended that such differences in structure and texture of apple fruits can influence the gas content of stored apples. He quoted the observation of Bigelow _et al_ that gas content increased while specific gravity decreased with mealiness and maturity in apples. Mohsenin _et al_ (1965) studied the influence of maturation on physical and mechanical properties in 6 apple varieties and found that specific gravity was significantly related to fruit maturation; and except for the Delicious and Stayman varieties, there was a slight decrease in specific gravity with maturation. Similar findings are reported by Westwood (1962) and by Blanpied (1966).

Williams (1961) concluded that high levels of CO_2 and low levels of O_2 in the internal atmosphere of Bartlett pears, stored over prolonged periods at 10-20% CO_2, did not give rise to anaerobic conditions inside the fruit. He attributed CO_2-induced core breakdown to the inhibition of certain normal biochemical processes, with concomitant production of certain metabolites in toxic amounts. Cerny _et al_ (1964) found that the susceptibility of apple fruits stored under regular storage at $1^{o}C$ to bitter pit, Jonathan spot and breakdown disorders were not related to the composition of the internal atmospheres. They further reported that in spite of wide differences observed in respiration rate, nitrogen content, cell volume, etc., in externally similar fruits from one tree, the differences in internal atmosphere were very small. They concluded that the internal atmosphere in Cox and Jonathan varieties was mainly controlled by the permeability of skin and flesh, and that this permeability tends to be lower in heavy crop fruits. Trout _et al_ (1942) examined the internal atmospheres of Granny Smith apples using intact fruits, peeled fruits, and fruits with the skin punctured or partly removed. The O_2 content of the internal atmospheres increased in direct proportion to the extent the skin was ruptured or removed, with that of peeled fruits closely resembling normal air. They observed that the gas composition throughout the flesh was rather uniform thereby suggesting the skin as the important barrier in gas exchange. Their other findings were that the respiration rate was related to the internal O_2 concentration; fruits exposed to air at 21°C had internal O_2 levels which declined greatly over 37-day period suggesting an increase in the resistance of the skin to O_2 diffusion. However, no marked changes were observed in the internal CO_2 concentration; this indicated a proportionately greater increase in the resistance to O_2 diffusion than to CO_2 gas. The investigations of Hackney (1944, 1944a) on internal atmospheres and on gas diffusion rates confirmed these findings, and showed that greater internal O_2 concentrations occurred in Granny Smith apples with open lenticels than in comparable apples with closed lenticels. He further suggested that investigations of respiratory metabolism should be augmented by studies of the resistance of fruits to gaseous diffusion. Clements (1935) reported that although environmental and cultural factors affect both the number and quality (open or closed) of the lenticels formed in a given apple variety, the CO_2 gas within apple tissues escaped with equal speed whether the fruit had many or few open lenticels. Marcellin (1956) had also presented evidence to show that CO_2 gas moves through the entire fruit surface and that the cuticular pathway appeared to be the most important with the lenticels playing no particular role. The observations of Hall et al (1955) and of Smith (1954) were in general agreement with these results. Burg and Burg (1965) showed the apple peel to be of major importance, offering considerably more resistance to gas diffusion than the apple flesh; but observed that gas exchange in apples was quantitatively accounted for by diffusion through lenticels alone. Hoff and Dostal (1966), on the other hand, reported great variability in fruit porosity between varieties and between fruits within a variety; fruits of Delicious variety after harvest were found to show extremely low porosity values whereas fruits of Jonathan exhibited very high values for gas flow. They believed fruit porosity to be a property of individual fruits rather than a function of unit weight or volume of fruit tissue and have shown that the resistance offered by the peel and pulp of a fruit to gas flow were additive in nature. The work of Wilkinson (1965) is significant because of a non-destructive technique used for studying the permeability to air in apple fruits stored under humid or dry conditions. Permeability to air was found to increase with time when the fruits were stored under humid conditions, while it declined sharply under dry conditions due to concomitant shrinkage of both skin and flesh tissues. Such changes in permeability suggest the need for taking into consideration many fruit factors including variety, size, and age in all studies pertaining to gas exchange

FRUIT DISORDER TERMINOLOGY AND RATING

The terms employed for the several disorders studied in the experiments reported here are as follows:

CO_2 Injury - includes the two disorders of:

Brown heart: Severe CO_2 injury manifested as chocolate colored browning of the core tissue which, in more severe cases, extends into the cortex. With prolonged storage "voids" usually develop in the core region (see Figure 1, A and B).

Simple CO_2 injury: A milder manifestation of the injury seen as slight brown discoloration of the core tissue (see Figure 1, C-F). In Figure 1D, both simple CO_2 injury and brown heart are seen.

Internal breakdown disorders - includes all senescence type of breakdown disorders other than CO_2 injury:

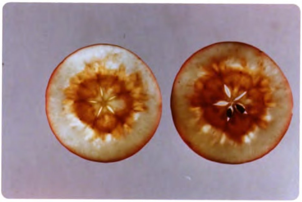
These are characterized by a general browning and softening of the cortical tissues mostly in the peripherial region (see Figure 1B). In severe cases the cortical tissues become spongy or soggy in texture.

Water core: A physiological condition observed in overmature fruits at harvest. Affected tissues in the core and the cortex are characterized by water congestion giving a glassy translucent appearance especially around the vascular bundles of the fruits. Figure 1A shows the CO_2 injury as brown heart of a water cored Jonathan apple. The translucent areas were congested with water.

Figure 1a. Carbon dioxide injury as brown heart in a water cored Jonathan apple after storage in 18% CO_2, 3% O_2 at 40°F. The severely browned area in the core and the adjacent cortical tissue was probably congested with water at the time of fruit harvest.

Figure 1b. Various manifestations of carbon dioxide injury as brown heart in Jonathan apples. Upper left: Slight injury; Upper right: Moderate injury; Lower left: Severe injury; Lower right: Severe injury plus severe internal breakdown.

Figure 1c. Simple carbon dioxide injury in the pith and cortex of Jonathan apple. The damage was rated as severe.



a

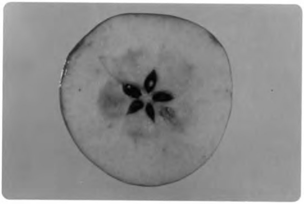
b

c

Figure 1d. Simple carbon dioxide injury in the pith and cortex of Jonathan apple. The damage was rated as severe. Brown heart, a more severe form of injury, is seen developing in one of the carpels.

Figure 1e. Simple carbon dioxide injury in the core tissue of Rome Beauty apples. Left to right, the damage was rated as slight, moderate, and severe.

Figure 1f. Simple carbon dioxide injury mostly in the core tissue of McIntosh apples. Left to right, the damage was rated as slight, moderate, and severe.



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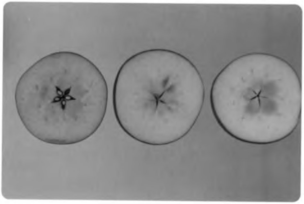

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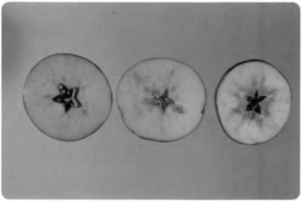

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Severity Index: The incidence and severity of these disorders were determined by making several transverse cuts through the fruit. The following score-card ratings were used for calculating the severity indices for the disorders:

	CO_2 Injury		
Severity Rating	Simple	Brown Heart	Internal Breakdown 1/
Sound fruits	0	0	0
Slight disorders	1	2	2
Moderate disorder	2	4	4
Severe disorder	4	8	8

1/ Same indice ratings were used for water core.

PART I. THE PREDISPOSITION OF APPLES TO CARBON DIOXIDE INJURY BY ENVIRONMENTAL FACTORS

Among the many factors of possible influence on the incidence or development of carbon dioxide injury in the apple fruit during CA storage are those of an environmental nature that occur in the orchard or prior to storage. Some of the factors observed or reported by fruit handlers and growers were examined in this respect.

A. The Influence of Fruit Size, Harvest Maturity and Water Core Condition at Harvest on the Incidence of CO_2 Injury: Exploratory studies made in 1964-65 had indicated that carbon dioxide injury was related to these factors. Apples with severe water core showed the initiation of carbon dioxide injury as typical dark brown discoloration in the water-conjested tissues (Figure 1A). Because water core is considered to be a disorder associated with late picking and over-maturity at harvest, these factors were considered for study together with water core in the fall of 1965.

It is not easy to assess accurately the effect of water core on CO_2 injury, primarily because of the lack of a good non-destructive technique to separate fruits with water core from those free of this condition, and to further categorize them into slight, moderate and severe water core. Brooks and Fisher (1926) and others (Carne, 1948; Lord and Southwick, 1964) noted that delaying apple harvest was conducive to the development of water core. A study was made in 1965-66 season to determine the relationship of water core condition to the development of CO_2 injury during storage.

<u>Experimental</u>: Time of harvest was used for obtaining fruits free of water core (early harvest) and with water core by delaying harvest. It was however recognized that in delaying the harvest the ill effects of fruit over-maturity get confounded; this is not desirable because earlier studies have shown susceptibility to CO_2 injury increases with over-maturity at harvest. But, the effect of water core with advancing fruit maturity was minimized as far as possible, by selecting trees at the MSU Horticulture Farm, fruits of which showed varying amounts of water core. Thus, fruit from tree 1 picked on October 14, 1965 was found to be free of water core (Lot A); whereas, with delayed picking on October 22, up to 17% of the fruits showed some degree of water core, with an index of 0.30 (Lot B). Fruits of Lot C, also picked on October 22, were from tree 2 and showed up to 55% water core condition, though the severity index was only 0.80 by a score rating described earlier. After each harvest, fruits were grouped into 2 lots of above 2 1/2 inches in diameter and of under 2 1/2 inches but not less than 2 inches in diameter. The data were analyzed as randomized block design with three factors replicated two times as follows:

A. Two fruit sizes...large and medium;

B. Three degrees of water core condition at harvest... none, slight and moderate;

C. Three examinations...80, 165 and 194 days in storage.

Fruits from the early harvest were held at 32°F until October 22, when they were removed for storage in metal chambers at 40°F. Since incidence of core browning was observed by Chace (1959) to be much higher at 13% level of CO_2 than at lower concentrations, 15% CO_2 was maintained in the storage atmospheres. Starting October 25, 1965, a prepared gas mixture of 15.0± 2.0% CO_2 and 17.8± 2.0% O_2 with the balance of the atmosphere being nitrogen was passed through each chamber at a constant rate of approximately o.75 cubic foot per hour. Each of these chambers held 2 bushel of apples and were sealed reasonably tight to permit the desired composition of atmosphere. At the end of 80, 165, and 194 days of storage, the fruits were examined for the incidence and severity of the storage disorders of CO_2 injury and internal breakdown.

The percentage fruits affected and the average severity of the disorders (index) in question were determined for each treatment. Arcsin $\sqrt{\text{percentage}}$ transformations were made in the case of percent fruits showing the disorders before the analysis of variance was made.

Results and Discussion: The results are summarized in Table 1. Duration of storage had a highly significant influence on the incidence and intensity of the two disorders; the greater the length of storage the greater was the incidence of brown heart and internal breakdown with the difference between 80 and 165 or 194 days and between 165 and 194 days being highly significant. Essentially the same trend was observed for severity (index) of these disorders. There was also a highly significant relation of brown heart to fruit size, being approximately three times more

Table 1. The effect of fruit size, water core and storage duration on brown heart and internal breakdown in Jonathan apples stored in 15% CO_2 and 18% O_2 at 40°F in 1965-66.

Factor	Brown heart Incidence Percent	Brown heart Incidence Severity index	Internal breakdown Incidence Percent	Internal breakdown Incidence Severity index
Fruit size:				
Large	29.9	2.0	32.0	2.4
Small	10.6	0.8	20.6	1.5
Significant values (Tukey's)...5%	4.0[a/]	0.4	N.S.	N.S.
...1%	5.5[a/]	0.6	N.S.	N.S.
Degree of Water core:				
None (Early harvest)	11.4	0.8	16.0	1.1
Slight (Late harvest)	26.1	1.7	29.7	2.1
Moderate (Late harvest)	21.7	1.7	33.7	2.6
Tukey's values...5%	6.0[a/]	0.6	11.4[a/]	1.3
...1%	7.8[a/]	0.8	N.S.	1.7
Duration of storage				
80 days	4.1	0.4	5.7	0.4
165 days	24.6	1.5	29.8	2.0
194 days	36.0	2.3	50.5	3.5
Tukey's values...5%	6.0[a/]	0.6	11.4[a/]	1.3
...1%	7.8[a/]	0.8	14.9[a/]	1.7

[a/] Values given in arcsin $\sqrt{\text{percentages}}$.

prevalent and severe in large than in small fruits; however, the differences in internal breakdown were non-significant.

The influence of water core condition at picking on the storage disorder incidence appears to be primarily an effect of over-maturity associated with late picking. Thus, no significant differences were observed with regard to both incidence and severity of brown heart and internal breakdown between the two lots of fruits picked late, but having slight and moderately high incidence of water core. However, significant differences were noted between the early picked lot (no water core) and the two late picked fruit lots. It is possible that the absence of significant differences in storage disorders in fruit lots having slight and moderately high incidence of water core were probably due to about the same indices of severity for water core condition, namely 0.30 and 0.80, respectively.

The only significant interaction observed for brown heart was fruit size with storage duration. From Table 2, it is apparent that both the quantity and severity of brown heart increased significantly with prolonged storage for the large fruits, but for small fruits there were no significant increases for 194 days of storage over 165 days.

Table 2. The interaction of storage duration on brown heart incidence or severity in large and small Jonathan apples stored in 15% CO_2 and 18% O_2 at 40°F in 1965-66.

Length of storage (days)	Brown heart Incidence Percent Large	Small	Severity Large (index)	Small (index)
80	8.4	1.3	0.6	0.1
165	32.9	17.1	2.0	0.9
194	54.4	19.5	3.4	1.2
(Tukey's) ...5%	10.5[a]		1.0	
...1%	N.S.		1.3	

[a]/Value given in arcsin $\sqrt{\text{percentage}}$

B. The Influence of Orchard Environments, Trees and Fruit Maturity Upon CO_2 Injury: The experimental trees of another experiment conducted by the department were utilized for purposes of this study. Fruits of 3 harvest dates from 2 trees in 7 orchards were used. In general, respiration rates were at the pre-climacteric minimum for fruits picked on September 26 (orchards 1 through 3) and September 29 (orchards 4 through 7); mid-climacteric for those picked on October 6 and 10; and at about the climacteric peak for those picked on October 17 and 20. A bushel of apples was harvested each time from each tree and stored in air at 32°F until October 21, when the room was sealed and the temperature raised to 40°F. The composition of the storage atmosphere was adjusted daily to approximately 18% CO_2 and 3% O_2. The room was opened on February 28, 1967 and the fruits were examined through March 3 for CO_2 injury and internal breakdown. Arcsin $\sqrt{\text{percentage}}$ transformations were made for percent fruits showing the disorders. The analysis of variance was made on the data recorded using a completely randomized design with the two trees as separate observations for each orchard location.

Results and Discussion: Both incidence and severity of internal breakdown were non-significant for orchard environments, trees, and fruit maturity. Significant differences occurred for incidence and severity of CO_2 injury and these data are summarized in Table 3.

Highly significant differences in the amount and intensity of CO_2 injury were found for orchard trees and time of harvest. The only significant interaction between orchards and harvest date observed was for percentage of affected fruit in respect to total CO_2 injuries;

Table 3. The incidence of CO_2 injury in Jonathan apples after storage in 18% CO_2 and 3% O_2 at 40°F in 1966-1967 according to orchard location and time of harvest.

	Orchard							
	1: Heuser	2: Mandigo	3: Stover	4: Graham Station	5: MSU Hort Farm	6: Klackle	7: Smith	Mean
	Total CO_2 Injury - Percent							
Harvest:								
Early	0.5	0.0	3.4	0.0	0.0	0.0	20.4	1.1
Mid-season	0.8	0.0	4.2	0.5	0.0	0.0	41.7	2.3
Late	24.4	6.9	7.6	1.8	0.0	0.5	58.7	9.0
Mean	5.0	0.8	4.9	0.5	0.0	0.1	39.6	
	Brown heart - Percent							
Early	0.0	0.0	2.6	0.0	0.0	0.0	9.0	0.4
Mid-season	0.2	0.0	3.6	0.0	0.0	0.0	9.5	0.6
Late	1.0	3.3	6.7	0.0	0.0	0.2	13.5	1.9
Mean	0.2	0.4	4.1	0.0	0.0	0.03	10.6	
	Total CO_2 Injury - Index							
Early	0.01	0.00	0.37	0.00	0.00	0.00	0.72	0.16
Mid-season	0.04	0.00	0.42	0.01	0.00	0.00	1.37	0.26
Late	0.46	0.26	0.85	0.04	0.00	0.03	1.82	0.50
Mean	0.17	0.09	0.55	0.02	0.00	0.01	1.31	
	Brown heart - Index							
Early	0.00	0.00	0.35	0.00	0.00	0.00	0.60	0.14
Mid-season	0.02	0.00	0.41	0.00	0.00	0.00	0.88	0.19
Late	0.12	0.21	0.83	0.00	0.00	0.02	1.04	0.32
Mean	0.05	0.07	0.53	0.00	0.00	0.01	0.84	

Continued on next page.

Table 3. Continued

	Orchard							
	1: Heuser	2: Mandigo	3: Stover	4: Graham Station	5: MSU Hort Farm	6: Klackle	7: Smith	Mean
	Total CO_2 Injury - Percent							
Tree 1	1.3	0.6	0.0	0.0	0.0	0.2	27.8	1.3
Tree 2	10.9	1.0	18.6	1.9	0.0	0.0	52.1	6.7
	Brown heart - Percent							
Tree 1	0.0	0.2	0.0	0.0	0.0	0.1	7.7	0.3
Tree 2	1.0	0.6	15.9	0.0	0.0	0.0	13.8	1.9
	Total CO_2 Injury - Index							
Tree 1	0.05	0.07	0.00	0.00	0.00	0.02	0.92	0.15
Tree 2	0.29	0.10	1.09	0.04	0.00	0.00	1.69	0.46
	Brown heart - Index							
Tree 1	0.00	0.05	0.00	0.00	0.00	0.01	0.65	0.10
Tree 2	0.09	0.09	1.06	0.00	0.00	0.00	1.03	0.32

Requirements for Significance (Tukey's Value):

	Total CO_2 Injury Arcsin $\sqrt{\text{percentage}}$		Brown heart Arcsin $\sqrt{\text{percentage}}$		Total CO_2 Injury Index		Brown heart Index	
	5%	1%	5%	1%	5%	1%	5%	1%
Orchard	8.8	11.0	6.8	8.6	0.58	0.73	0.50	0.63
Harvest	4.4	5.8	3.4	N.S.	0.27	N.S.	N.S.	N.S.
Orchard x harvest	18.9	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Tree	2.9	4.1	2.3	3.2	0.19	0.27	0.17	N.S.
Orchard x tree	14.3	17.5	11.2	13.9	0.96	N.S.	0.83	N.S.

whereas the interaction between orchards and trees were significant both for total CO_2 injury and brown heart incidence at the 1% level, and intensity at the 5% level.

The mean CO_2 injury recorded for all fruits stored from the 7 orchards was only about 9% even though the CO_2 level was quite high (approximately 18%); only the Smith Orchard showed excessive injury and these also had extensive water core at harvest. Though significant increases in the incidence of CO_2 injury were recorded with advancing fruit maturity, it is noteworthy that fruits from as many as 5 of 14 trees failed to show CO_2 injury and in 4 of the remaining 9 trees the injury was a mere 2 to 9%. It was not possible, however, to relate differences in susceptibility to CO_2 injury to differences, if any, in the respiratory climacteric of fruits from different trees, since the data on respiration rates of individual trees were not maintained, though data for each orchard are available. This suggests that fruit maturity *per se* does not directly influence the development of CO_2 injury though in some cases it seems to have a predisposing influence.

C. The Influence of Cultural Practices Upon the Incidence of CO_2 Injury: Jonathan fruits from the Concord and Gibson Blocks at Graham Station were used. Trees in the Concord Block had received applications of nitrogenous fertilizers each year and were of vigorous growth with dense green foliage, and in general had heavy crops of 15 to 20 bushels or more per tree; whereas, the trees in the Gibson Block had received no fertilizer applications during the past two years and were decidedly poor in vigor, with yellowish foliage, and with low yields.

Experimental: A bushel of apples was harvested on October 10, 1966 from each of two trees randomly selected in the Concord Block at the Graham Experiment Station. Similarly, two bushels were taken from the Gibson Block - a bushel, which was all that could be picked from two trees and another bushel from a tree bearing a fairly good crop of about 10-12 bushels. Fruits were taken the same day to a small CA room and held at 32°F until October 21, when the room was sealed and the temperature raised to 40°F. They were held under approximately 18% CO_2 and 3% O_2 atmospheres until February 28, 1967 when examined for storage disorders. The data were tested for significant differences by the chi-square test for independence (Snedecor, 1962).

Results and Discussion: Testing of the null hypothesis showed the storage behavior of fruits due to fertilizer pre-treatment was not independent. It will be seen from Table 4 that greatest single contribution seems to have been the yield of the trees as demonstrated by the highly significant chi-square values obtained for the heavy and the light producing trees from the Gibson Block (no fertilizer applications). Yet, when the chi-square test was made for the heavy cropping trees from the Concord Block (fertilizers applied) and that from the Gibson Block (no fertilizers applied) the values for simple CO_2 injury and total CO_2 injury were non-significant. However, the value was highly significant for incidence of brown heart.

General Discussion: The evidence indicates that fruit size is perhaps the most important factor affecting CO_2 injury, with the large fruits being more susceptible to damage than small fruits. Fruit maturity, orchard location, trees, and the cropping levels are among

Table 4. The incidence of storage disorders in Jonathan apples from orchards of different fertility levels after storage in 18% CO_2 and 3% O_2 at 40°F in 1966-67.

Orchard Practice	Number of fruits examined	Fruit disorders: Simple CO_2	Brown heart	Total CO_2 injury	Internal breakdown
		(No. of affected fruits)			
Orchard					
A [1/]...Fertilizers applied	200	12	0	12	0
B [2/]...No fertilizers applied	200	6	42	48	38
Chi-square test and significance		(1.1)N.S.	(46.7)**	(26.8)**	(42.5)**
B...No fertilizer applied:					
Heavy cropping trees	100	2	6	8	0
Light cropping trees	100	4	36	40	38
Chi-square test and significance		(4.23)*	(36.5)**	(36.5)**	(54.4)**
A...Fertilizers applied:					
Heavy cropping trees	200	12	0	12	0
B...No fertilizer applied:					
Heavy cropping trees	100	2	6	8	0
Chi-square test and significance		(1.35)N.S.	(14.9)**	(0.8)N.S.	----

[1/]A-Concord Block

[2/]B-Gibson Block

the other factors affecting the incidence of CO_2 injury. Fruit size is conceivably affected by most of these factors.

PART II. PHYSICAL CHARACTERISTICS OF APPLE FRUITS AS RELATED TO FRUIT SIZE AND TO THE INCIDENCE OF CO_2 INJURY

Since a significant effect of fruit size on the development of CO_2 injury was established, it was desirable to study the physical properties of the apples as related to fruit size and to the incidence of CO_2 injury. This problem was initiated by a study of some commercially important apple varieties, particularly because Carne (1948) and Smith (1963) found apple varieties to differ greatly in their tolerance to CO_2 levels in storage. McIntosh and Jonathan varieties are known to be susceptible to CO_2 injury; whereas, Delicious and Golden Delicious are not as prone to injury (Smock, 1949; Bunemann, 1963).

A. <u>The Incidence of CO_2 Injury as Related to Apple Variety</u>: Storage studies for the six varieties of Foster Jonathan, King Jonathan, Rome Beauty, Delicous, Golden Delicious and McIntosh were carried out under high CO_2 atmospheres. These studies were followed by a study of physical characteristics of Rome Beauty, Delicious, Golden Delicious and McIntosh fruits to determine if there were any characteristic differences related to CO_2 injury.

<u>Experimental</u>: Fruit samples of all varieties except McIntosh were obtained from the Graham Station; McIntosh fruits were obtained from the Smith Orchard near Greenville. The harvests were made on October 10, 1966 in one bushel quantities of apples from each of two trees selected at random. They were stored immediately at 32°F until October 21 when the room was sealed for CA and the temperature raised to 40°F and the

composition of the storage atmosphere adjusted to approximately 18% CO_2 and 3% O_2.

Two lots of McIntosh were obtained from the Smith Orchard; one bushel came from a tree that had been sprayed with 2,000 ppm of Alar (N-dimethyl amino-succinamic acid) on July 20, 1966; the other bushel was picked from an unsprayed comparable tree.

Two bushels of severely russetted Golden Delicious, picked from two trees at the Graham Station, were also stored for observation purposes.

The fruits were examined in early March for storage disorders. Percent and severity of CO_2 injury and internal breakdown were recorded. The data for percent fruits with disorders was first transformed to arcsin $\sqrt{\text{percentage}}$ for analysis of variance. A completely randomized design with apple varieties as treatments and the two trees for each variety considered as observations was employed.

Results and Discussion: The results are summarized in Table 5. Significant differences occurred between varieties for incidence and severity of CO_2 injury; differences in internal breakdown were non-significant. Of the varieties, Golden Delicious fruits (non-russetted*) showed the lowest incidence of disorders while the fruits of McIntosh, followed by King Jonathan and Rome Beauty had very high incidences of disorders. Fruits of McIntosh, Delicious and Rome Beauty varieties had significantly greater incidences of simple CO_2 injury than Golden Delicious. Fruits of Foster Jonathan also had a low incidence of simple CO_2 injury

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* The russetted fruits of Golden Delicious remained free of storage disorders.

Table 5. The incidence of CO_2 injury and internal breakdown in several apple varieties stored in 18% CO_2 and 3% O_2 at 40°F in 1966-67.

Storage disorder	Rome Beauty	McIntosh	Delicious	Golden Delicious	King Jonathan	Foster Jonathan	Significance Tukey's Values for: 5% (1%)
Simple CO_2 injury percent	64.50	69.40	43.50	0.50	22.30	5.30	35·9 (50·8) a/
Total CO_2 injury percent	64.50	88.60	43.50	0.50	62.90	11.50	40·6 (57·5) a/
Internal breakdown percent	0.00	15.40	8.30	0.00	54.30	11.80	N.S.
Simple CO_2 injury index	1.34	1.44	0.84	0.02	0.34	0.20	0·52 (0·74)
Total CO_2 injury index	1.34	2.92	0.84	0.02	2.38	0.50	N.S.
Internal breakdown index	0.00	1.22	0.38	0.00	3.56	1.03	N.S.

a/ Values given in arcsin $\sqrt{\text{percentages}}$.

(5·3%), the difference being significant from McIntosh and Rome Beauty and Delicious varieties. Though the simple CO_2 injury incidence for King Jonathan was also significantly less as compared to that observed for McIntosh and Rome Beauty, it had the highest recorded incidence for brown heart (about 39%). Even so, the amounts of brown heart were not significantly different.

In general, the fruits of McIntosh and Rome Beauty were more susceptible to CO_2 injury than the Golden Delicious and Foster Jonathan frui under the conditions of this study.

B. The Incidence of CO_2 Injury as Related to the Size of Fruits of Some Apple Varieties: The physical properties of fruits of some apple varieties were examined to see if they were related to fruit size and storage disorder incidence.

Experimental: In view of the marked differences within the varieties with regard to CO_2 injury during storage, fruits with as wide as possible a range in weights were selected by variety in the following quantities:

Variety	Tree 1	Tree 2	Total
	No. of fruits		
Delicious	33	13	46
Golden Delicious	6	6	12
Rome Beauty	22	18	40
McIntosh	21	16	37

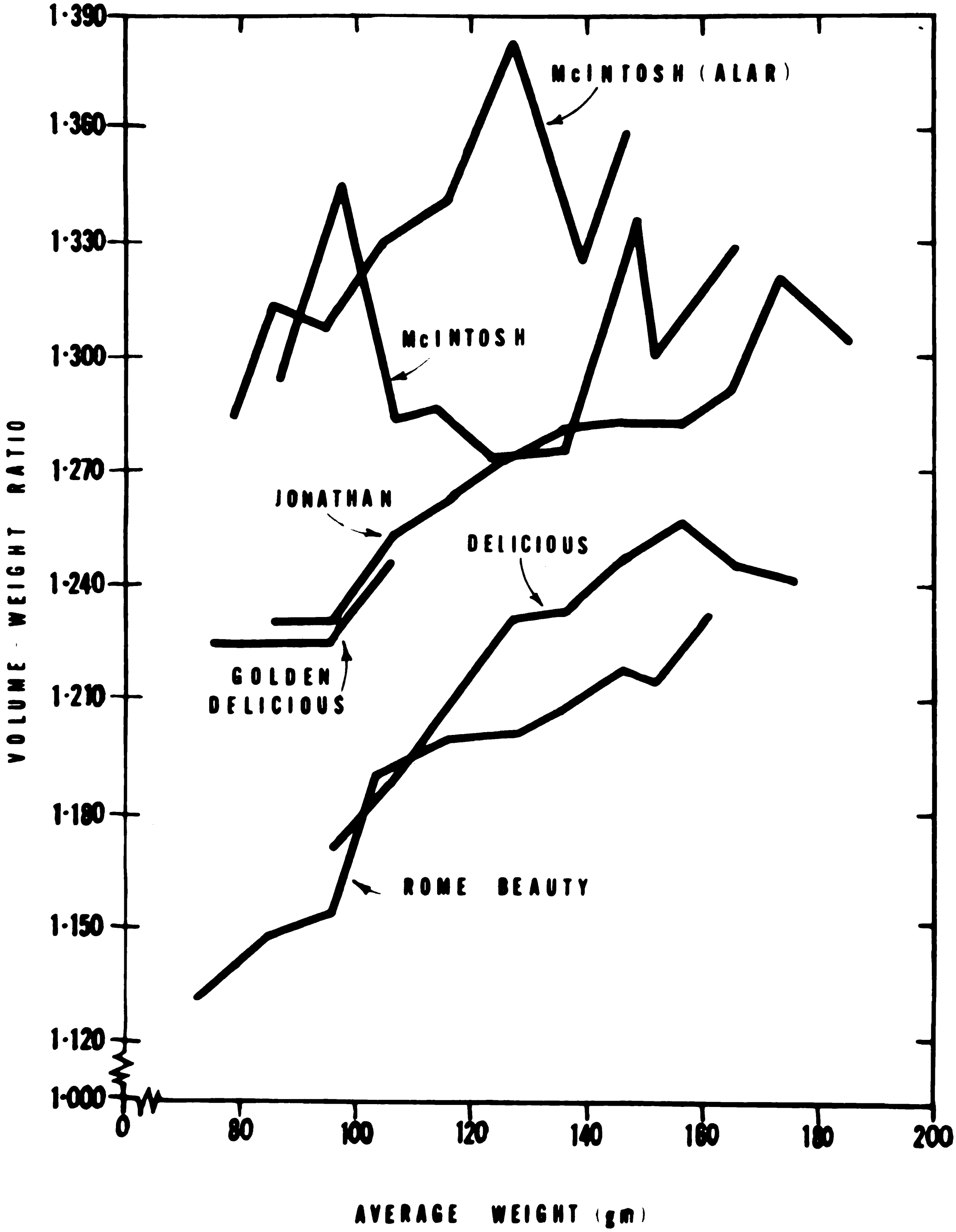
Individual fruit volumes were obtained by measuring the buoyancy force, recorded as the gain in weight when the fruit was suspended and submerged in water (Mohsenin, et al 1965). Also, the fruit was weighed for a calculation of volume-weight ratio for each fruit. They were then examined individually for internal disorders. The coefficients of correlation were calculated using individual fruit observations for each variety for CO_2 injury and internal breakdown.

Results and Discussion: The volume-weight ratios plotted in Figure 2 differed by variety. Rome Beauty had the lowest ratio and McIntosh the greatest. For Rome Beauty, Delicious, and Jonathan (Anderson strain) the ratios became greater with increased fruit weight. Varietial differences in these ratios were also reflected in the percentage of fruits showing simple CO_2 injury or brown heart and in the severity of CO_2 injury (Table 5); thus, Golden Delicious, Delicious and Rome Beauty, with relatively low volume-weight ratios had only simple CO_2 injury; whereas, McIntosh apples showed more severe browning of the core tissue.

Furthermore, except in the case of the McIntosh variety, there was no significant correlation between the trees of fruit origin and storage disorders (Table 6). The significant relationship observed for McIntosh was indicated in the amount of storage disorders observed in fruits from the two McIntosh trees:

Disorder Incidence	Tree 1 (Control)	Tree 2 (Alar-2000 ppm)
Sound (number)	17	0
Simple CO_2 injury (number)	78	60
Brown heart (number)	2	35
Internal breakdown (number)	3	35
CO_2 injury (Index)	1.36	1.51
Brown heart (Index)	0.16	2.80
Internal breakdown (Index)	0.16	2.28

Figure 2. The volume-weight ratios of several apple varieties as related to fruit weight.



McINTOSH (ALAR)
McINTOSH
JONATHAN
DELICIOUS
GOLDEN DELICIOUS
ROME BEAUTY
VOLUME - WEIGHT RATIO
1·390
1·360
1·330
1·300
1·270
1·240
1·210
1·180
1·150
1·120
1·000
0
80
100
120
140
160
180
200
AVERAGE WEIGHT (gm)

Table 6. Coefficients of correlation between fruit source (tree), weight, volume or volume-weight ratios and CO_2 injury or internal breakdown for several apple varieties stored in 18% CO_2 and 3% O_2 at 40°F in 1966-67.

Source of physical property	McIntosh		Rome Beauty		Delicious		Golden Delicious	
	'r'	Significance	'r'	Significance	'r'	Significance	'r'	Significance
				CO_2 Injury				
Tree source	0.46	**	0.23	N.S.	0.23	N.S.	0.30	N.S.
Fruit weight	0.14	N.S.	0.48	**	0.35	*	0.02	N.S.
Fruit volume	0.02	N.S.	0.45	**	0.38	**	0.07	N.S.
Volume-weight Ratio	0.66	**	0.20	N.S.	0.52	**	0.24	N.S.
				Internal Breakdown				
Tree source	0.38	*	0.18	N.S.	0.06	N.S.	--a/	
Fruit weight	0.16	N.S.	0.10	N.S.	0.37	**		
Fruit volume	0.08	N.S.	0.11	N.S.	0.38	**		
Volume-weight Ratio	0.44	**	0.15	N.S.	0.27	N.S.		

a/ No internal breakdown

Marked differences in storage disorder incidences can probably be attributed to the fact that fruit sprayed with Alar had, in general, a greater volume-weight ratio than those not similarly sprayed (Figure 2). It is not known whether this was a direct effect of Alar or a chance effect of other tree differences. Since Alar is reported to reduce fruit size if applied within one month of bloom (Batjar et al, 1964; Edgerton et al, 1965) one would not expect a reduction in size of fruits in this experiment. Further experimental work is needed to confirm this possible effect of Alar. However, several workers (Sharples, 1966; Blanpied et al, 1967) have observed that apple fruits sprayed with Alar were prone to develop core flush during storage. Under the circumstances, it is quite possible that the greater prevalence and severity of CO_2 injury observed in the Alar-sprayed McIntosh may have been contributed, at least, in part, by this increased susceptibility to core flush, and if this was the case there would be difficulty to distinguish CO_2 injury from core flush. Finally, though the relationship between volume-weight ratio and CO_2 injury was significant, the injury was not observed to be related to either fruit weight or fruit volume separately for this variety.

In Rome Beauty which had the highest density of the fruits studied, CO_2 injury was significantly related to fruit weight and to fruit volume, but not to the volume-weight ratio. In the Delicious variety which had fruits of intermediate density the CO_2 injury was significantly related to fruit weight, fruit volume, and volume-weight ratio. Similarly, significant relationships were observed between the fruit physical properties and the amount of internal breakdown for McIntosh and Delicious varieties but not for Rome Beauty.

These correlations indicate that fruit structure has an important bearing on susceptibility to CO_2 injury and to internal breakdown.

C. The incidence of CO_2 Injury to Jonathan Apples as Related to Fruit Size: Evidence presented in respect to apple varieties has shown significant correlations between the amount of internal disorders and several of the physical characteristics of fruits. It was therefore of particular interest to relate the physical characteristics of Jonathan apples to the development of CO_2 injury and internal breakdown.

Experimental: Jonathan apples (Anderson strain) harvested October 10, 1966 from Block 11 at the Graham Station were sorted into groups of small, medium and large sized fruits. Two bushels of each size were removed to CA room and stored at 32°F until October 21, when the room was sealed and temperature raised to 40°F. The composition of the storage atmosphere was maintained at approximately 18% CO_2 and 3% O_2. The CA room was opened on February 28, 1967 when fruits were examined for storage disorders. All fruits were numbered and weighed, and volume was obtained by weighing under water as described earlier; volume-weight ratios for each fruit were also calculated. Thus, data on the physical properties and disorder status of each fruit was available for:

169 small size fruits (80-110 gram weight);
116 medium size fruits (111-130 gram weight);
and 149 large size fruits (131 grams and above).

The data on these 434 fruits were grouped into 20 categories, each increasing in increments of 5 grams weight, starting with the 80 to 85 gram category and ending with fruits falling in 180 to 187 gram-weight category. From this the average fruit weight, volume and volume-weight ratios were calculated for each category along with percent fruits showing the two disorders; the index for severity of CO_2 injury and internal breakdown were also determined using the score-card ratings given earlier.

Results and Discussion: The most important differences were for volume-weight ratios (Table 7). Using a one-way analysis of variance with unequal number of replications the 'F' value was determined to test significance of differences in volume-weight ratios between different fruit weight categories or sizes. The 'F' value was highly significant; thus, as fruit weight increases a significant decrease in the specific gravity is observed. This observation is in agreement with that of Bain and Robertson (1951).

It is further evident that the storage disorders of simple CO_2 injury, CO_2 injury manifested as brown heart and internal breakdown were definitely related to fruit size. This was shown by the significant correlations (calculated from individual fruit observations) of the physical properties with the incidence of CO_2 injury or internal breakdown, see Table 8. Thus, the correlation coefficients obtained show that about 61% of the variation observed in CO_2 injury incidence may be ascribed to the effects of fruit weight, 62% as due to fruit volume, and 26% as due to volume-weight ratio; for internal breakdown, the respective figures were 37, 36, and 7%.

According to the data of Table 7 and Figure 3, fruits up to 105 grams in weight failed to develop CO_2 injury; fruits of 106-110 grams in weight had 1.4% injury and this increased to about 9.0% and 8.0% in the

Table 7. The influence of fruit weight and volume on the development of CO_2 injury and internal breakdown in Jonathan apples during storage in 18% CO_2 and 3% O_2 at 40°F in 1966-67.

Weight category	Fruit frequency	Mean wt	Mean vol	Mean vol-wt ratios	Percent fruits: Total CO_2 injury	Percent fruits: Brown heart	Percent fruits: Internal breakdown	Index: Total CO_2 injury	Index: Internal breakdown
(gram)	Nos.	(grams)	(c.c.)						
80 - 85	5	83.7	102.9	1.2294	0.0	0.0	0.0	0.00	0.00
86 - 90	11	89.0	109.5	1.2309	0.0	0.0	0.0	0.00	0.00
91 - 95	21	93.1	114.4	1.2284	0.0	0.0	0.0	0.00	0.00
96 -100	15	98.8	121.7	1.2318	0.0	0.0	0.0	0.00	0.00
101 -105	44	103.5	129.2	1.2479	0.0	0.0	0.0	0.00	0.00
106 -110	73	108.4	136.6	1.2601	1.4	0.0	0.0	0.02	0.00
111 -115	57	113.2	142.9	1.2616	3.5	0.0	0.0	0.04	0.00
116 -120	33	118.2	149.4	1.2640	9.1	0.0	0.0	0.09	0.00
121 -125	26	122.9	156.1	1.2701	7.7	3.8	0.0	0.12	0.00
126 -130	24	128.2	163.8	1.2778	45.8	16.7	4.2	1.20	0.33
131 -135	22	133.2	170.7	1.2818	45.4	36.4	18.2	2.14	1.18
136 -140	16	138.3	177.1	1.2802	68.8	62.5	43.8	4.10	3.25
141 -145	21	143.6	184.6	1.2857	71.4	66.7	47.6	4.00	2.67
146 -150	18	147.9	189.5	1.2806	77.8	72.2	55.6	4.50	2.90
151 -155	18	153.2	197.1	1.2866	94.4	94.4	50.0	5.33	3.00
156 -160	12	158.4	202.4	1.2776	91.7	83.3	58.3	5.75	3.50
161 -165	6	162.0	210.5	1.2993	100.0	100.0	50.0	7.33	4.00
166 -170	5	167.9	215.3	1.2824	100.0	80.0	40.0	6.40	3.20
171 -175	4	172.9	228.4	1.3211	100.0	100.0	50.0	8.00	4.00
180 -187	3	184.7	241.0	1.3047	100.0	100.0	66.7	8.00	5.33

Figure 3. The relation of fruit weight to volume-weight ratio and to the development and incidence of disorders in Jonathan apples stored in 18% carbon dioxide and 3% oxygen atmospheres at $40^{\circ}F$.

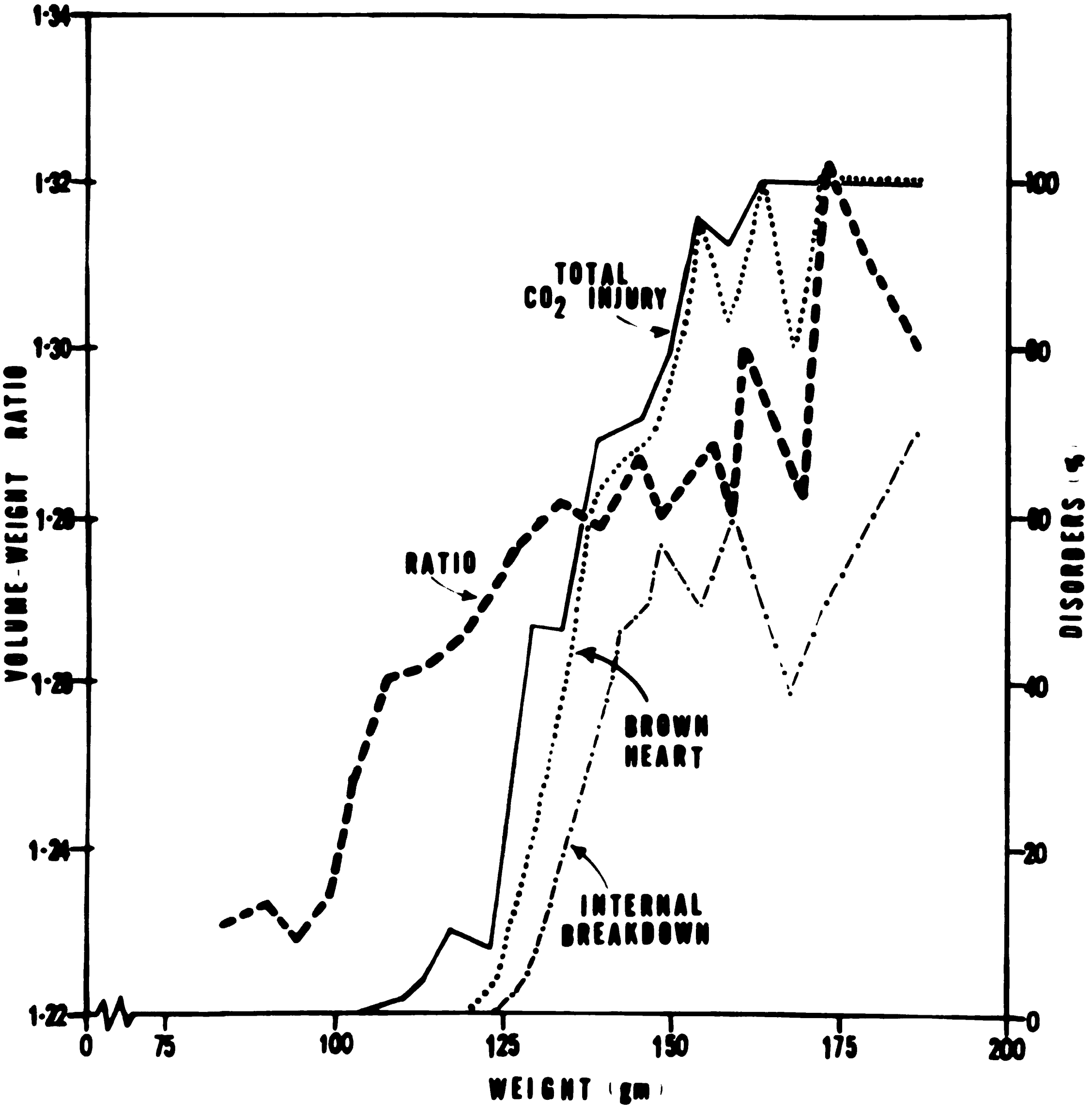

VOLUME-WEIGHT RATIO
1·34
1·32
1·30
1·28
1·26
1·24
1·22
DISORDERS (%)
100
80
60
40
20
0
TOTAL
CO_2 INJURY
RATIO
BROWN
HEART
INTERNAL
BREAKDOWN
0
75
100
125
150
175
200
WEIGHT (gm)

Table 8. The incidence of storage disorders of Jonathan apples as related to the physical properties of fruits stored in 18% CO_2 and 3% O_2 atmospheres at 40°F in 1966-67.

Independent variable	Coefficients of correlation [a] for dependent variable: CO_2 injury R	Significance	Internal breakdown R	Significance
Fruit weight	0·78 (3°)	**	0·60 (3°)	**
Fruit volume	0·79 (3°)	**	0·60 (3°)	**
Volume-weight ratio	0·51 (2°)	**	0,26 (2°)	**
Total air space	0·77 (3°)	**	0·56 (3°)	**
Percent air space	0·51 (2°)	**	0.27 (2°)	**

[a] Non-linear terms: 2°...Quadratic term
3°...Cubic term

next two fruit weight categories of 116 - 120 and 121 - 125 grams, respectively. But in the next two larger fruit weight categories 126 - 130 and 131 - 135 gram weights, the injury was slightly over 45%, with steady increases for the next higher fruit weight categories reaching 100% in fruits weighing above 160 grams.

Though the incidence and severity of CO_2 injury increased markedly in fruits weighing 130 grams and more, the possible association of concomitant increases in cell number (average) and cell size (average) with CO_2 injury cannot be ruled out. Additional work on these factors is necessary.

General Discussion: The basis for the high susceptibility of large sized fruits is not clear. Weight and volume are the two important parameters directly affecting fruit size; it is however the volume-weight ratio which is descriptive of fruit structure. Fruits having a large ratio would have a large amount of air space for each unit of fruit tissue. Bain and Robertson (1951) also observed that air space as per cent of fruit volume increased rapidly with increases in fruit weight levelling off at about 27% for larger fruits (150 grams or more in weight).

Employing their method, the total air space and air space as percent of fruit volume was calculated for all the 434 fruits in the present study. The total air space in fruits was found to be related linearly with fruit weight with an 'r' value of 0·96; but per cent air space, though significantly correlated had an 'r' value of 0·54. Fruits of 80-85 grams in weight had an air space of about 26cc, which increased to 74-78cc for fruits weighing 180-187 grams. Air space, expressed as per cent of fruit volume, however, increased by about 6% (over the same fruit weight range) to 32%, though the incidence of CO_2 injury increased from 0 to 100 per cent.

Another possibility is that the increase in the volume-weight ratios of large fruits had no significant influence on the incidence of storage disorders, and that the effect of increased fruit size was primarily on the peel structure. Since Burg and Burg (1965) have reported that the apple peel offers considerably more resistance to gas exchange than the flesh, it is reasonable to suppose that a change in the peel structure can affect the gas exchange in apples and therefore their storage performance.

It is therefore necessary to consider the significant correlations between the physical properties of the apples of different varieties and the incidence of CO_2 injury in light of these possibilities. For varieties of moderate fruit densities or volume-weight ratios, like Delicious and Jonathan (Anderson strain), CO_2 injury was significantly related with fruit weight, fruit volume or volume-weight ratios; in Rome Beauty, the most dense variety studied, the injury was related to fruit weight or fruit volume, but not to the volume-weight ratios; whereas, in McIntosh, the least dense variety studied, the injury was related only to volume-weight ratio. This suggests an association of physical properties of fruits to gas exchange; the relative importance of weight, volume or volume-weight ratio differ according to variety and fruit size within a variety.

These relationships of fruit structure help to explain why over-maturity due to late picking and low tree yields significantly influence the susceptibility of fruits to CO_2 injury.

PART III. PRE-STORAGE TREATMENTS OF FRUITS AFFECTING GAS EXCHANGE AND DEVELOPMENT OF CO_2 INJURY AND INTERNAL BREAKDOWN

It has been shown that fruit size has a direct bearing on both the total air space and percentage of air space in an apple, and also affects its susceptibility to CO_2 injury. Possibly the effect of air space in fruit tissues is incidental and the increased susceptibility of large fruits to disorders is due to the concomitant increase in the average number of cells and volume of individual cells. Therefore, two separate studies were initiated to ascertain if gas exchange in fruits is of significant effect on the development of storage disorders.

A. The Internal Atmospheres of CA Stored Jonathan Apples in Relation to Storage Disorders: Evidence has been presented indicating the possible involvement of gas exchange in fruits to storage disorders. The next step was to consider the composition of the internal atmospheres of apples stored under high CO_2 atmospheres and to examine its possible bearing on these disorders.

Experimental: Jonathan apples, stored in a prepared gas mixture of approximately 15% CO_2 and 18% O_2, from the 1965-66 storage trial (Part 1, A) were used in this study. Composition of the internal respiratory gases in the fruits, removed at end of 165 days storage, were determined by the procedure described by Williams and Patterson (1962). Six firm fruits were removed at a time from the storage atmospheres and sampled under water for internal atmospheres by drawing up to 1 ml of gas from the core cavity into hypodermic glass syringes. All 6 fruits were sampled within 12-18 minutes upon removal from storage, thus, insuring that no marked changes had occurred in the composition of their internal atmospheres due to gas diffusion. The samples were then analyzed, one by one, for percentages of CO_2 and O_2 using a micro-gas analyzer (Scholander, 1947).

Results and Discussion: The data of Table 9 show that the average composition of the internal atmospheres of sound fruits and of those with storage disorders were practically the same for each fruit size. Furthermore, the composition of the internal atmospheres of the fruits closely approximated the composition of the gas mixture external to the fruits (14.5% CO_2 and 17.6% O_2). Williams (1961) reported that Bartlett pears upon removal from CA storage had an internal level of CO_2 only slightly higher than the storage atmosphere.

It is noted that in large fruits the CO_2 level was slightly lower than the small sized Jonathans and possibly had better gas exchange.

These results appear to suggest that the increased susceptibility of large sized fruits to CO_2 injury and internal breakdown (Part II) is not dependent upon the concentration of the respiratory gases in the internal atmospheres of the fruits at least at this time.

B. The Rate of Gas Exchange in CA Stored Jonathan Apples in Relation to Storage Disorders: Two separate tests were made to ascertain if the rate of gas exchange in CA stored apples was related to the storage disorders.

(i). The rates of equilibration of the internal atmosphere of several CA stored fruits were studied with time-course determinations on the changes occurring in the concentration of CO_2 and O_2 gases of the internal atmosphere by diffusion.

Experimental: As in the previous experiment Jonathan apples, stored in high CO_2 atmospheres from the second examination lot (165 days in storage) were used. Fruits were selected at random and

Table 9. The average composition of the internal atmospheres of large and small sized Jonathan apples stored in approximately 15% CO_2 and 18% O_2 at 40°F in 1965-1966.

Fruit condition	Large Fruits No. fruits sampled	Large Fruits CO_2 (%)	Large Fruits O_2 (%)	Small Fruits No. fruits sampled	Small Fruits CO_2 (%)	Small Fruits O_2 (%)
Sound	15	13.1	17.6	20	14.6	17.5
Brown heart	7	13.1	17.2	16	14.4	17.5
Brown heart plus Internal breakdown	7	13.8	17.4	4	14.6	17.3

determinations of the composition of the internal atmospheres of each of 8 fruits were made at 0, 2, 4, 6, 24 and 48 hours after removal to air.

Results and Discussion: The data summarized in Table 10 indicate slightly higher rates of gas exchange in fruits with CO_2 injury as compared to sound apples. Moreover, it is interesting to note that the rate of CO_2 decline in the internal atmospheres was quite rapid in all apple fruits, reaching about one-third of their initial concentration at the end of 4 hours. The internal O_2 levels never exceeded 19% (averaged 18.3%) even after 24 and 48 hours in air. Indeed, at the end of 24 hours in air, the internal O_2 levels showed slight decreases perhaps because of

Table 10. The composition of internal atmospheres of Jonathan apples after removal to air at room temperature, from storage atmospheres of approximately 15% CO_2 and 18% O_2 at 40°F in 1965-1966.

Storage Disorder Status	Hours in Air 0	2	4	6	24	48
			Percent CO_2			
Sound fruits (Av of 5)	16.3	11.8	6.2	4.4	3.9	5.1
Brown Heart (severe) (Av of 2)	16.2	9.2	4.4	3.4	3.2	4.7
Brown Heat (severe) plus Internal Breakdown (1 fruit)	15.5	9.9	5.2	4.0	4.8	6.1
			Percent O_2			
Sound fruits (Av of 5)	17.2	17.4	18.2	18.4	16.4	16.3
Brown Heart (severe) (Av of 2)	17.5	18.0	18.7	18.6	17.6	16.6
Brown Heart (severe) plus Internal Breakdown (1 fruit)	17.4	17.1	18.0	17.8	16.6	15.9

increased resistance to gas exchange as a result of shrinkage due to moisture losses. Further study of this observation is needed, however.

(ii). In another experiment the rate of O_2 diffusion in apple fruits was studied. Though several techniques have been reported (Burg and Burg, 1965; Burton, 1965; Marcellin, 1956; Wilkinson, 1965), for the measurement of O_2 gas exchange rates in fruits, diffusion was measured here with platinum micro-electrodes (Lemon and Erickson, 1952).

<u>Experimental</u>: Jonathan apples from the third examination (194 days storage) were used. Fruits firm in texture were selected and equilibrated to room temperature (85-90^{o}F) overnight. Measurements of O_2 diffusion rates were made by inserting 3 marked platinum micro-electrodes to approximately the same depth in each fruit. At the start, the O_2 diffusion meter was set to a standard potential of -0.65 volts as read on the voltmeter, then by means of a selector switch the micro-meter was connected in series with individual platinum electrodes inserted into the fruit flesh. The readings were recorded after the current dropped to a fairly steady state after 2 or 3 minutes.

In order to obtain uniform readings, the individual fruits were placed on a cheese cloth kept moist by sprinkling saturated solution of KCl as an electrolite. Determinations of the O_2 diffusion rates were made at about 85-90^{o}F, after which each fruit was examined for storage disorder incidence. From the O_2 diffusion data recorded for the 3 micro-electrodes, the average rate was calculated for each fruit. A one-way analysis of variance with unequal number of replications was employed to evaluate differences between fruit sizes and fruits showing different disorders.

<u>Results and Discussion</u>: The results are recorded in Table 11. It was found that the rate of O_2 diffusion in large fruits was significantly greater than for small fruits. Similarly, fruits with brown heart or brown heart plus internal breakdown showed significant differences in O_2 diffusion rates over those of sound fruits.

Thus, it seems that large fruits or fruits with internal disorders

Table 11. The oxygen diffusion rates in Jonathan apples as related to fruit size and storage disorders. in 1965-66.

Fruit Category	No. of fruits	Micorgram O_2 per minute per cm^2
Small size	28	0.81
Large size	38	1.03
Significance		**
Sound	27	0.84
Brown heart	23	1.00
Significance		**
Sound	27	0.84
Disorders	39	1.01
Significance		**

had superior gas exchange. However, it is possible that the higher rates of gas diffusion observed in fruits with CO_2 injury or other internal disorders was primarily a consequence of the 'voids' developed, or tissue necrosis rather than the cause of these two disorders.

C. <u>The Effect of Epidermal Punctures on the Development of CO_2 Injury in Jonathan Apples</u>: Since it was observed apple fruits showing brown heart have higher rates of gas exchange, it was decided to examine further if CO_2 injury could be induced in apple fruits by making epidermal punctures thereby enhancing their rate of gas exchange.

<u>Experimental</u>: Small sized Jonathan apples, 2 1/4 inches in diameter, held in cold storage at 32°F for ten months were selected. The

epidermis of the fruits was punctured with a hypodermic needle of 18 or 24 gauge. Fruits with 0 (control), 25, 50 and 100 epidermal punctures over the entire fruit surface were stored in plastic buckets at 40°F through which a gas mixture of 60.0 ± 3.0% CO_2 and 21 to 23% O_2 was passed at a constant rate of 0.6 cubic foot per hour. A control lot of fruits similarly treated were stored in air at the same temperature.

Results and Discussion: The data obtained upon examination for CO_2 injury at the end of 24 days of treatment are presented in Table 12. The incidence of CO_2 injury was observed to be significantly correlated with both the number and the size of epidermal punctures. None of the fruits having epidermal punctures showed CO_2 injury when stored in air.

These results indicate that gas exchange in fruits is a primary factor of injury and suggest skin porosity is of significance to the development of CO_2 injury during storage.

D. The Effect of Waxing on the Development of Storage Disorders in Jonathan Apples: In view of the significant relationship between epidermal punctures and CO_2 injury, it was logical to study the effect of fruit waxing on the development of CO_2 injury.

Experimental: Large, medium and small sized Jonathan apples from the same fruit lots used in the storage study on fruit size and CO_2 injury were used in this experiment. A randomized block design with 3 factors replicated two times was used:

A. 3 fruit sizes ... large, medium and small;
B. 2 pre-treatments ... waxing, no waxing (control);
C. 4 examinations ... 75, 110, 140 and 175 days in storage.

Table 12. The effect of epidermal punctures on the development of CO_2 injury in Jonathan apples stored in 60% CO_2 and 21% O_2 at 40°F in 1965-66.

Epidermal Punctures (number & size)	Stored in CO_2 atmospheres				Stored in air	
	Total fruits	Nos. sound	CO_2[a/] injury %	Index	Total fruits	Nos. sound
0 ...(Control)	12	9	33.3	0.33	9	9
25 ... Gauge 24	3	3	0.0	--	--	--
Gauge 18	9	2	77.8	1.44	--	--
50 ... Gauge 24	9	5	44.4	1.06	6	6
Gauge 18	15	6	60.0	1.43	6	6
100 ... Gauge 24	12	2	83.3	1.50	9	9
Gauge 18	3	1	66.7	2.00	3	3

Coefficients of Correlation:

CO_2 injury as related to:	r	Significance
Number of epidermal punctures	0.2685	*
Number plus size of epidermal punctures	0.3473	*

a/ The injury was always simple CO_2 injury.

Half of the fruits under each treatment were stored without wax treatment (control), and one half were coated by submerging the fruits in Waxol-12[a/], a wax emulsion of 12% solids, the principal constituents of which were sugarcane and carnabua waxes and paraffin wax. The fruits were allowed to dry before placement into storage.

The apples were placed into metal chambers held at 40°F. Care was taken to keep the respective treatment lots of waxed and control fruits in

a/ Supplied by the Central Food Technological Res. Inst., Mysore, India.

the same chamber. The chambers were closed tightly on October 30 and a prepared gas mixture of 20 ± 2% CO_2 and 20 ± 2% O_2 was passed over the fruits at a constant rate of about 0.8 cubic foot per hour. The fruits were examined for storage disorders after 75, 110, 140 and 175 days in storage. Arcsin $\sqrt{\text{percentage}}$ transformations were made in the case of percent fruits showing disorder before analysis of variance was made.

Results and Discussion: It will be seen from Table 13 that the incidence of brown heart and internal breakdown were significantly related in a positive manner to fruit size and storage duration.

The incidence and severity of brown heart were not affected by waxing, but there was a significant reduction of internal breakdown due to waxing. These results suggest that internal breakdown, a senescence disorder, can be significantly reduced by waxing the fruits so as to limit the O_2 supply. Waxing had little effect on the incidence of brown heart and this was possibly due to the high concentration of O_2 (20 ± 2.0%) used in the storage atmospheres. It is suspected that O_2 in limited supply would have affected the results in that the severity, if not the incidence, of CO_2 injury would have been reduced.

In the interaction for size and duration of storage the index for brown heart was significant, but the incidence was non-significant. The interaction of waxing and fruit size was non-significant for brown heart incidence, but significant for the internal breakdown incidence. The significance observed was probably due to the relatively large numerical value recorded for large fruits without wax. The interaction between fruit coat and duration of storage shows that brown heart

Table 13. The effect of waxing on storage disorders of large, medium and small sized Jonathan apples stored in 20% CO_2 and 20% O_2 at 40°F in 1966-67.

	Brown heart				Internal breakdown			
	Percent		Index		Percent		Index	
Fruit size:								
Large	32.2		1.83		15.7		1.22	
Medium	2.3		0.24		1.0		0.23	
Small	0.1		0.04		0.03		0.02	
Tukey's value ...5%	5.4[a]		0.27		6.0 [a]		0.39	
...1%	7.0[a]		0.35		7.7 [a]		0.50	
Skin-coat:								
No wax	6.6		0.70		8.8		0.90	
Wax	7.9		0.71		0.5		0.08	
Tukey's value ...5%	N.S.		N.S.		4.1 [a]		0.26	
...1%					5.5 [a]		0.36	
Storage duration:								
75 days	3.5		0.46		1.3		0.35	
110 "	3.2		0.54		2.4		0.47	
140 "	9.9		0.73		2.2		0.38	
175 "	12.6		1.08		7.7		0.76	
Tukey's value ...5%	6.9 [a]		0.34		7.6 [a]		N.S.	
...1%	8.7 [a]		0.43		9.6 [a]			
Interactions:	No wax	Wax	No wax	Wax	No wax	Wax	No wax	Wax
Fruit size X waxing:								
Large...	31.5	33.0	1.77	1.89	33.1	4.1	2.20	0.23
Medium...	3.4	1.4	0.33	0.16	4.0	0.0	0.47	0.00
Small...	0.0	0.4	0.00	0.08	0.1	0.0	0.03	0.00
Tukey's value...5%	N.S.		N.S.		10.5 [a]		0.68	
...1%					13.0 [a]		0.86	
Storage duration X waxing:								
75 days	4.6	2.6	0.48	0.44	3.4	0.2	0.67	0.04
110 "	3.4	3.0	0.60	0.48	4.3	1.0	0.77	0.17
140 "	12.2	7.7	0.98	0.48	4.6	0.7	0.67	0.08
175 "	7.8	18.3	0.73	1.44	24.7	0.2	1.50	0.02
Tukey's value...5%	11.8 [a]		0.58		13.0 [a]		0.85	
...1%	N.S.		0.71		15.9 [a]		1.03	
Storage duration X Fruit size:								
Significance...	N.S.		**		N.S.		N.S.	
Storage duration X Fruit size X Skin-coat:								
Significance...	N.S.		N.S.		N.S.		N.S.	

[a] Values given in arcsin $\sqrt{\text{percentages}}$.

incidence was slightly less in waxed fruits at each of the first three examinations; however, at the end of 175 days of storage, the non-waxed fruits showed a sharp decline in brown heart incidence. This effect was not observed for internal breakdown. The interaction between fruit coat, fruit size and length of storage was non-significant for both disorders.

It appears that skin coatings have a modifying effect on gas exchange in fruits, the effect possibly being more pronounced for O_2 than CO_2.

E. The Effect of Fruit Finish on the Development of CO_2 Injury in Jonathan Apples: Apples with russetted skin and those with good skin finish were used in the storage study to further elucidate the role of gas exchange in the fruits and their possible effects on the development and incidence of storage disorders.

Experimental: Jonathan apples from Blocks 10, 11 and 12 of the Graham Station in which the Horticulture Department was evaluating the effect of fungicides and pesticides on fruit finish were used in this study.

Three bushels of medium-large fruits, 2 1/2 to 2 3/4 inches in diameter, were harvested on October 10. These were badly russetted due to pre-harvest sprays of Glyodin. Similarly, 3 bushels of fruit with fairly good finish due to the use of pre-harvest sprays of Captan were obtained. All lots were held at $32^{o}F$ until October 21 when the experimental room was sealed and the temperature raised to $40^{o}F$. The storage atmosphere was maintained at approximately 18% CO_2 and 3% O_2. The fruits were examined in early March for storage disorders. The data on percent disorders was first transformed to arcsin $\sqrt{\text{percentages}}$ before an analysis of variance was made employing a completely randomized design with fruit finish as treatments and 3 observations.

Results and Discussion: The results are summarized in Table 14. The russetted fruits in general were slightly shrivelled in appearance, but otherwise compared favorably with the non-russetted fruits. Differences in the incidence of total CO_2 injury due to skin finish were non-significant, yet differences in severity of total CO_2 injury were significant with the average index being 1.03 for the russetted fruits as against 3.24 for the non-russetted fruits. The effect of fruit finish seems to be primarily on the type of CO_2 injury that developed during prolonged storage under high CO_2 levels. This is evident in the fact that for fruits with good finish brown heart was approximately 21 times more prevalent than in russetted fruits. On the other hand, the incidence of simple CO_2 injury was almost 7 times higher in the russetted fruits than in the non-russetted fruits.

Fruit finish apparently influences gas exchange in fruits, but in absence of precise experimental data it is difficult to define the relationship between gas exchange in fruit and CO_2 injury. Possibly, the dark fixation of CO_2 within the fruit tissues and particularly in the enzyme-rich area is enhanced by the greater freedom of gas exchange.

The literature suggests that the dark fixation of CO_2 is O_2 dependent (Miyachi et al., 1955; Rhoads and Wallace, 1960). Biale (1962), working with lemons, and Thornton (1933), working with several commodities, also report a spurt in the O_2 uptake under high CO_2 atmospheres.

Table 14. The effect of skin russetting on the development of CO_2 injury in Jonathan apples stored in approximately 18% CO_2 and 3% O_2 at 40°F in 1966-67.

Storage disorder status	Non-russetted	Russetted	Significance
Simple CO_2 injury - Percent	8.8	59.0	**
Brown heart - Percent	46.0	2.2	**
Total CO_2 injury - Percent	55.1	62.4	N.S.
Internal breakdown - Percent	5.6	0.0	**
Simple CO_2 injury - Index No.	0.11	0.81	**
Brown heart - Index No.	3.13	0.23	**
Total CO_2 injury - Index No.	3.24	1.03	**
Internal breakdown - Index No.	0.45	0.00	**

This then would suggest that fruits having less obstruction to oxygen movement should show an earlier manifestation of the injury when stored under high levels of CO_2 and low concentrations of O_2 as was the case in this study.

In light of the above hypothesis, the markedly low incidence of brown heart in the russetted fruits was possibly due to the shrinkage that occurred in these fruits because of an excessive rate of moisture loss during storage. That such shrinkage in apple fruits may alter the permeability to gas exchange has been reported by Wilkinson (1963). He found that Cox Orange Pippin fruits stored in humid atmospheres increased in volume by 3% without a corresponding increase in fruit weight and became progressively more permeable to the passage of air. Conversely, he found fruits stored under dry conditions became steadily less permeable to air because of considerable shrinkage of the fruit with a concomitant contraction of both skin and flesh.

The fact that the russetted fruits also failed to show internal breakdown which is usually associated with a higher availability of O_2 lends further support to the hypothesis that the development of CO_2 injury is related to the availability of O_2 to the internal tissues.

General Discussion: Experimental evidence has been obtained showing fruits with higher rates of gas exchange were more likely to develop CO_2 injury in CA storage. These results, together with those of fruit coatings and fruit finish seem to suggest the likelihood that in each fruit some sort of a "predisposing" condition is operative, which modifies the rates in which O_2 and CO_2 gases diffuse in and out of the fruit tissue. This, then, would greatly influence the development and incidence of CO_2 injury and internal breakdown. This hypothesis would explain the significant positive correlation observed for the number of epidermal punctures with CO_2 injury in small sized Jonathan apples. As reported by Burg and Burg (1965), the skin appears to be the most effective barrier to gas exchange of CO_2 and O_2; thus, the epidermal punctures greatly reduce the efficacy of this natural barrier to bring about an inbalance of gas exchange and cause CO_2 injury. A detailed study of porosity in Jonathan apples was therefore undertaken.

PART IV. FRUIT POROSITY IN RELATION TO THE DEVELOPMENT OF CO_2 INJURY AND INTERNAL BREAKDOWN

The evidence already presented suggests that gas exchange in fruits plays an important role in influencing the storage physiology of the fruits. It would be logical to believe that the most important factor affecting gas exchange would be fruit porosity. Its measurement should offer an index of the fruit structure and, therefore, its susceptibility to damage by high levels of CO_2 in the storage atmosphere. During the 1966-67 season, a detailed study of porosity of Jonathan apples was undertaken.

A. <u>The Effect of Harvest Maturity on the Porosity of Jonathan Apples</u>: Storage studies made during 1965-66 and 1966-67 seasons indicated that late picked or over-mature fruits were highly susceptible to CO_2 injury. It was therefore decided to examine the influence of harvest maturity on fruit porosity. The technique developed by Hoff and Dostal (1967) for the measurement of gas movement in the apple was adopted.

<u>Experimental</u>: Fruits from the two trees in the Stover Orchard near Berrien Springs, harvested on September 26, October 6 and 17, 1966, and held at 32°F until November 27 were used in this study (see Part I, B). Ten fruits were randomly selected from each harvest for each tree and the 30 fruits numbered and weighed individually. The time required in seconds for the manometer column to fall from 40 to 30 cm (PO/P) was recorded. The values indicate the rate of gas flow or conductivity from the core of the fruit to its surface; flow resistance is the time required to attain this drop or the reciprocal of conductivity. Porosity, a proportionality factor constant for each

fruit, is expressed as ml per second.

Results and Discussion: It may be observed in Table 15 that fruit porosity differed greatly by trees for a given harvest date with fruits of tree 1 having lower porosities than those from tree 2. The time of harvest had a profound effect on fruit porosity; each delay in harvest resulted in an increase in fruit porosity with the increase for tree 2 being considerably greater than for tree 1.

Table 15. The effect of trees and harvest maturity on the porosity of Jonathan apples in 1966-67. (Average values, based on 10 fruits).

	Tree 1			Tree 2		
Harvest (date)	Mean Fruit Wt (gm)	PO/P 40/30 cm drop (seconds)	Porosity (ml/sec)	Mean Fruit Wt (gm)	PO/P 40/30 cm drop (seconds)	Porosity (ml/sec)
Early Harvest (9/26/1966)	75.0	326.6	1.11	94.5	167.3	2.13
Mid-season Harvest (10/6/1966)	82.2	246.4	1.24	91.2	192.1	1.88
Late Harvest (10/17/1966)	84.3	169.7	1.94	91.6	133.1	2.85

Only the fruits from tree 1 recorded slight increases in weight with fruit ripening; the average weight recorded for the third harvest was about 84 grams compared to 75 grams for the first harvest, while the average weights recorded for fruits from tree 2 were 94.5 and 91.6 grams, respectively. Though this can very well be an effect of random sampling, the increases in porosity for tree 2 fruits may possibly have been due to differences in fruit volume. The coefficients of correlation, calculated from the data for 60 individual fruits, are given in Table 16. Fruit weight was affected by the tree from which the fruit was harvested, while the time of harvest was of no effect on fruit weight.

Table 16. Porosity of Jonathan apples as related to tree, harvest maturity, and fruit weight in 1966-67.

	Fruit Weight		Fruit Porosity	
	r	Significance	r	Significance
Tree (fruit source)	0.45	**	0.43	**
Harvest maturity (time of harvest)	0.10	N.S.	0.32	*
Fruit weight	---	---	0.65	**
Tree plus harvest maturity	0.46	**	0.54	**
Harvest Maturity plus fruit weight	---	---	0.70	**
Tree plus harvest maturity plus fruit weight	---	---	0.72	**

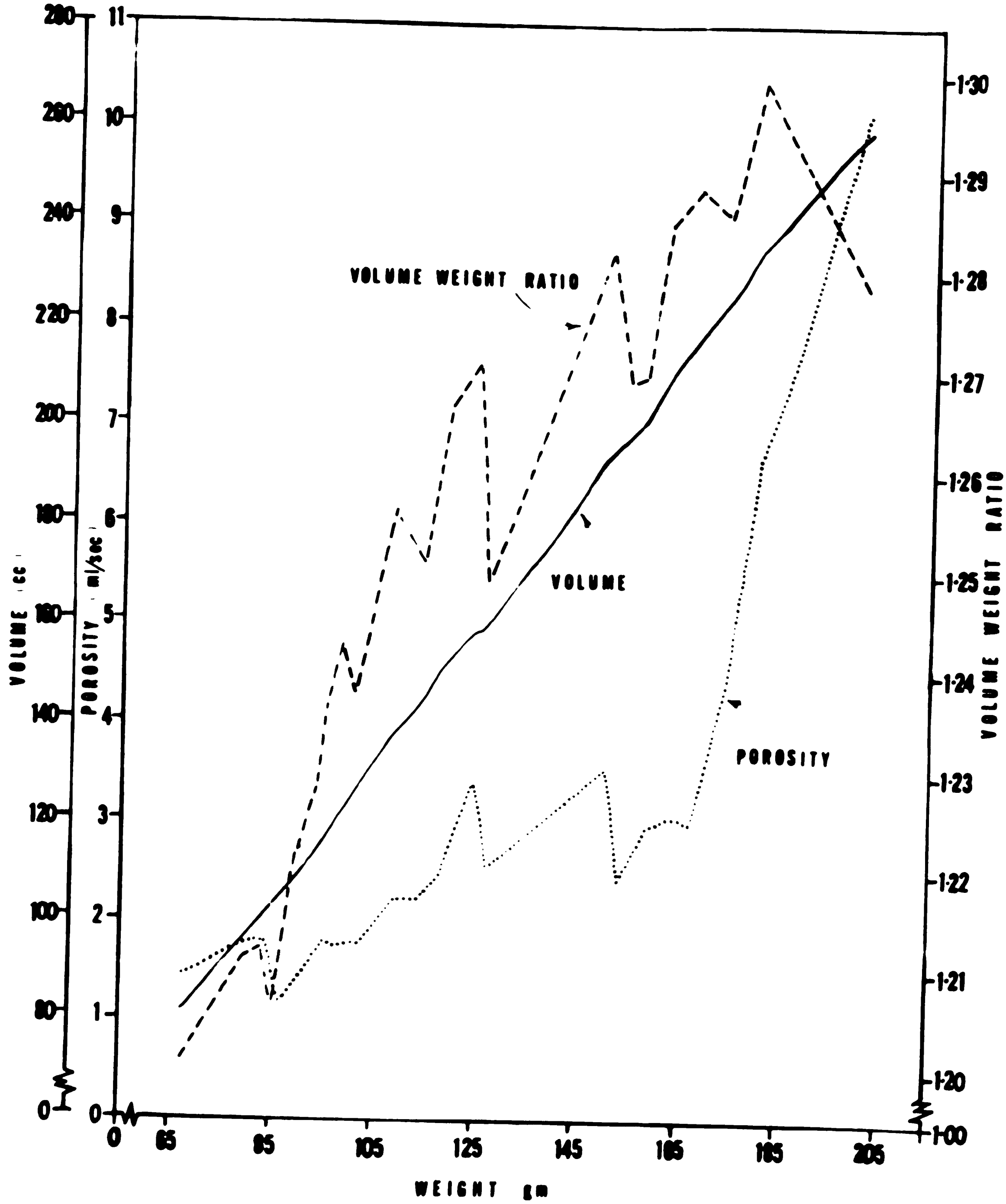
Fruit porosity was observed to be highly significantly correlated with: fruit weight, porosity increasing with increase in fruit weight; tree from which fruit was obtained, and fruit maturity, with porosity increasing with over-maturity at harvest. About 43% of the variation observed in fruit porosity can be ascribed to fruit weight alone; whereas, only about 19% and 10% can be ascribed to the tree of fruit source and to fruit maturity, respectively. This value was improved from 43 to 49% when fruit weight and harvest maturity were considered together, and further increased to 52% when fruit source was also included.

B. The Effect of Fruit Size on the Porosity of Jonathan Apples: The relationships between fruit weight, volume, volume-weight ratio and fruit porosity were studied using small, medium and large sized Jonathan apples.

Experimental: Jonathan apples, harvested October 10, 1966 from Block II, at the Graham Station were sorted into groups of small, medium and large size (see Part II, C) and held at 32°F until December 4, 1966 when 20 fruits of each size were randomly selected, numbered and weighed individually. Fruit volume was obtained by weighing under water, after which porosity was determined. The data on the physical properties recorded for the 60 fruits were grouped into 20 weight categories in increments of 5 grams starting with a 66-70 gram group. From this the average fruit weight, volume, volume-weight ratio and fruit porosity were calculated for each fruit weight category.

Results and Discussion: Fruit volumes and volume-weight ratios, recorded in Table 17 and depicted in Figure 4, increased rather consistently with weight-class increases. Fruit porosity, however, showed little increase for fruits weighing up to 105 grams; thereafter, it increased gradually with weight-class increases up to about 120 grams, but for fruits in the weight-classes of 125 to 170 grams the increases were, in general, of greater magnitude. In fruits above 170 grams in weight, porosity, however, was observed to increase tremendously as size increased. The coefficients of correlation were calculated from individual fruit observations for these several measurements and porosity,

Figure 4. The average values for fruit volume, volume-weight ratio, and porosity of Jonathan apples plotted by fruit weight groups.



VOLUME WEIGHT RATIO
VOLUME
POROSITY
VOLUME (cc)
POROSITY (ml/sec)
VOLUME WEIGHT RATIO
WEIGHT gm
0
65
85
105
125
145
165
185
205
0
80
100
120
140
160
180
200
220
240
260
280
0
1
2
3
4
5
6
7
8
9
10
11
1·00
1·20
1·21
1·22
1·23
1·24
1·25
1·26
1·27
1·28
1·29
1·30

Table 17. The effect of fruit weight and volume on fruit porosity in 1966-67.

Fruit weight category	Fruit Nos.	Weight (Av) gram	Volume (Av) c.c.	Mean Volume: weight ratio	Time in seconds for manometer column to fall thru 40/30cms (PO/p)	Fruit porosity ml/sec
Small size:						
	3	67.6	81.2	1.2007	753	0.92
	2	79.7	96.5	1.2108	540	1.28
	6	82.9	100.5	1.2121	545	1.27
	1	86.2	104.0	1.2065	1089	0.64
	4	93.6	115.0	1.2293	553	1.25
	2	97.0	120.5	1.2429	574	1.21
	1	100.6	124.5	1.2376	566	1.22
Medium size:						
	6	108.2	135.8	1.2554	409	1.69
	4	113.6	142.0	1.2497	411	1.69
	3	118.2	149.7	1.2659	360	1.93
	6	123.1	156.4	1.2712	237	2.92
	2	126.8	158.3	1.2485	345	2.01
Large size:						
	1	149.8	192.0	1.2817	231	3.00
	3	153.5	194.7	1.2685	363	1.91
	3	157.2	199.5	1.2688	286	2.42
	3	161.9	208.0	1.2845	276	2.51
	5	167.0	215.1	1.2880	279	2.48
	2	173.5	223.0	1.2853	181	3.83
	2	179.4	233.0	1.2988	116	5.98
	1	200.2	256.0	1.2787	73	9.50

for the three fruit sizes together and separately for each size. These are presented in Table 18. Up to 99.7% of the variation in fruit volume were ascribed to the effect of fruit weight when all sizes were considered together; though for large fruits this value was somewhat lower, 95.5%. This suggests that there are greater volumetric variations for large than for small apples. Similarly, when all fruit sizes were considered

Table 18. Coefficients of correlation between fruit weight, volume or volume-weight ratios and porosity of Jonathan fruits in 1966-67.

Physical property	All fruit Sizes together R	Signi-ficance	Small R	Signi-ficance	Medium R	Signi-ficance	Large R	Signi-ficance
					FRUIT VOLUME:			
Fruit Weight	0.999(1°)	**	0.994(1°)	**	0.986(1°)	**	0.977(1°)	**
					VOLUME-WEIGHT RATIO:			
Fruit Weight	0.80(1°) 0.84(2°)	** **	0.63(1°)	**	0.29(1°) 0.66(3°)	N.S. *	0.25(1°)	N.S.
Fruit Volume	0.82(1°) 0.86(2°)	** **	0.72(1°)	**	0.45(1°)	*	0.45(1°)	*
					FRUIT POROSITY:			
Fruit Weight	0.66(1°) 0.71(2°)	** **	0.11(1°)	N.S.	0.57(1°)	**	0.60(1°)	**
Fruit Volume	0.67(1°) 0.76(3°)	** **	0.12(1°)	N.S.	0.57(1°)	**	0.62(1°)	**
Volume-weight ratio	0.54(1°) 0.63(4°)	**	0.14(1°)	N.S.	0.22(1°)	N.S.	0.31(1°)	N.S.

N.B. 1°... Linear term.
2°... Quadratic term.
3°... Cubic term.
4°... Quartic term.

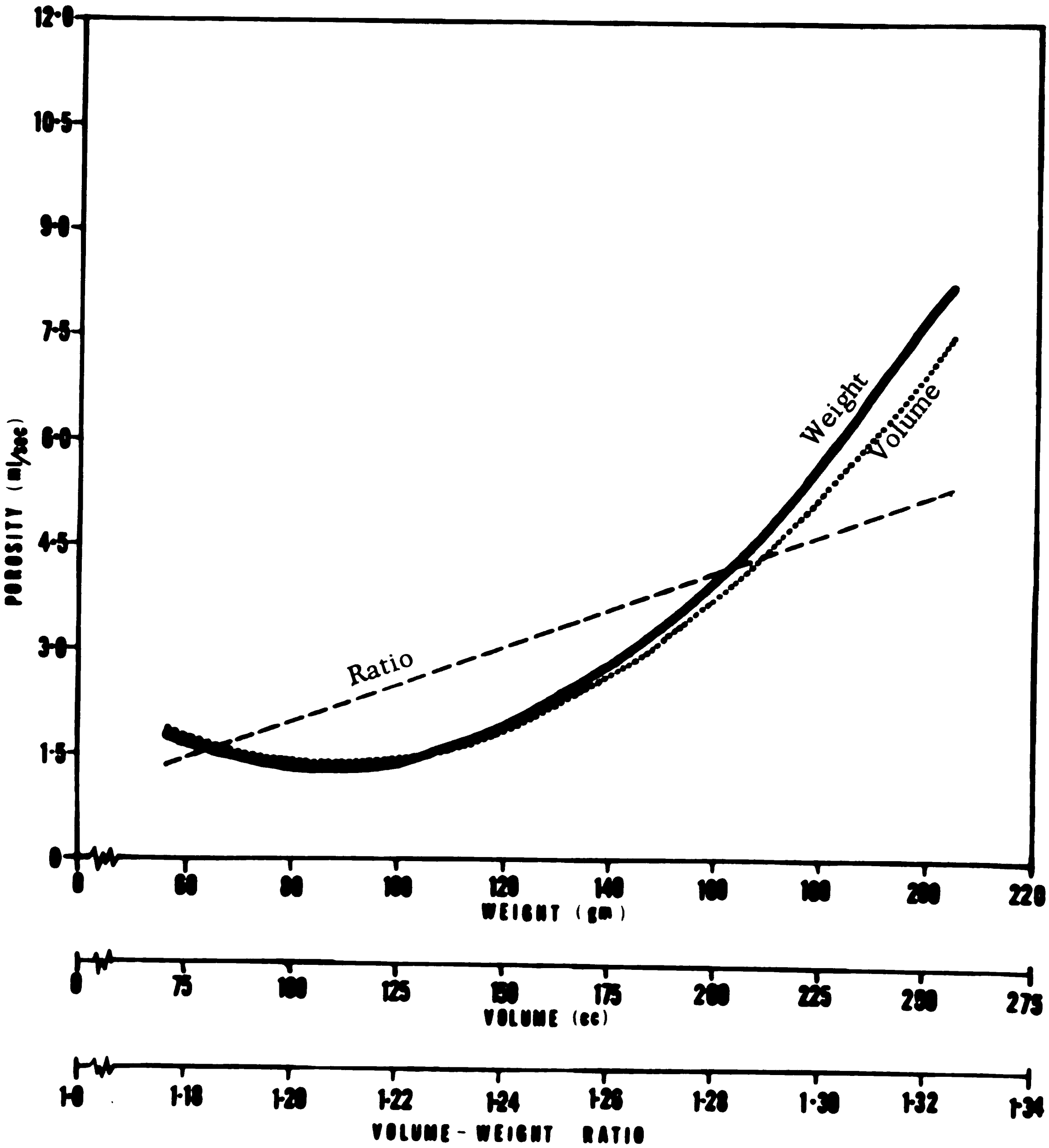
together about 70 to 77% of the observed variation in volume-weight ratios were ascribed to the effect of fruit weight or fruit volume. These highly significant correlations noted between fruit weight, fruit volume, or volume-weight ratio and fruit porosity are depicted in Figure 5. Up to

Figure 5. The regression of fruit porosity with fruit weight, volume, or volume-weight ratio for Jonathan apples. The regression coefficients are:

Fruit weight: $5.0633-0.0837\ (\text{weight}) + 0.0005\ (\text{weight})^2$

Fruit volume: $4.6648-0.0599\ (\text{volume}) + 0.0003\ (\text{volume})^2$

Volume-weight Ratio: $-38.7872 + 32.9960\ (\text{ratio})$

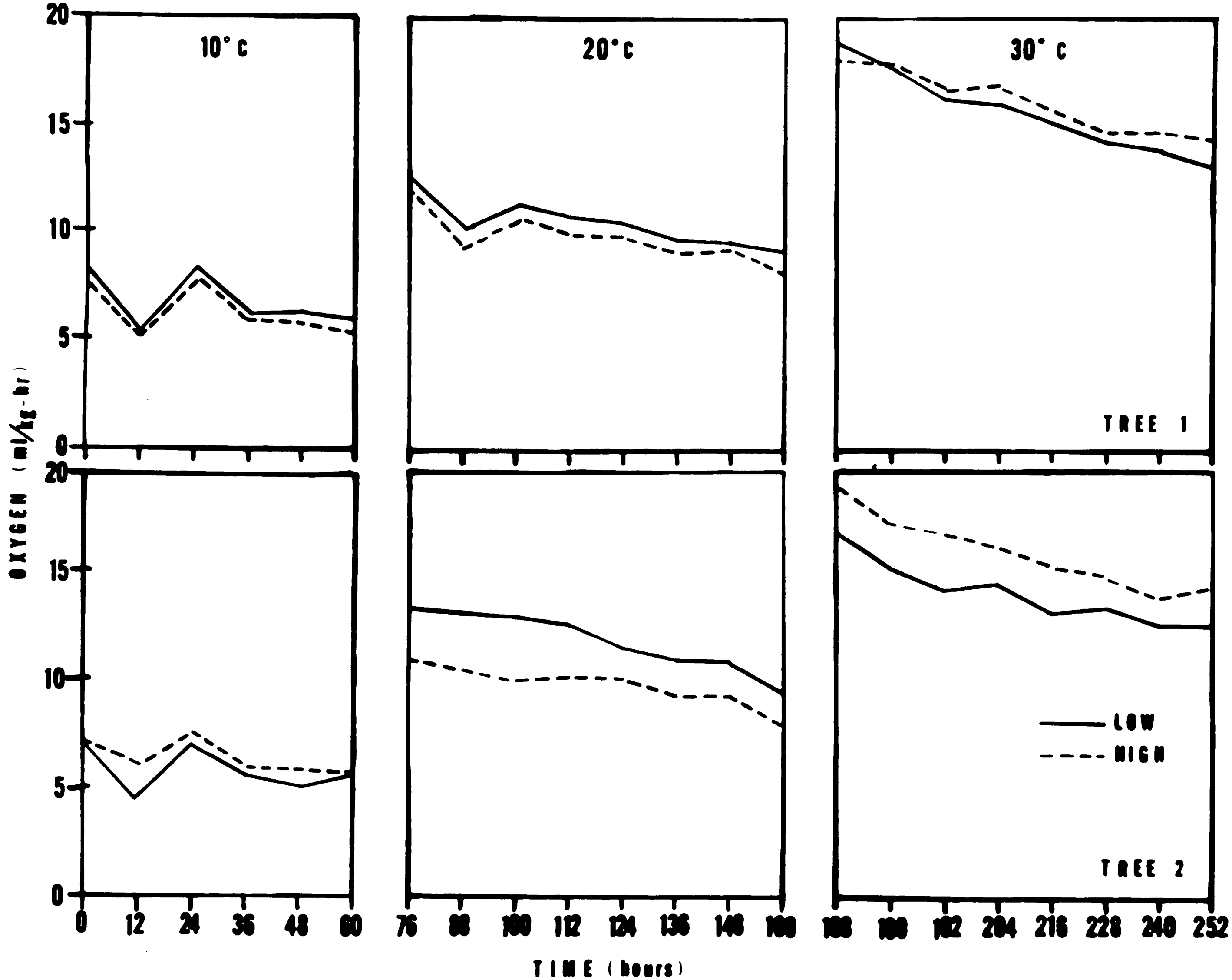

12·0
10·5
9·0
7·5
6·0
4·5
3·0
1·5
0
POROSITY (ml/sec)
Ratio
Weight
Volume
0
60
80
100
120
140
200
220
WEIGHT (gm)
0
75
100
125
150
175
200
225
250
275
VOLUME (cc)
1·0
1·18
1·20
1·22
1·24
1·26
1·28
1·30
1·32
1·34
VOLUME - WEIGHT RATIO

51 to 57% of the observed variation in fruit porosity can be ascribed to either fruit weight or fruit volume, while about 39% to the volume-weight ratio.

The correlations between the several size measurements showed that for large fruits the volume-weight ratio was not correlated with fruit weight; whereas, for medium sized apples the correlation was non-significant for the linear term, but significant for a cubic term; while for small fruits, the linear term alone was significant. Because of the large variations in the volume-weight ratios of large fruits any possible relationship may have been obscured; thus, in both large and medium sized fruits porosity was not significantly related with volume-weight ratios, though it was correlated with fruit weight and volume. On the other hand, the absence of significant correlations between porosity and fruit weight, volume, or volume-weight ratio of small fruits may be due to the similar values of fruit porosity noted for fruits of 65 to 105 grams weight.

C. <u>The Effect of Fruit Porosity on the Respiratory Activity of Jonathan Apples</u>: Fruits of trees 1 and 2, Stover Orchard (see Part IV, A) of three harvest dates, for which porosity readings were available were used in this study. Fruits of approximately similar weights (maximum difference 4 grams) but differing greatly in their porosity values were paired for low and high porosity readings. 13 such pairs were obtained from the 30 fruits of each tree. The respiration rates for all samples were measured first at 10°, then at 20°, and finally at 30°C beginning December 1, 1966. These are depicted in Figure 6. Fruits from trees 1 and 2 had similar rates of respiration (O_2 uptake). Further, the low and high porosity fruits from tree 1 did not show as wide a

Figure 6. The effect of fruit porosity on the respiration of Jonathan apples at 10°, 20° and 30°C.


10°C
20°C
30°C
TREE 1
TREE 2
LOW
HIGH
OXYGEN (ml/kg-hr)
TIME (hours)
20
15
10
5
0
0
12
24
36
48
60
76
88
100
112
124
136
148
160
168
180
192
204
216
228
240
252

difference as was seen for low and high porosity fruits of tree 2, due perhaps to fruits from tree 1 having lower porosities than those of tree 2.

The low and high porosity fruits from tree 2 showed marked differences in their respiration rates at 20° and 30°C. Thus, at 20°C when the respiratory metabolism was under no stress, the low porosity fruits had a slightly higher rate of O_2 uptake; but at 30°C when the respiratory metabolism would be under some degree of stress a reversal of the situation at 20°C was observed, in that the high porosity fruits now had a higher rate of O_2 uptake. The low and high porosity fruits of tree 1 show a similar relationship to temperature changes, though the differences were slight in comparison to those observed for fruits of tree 2.

The changes in relative respiration rates observed for high and low porosity fruits at 20° and 30°C suggest that gas movement was apparently not a limiting factor in high porosity fruits. At the higher temperature, the respiratory activity of low porosity fruits was depressed perhaps because of an oxygen shortage or a build-up of CO_2 within the tissues or both. Studies of Trout <u>et al</u> (1942) showed that at higher temperatures the internal O_2 in Granny Smith apples was consumed faster than the rate at which it diffuses. Thus, at 7°C they found the internal O_2 to be 17% but at 29°C it was only 2%. Similarly, the internal CO_2 levels observed by them were 2% at 7°C and 17% at 29°C. Moreover, they found the respiration rate was related to internal O_2 concentration.

The lower rate of respiration observed for high porosity fruits at 20°C is not clearly understood. The age of the fruit may have been a factor since 50% of the high porosity fruits were from the third harvest of October 17, whereas, 42% of the fruits in the low porosity group were from the first harvest of September 26.

Finally, the rates of CO_2 production by the low and high porosity fruits of trees 1 and 2 closely paralleled those for O_2 uptake, the respiratory quotients for the low and high porosity fruits being similar (approximately 1·04).

D. The Effect of Skin-coating on Fruit Porosity and Respiration of Jonathan Apples: The influence of porosity on the respiratory activity of fruits was further studied using skin coatings of wax.

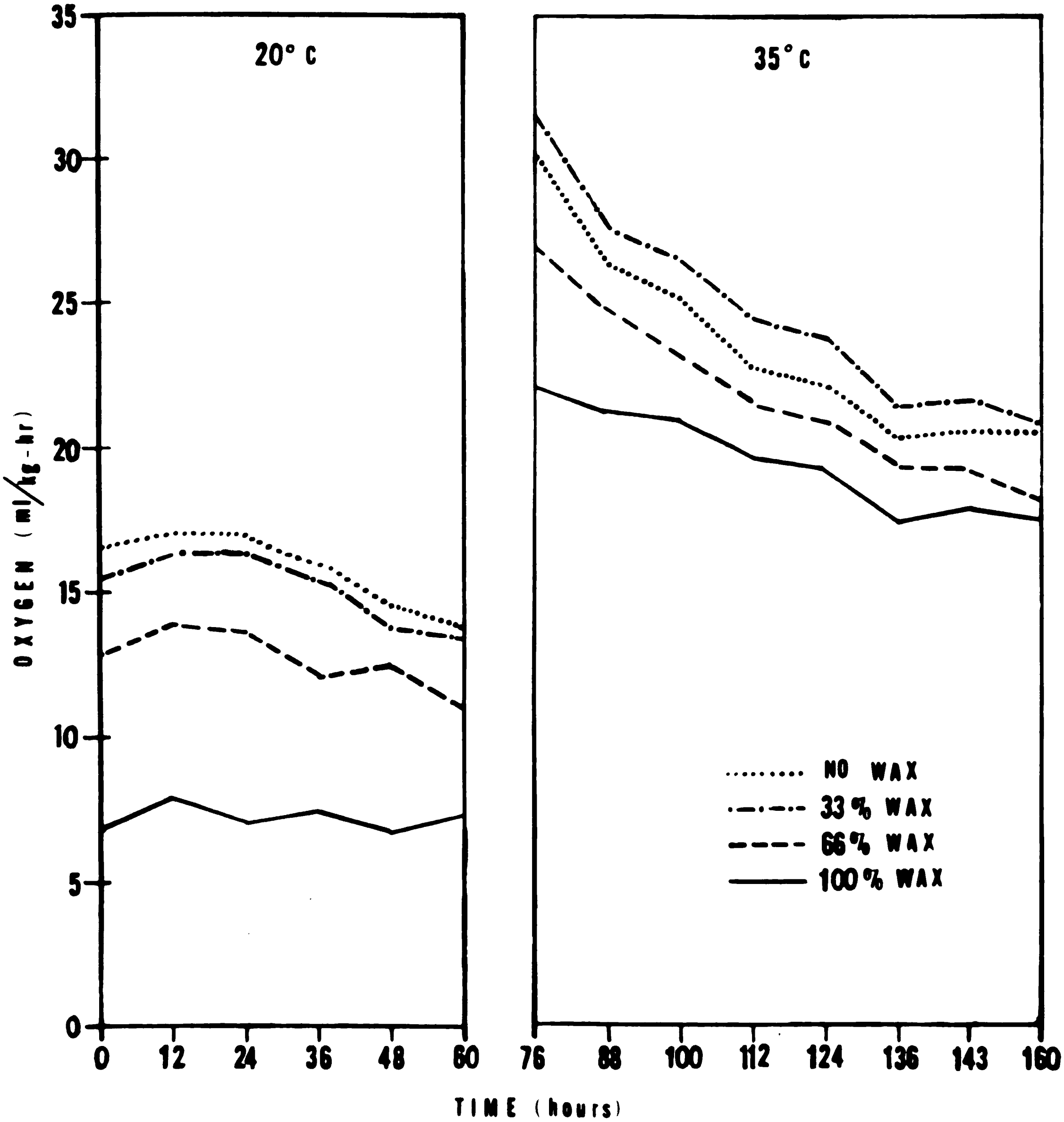
Experimental: Medium sized Jonathan fruits harvested October 10, 1966 at the Graham Station were tested for porosity by the Hoff and Dostal (1967) method using 8 fruits. Porosity readings were taken before and immediately after waxing with 100% concentration of Waxol-12.

In addition, several lots of fruits were similarly prepared by waxing using concentrations of 100%, 66%, 33% and 0% (control) Waxol-12 as a skin-coat. The respiration rates for all samples were measured first at 20° and then at 35°C.

Results and Discussion: The data presented in Table 19 show that porosity was markedly reduced for all but one apple by waxing. The high initial gas movement rate in this particular fruit suggested more or less free passage of the gas and this was confirmed by subsequent examination. Since the calyx canal was not fused, there was opportunity for direct passage of air to the interior of the core.

The respiration rates (O_2 uptake) of fruits, shown in Figure 7, were markedly affected by waxing, particularly at 20°C. At this temperature the apples coated with 100% Waxol-12 had a rate less than one-half the unwaxed; those with 33% concentration were similar in rate

Figure 7. The effect of skin coatings on the respiration of Jonathan apples at 20° and 35°C.



20° C
35° C
OXYGEN (ml/kg-hr)
35
30
25
20
15
10
5
0
0
12
24
36
48
60
76
88
100
112
124
136
143
160
TIME (hours)
NO WAX
33 % WAX
66 % WAX
100 % WAX

to the controls. The differences were considerably reduced when the apples were transferred to 35°C, however, the rates were dependent upon the amount of wax applied for the duration of the experiment.

Table 19. The effect of waxing on the porosity of Jonathan apples in 1966-67.

Fruit weight gram	Before waxing PO/p 40/30 cm sec	Before waxing Porosity ml/sec	After waxing[1/] PO/p 40/30 cm sec	After waxing[1/] Porosity ml/sec
114	140	2.05	746	0.38
117	115	2.50	563	0.51
123	123	2.34	1617	0.18
126	360	0.80	640	0.45
128	5	57.50	5	57.50
132	56	5.13	142	2.02
133	83	3.46	215	1.34
135	45	6.39	330	0.87

[1/] 100% concentration of Waxol-12 was used.

Finally it may be pointed out that the amount of CO_2 produced by fruits of different treatment lots was essentially the same as those observed for O_2 uptake, the respiratory quotients being very close to 1.05 in all the four treatment lots at both 20° and 35°C.

Fruit porosity obviously affects gas exchange and is important to the respiratory metabolism of the apple fruit. It is likely that fruits of different porosities respond differently when stored under atmospheres of high CO_2 and low O_2 levels. In view of the findings of Trout et al (1942) and of Hackney (1944) on the internal O_2 levels of apples, it would appear that fruits of higher porosities were better able to obtain their O_2 requirements than the fruits of low porosities, thus affecting their respiratory metabolism.

General Discussion: In Part II it was shown that CO_2 injury and internal breakdown were positively correlated with fruit weight, volume, and volume-weight ratio, while here, it has been shown that fruit porosity was similarly correlated with these physical characteristics of fruits; the coefficients of correlation between fruit size and CO_2 injury, and between fruit size and fruit porosity being about the same magnitude. That fruit porosity is a likely predisposing factor to CO_2 injury is evidenced by (Table 20) the results recorded for the Stover orchard's Jonathan apples in the storage trial (see Part I, B).

Table 20. The effect of fruit maturity on fruit porosity and CO_2 injury of Jonathan apples from the Stover orchard stored for 130 days in 18% CO_2 and 3% O_2 at 40°F in 1966-67.

Harvest	Average wt (gm)	Average porosity (ml/sec)	CO_2 Injury (%)	Average wt (gm)	Average porosity (ml/sec)	CO_2 Injury (%)
	Tree 1			Tree 2		
Early	75.0	1.11	0.0	94.5	2.13	13.0
Mid-season	82.2	1.24	0.0	91.2	1.88	16.0
Late	84.3	1.94	0.0	91.6	2.85	28.0

Fruits of tree 1 in general had slightly lower fruit porosities than fruits from tree 2. Tree 1 fruits failed to show CO_2 injury even though there was a progressive increase in porosity as the harvests progressed. This suggests the possibility of a 'threshold' level for fruit porosity which if exceeded results in CO_2 injury. Large fruits have been shown to be susceptible to CO_2 injury, while on the other hand, Denne, 1960 and Smith, 1950 have indicated that large fruits, in general, have more cells of larger average size than small fruits. Since experimental data on cell number and cell size for these large sized Jonathan apples were not obtained, it is difficult to specifically relate the incidence and severity of CO_2 injury to individual effects of higher fruit porosities or gas exchange, greater cell number, or increased cell size. Nevertheless, experimental evidence presented in Part III have indicated that increased gas exchange in fruits increases the incidence and severity of the storage disorders. This then suggests high porosities of large fruits is an important factor favoring gas exchange. Thus, it appears that high porosities in apple fruits allow the accumulation of CO_2, yet permit an adequate supply of O_2 so as to favor the development of CO_2 injury in the core region.

GENERAL DISCUSSION

Brown heart of apples, which is often referred to as CO_2 injury, is generally considered to develop in fruits of under conditions which cause a toxic build-up of CO_2 gas within the fruit. This belief probably originated when the disorder was first recognized to be of commercial importance in overseas shipments of apples from Australia to England and related to conditions of poor ventilation in ships' holds (Kidd and West, 1923). Even at that time it was recognized that high CO_2 did not always cause brown heart and that some apples remained entirely free of it. The results of this study, made over a period of two years, helps to account for these variable responses and manifestations of the brown heart disorder. Tests made on apples stored in high CO_2 atmospheres had indicated that fruit which had developed brown heart had higher, rather than lower, rates of gas exchange over fruits of comparable size and free of brown heart. In a subsequent study, it was further demonstrated that CO_2 injury could be induced, even in small sized Jonathans, by facilitating gas exchange with epidermal punctures. This finding was examined in detail by a study of porosity of Jonathan apples, especially as related to tree source of the fruits, harvest time over-maturity, and fruit size.

Of the factors studied, fruit size was found to be of foremost importance. There was a consistent increase in porosity as determined by gas flow characteristics with increases in fruit size. There is a highly significant positive correlation between fruit porosity and fruit weight, volume, or volume-weight ratio when all sizes of fruit are considered together. Further, fruit porosity was related significantly to the tree source and maturity with fruits picked late in the season having higher porosities than earlier-picked fruit. It is probable that these factors affect porosity because of their effects on fruit size. In small sized Jonathan (105 grams and less), no significant correlations were observed for fruit porosity, though in medium and large sized Jonathans porosity was positively correlated with fruit weight or fruit volume, but not with volume-weight ratios.

Interestingly, the significant correlation coefficients between fruit porosity and fruit weight or volume were about the same as those between fruit porosity and total air space, while the correlation coefficients between porosity and volume-weight ratio, and between porosity and air space as percent of fruit volume were also of about the same magnitude. In this connection, not only fruit volume and total air space are related linearly to fruit weight (with r values above 0.96), but also with each increase in fruit weight both increased at slightly greater rates; consequently there occurred slight increases in air space expressed as percent of volume or volume-weight ratios with increases in fruit weight. This observation is in agreement with the observations of Bain and Robertson (1951). Hoff and Dostal (1967) believe porosity to be a property of fruits with both peel and flesh influencing its value. Since, in the present study porosity determinations were made for the 'whole' fruits, the influence of flesh, if any, would be difficult to establish. The modifying influence of the peel on fruit porosity is important, since the number of lenticels or the free passages would greatly influence the rate of gas leakage through the fruit. Burg and Burg (1965) have also considered the apple peel to be of major importance, offering considerably more resistance to gas exchange than the flesh. Further, they found gas exchange in apples was quantitatively accounted for by diffusion through the lenticels. The significant increases observed in the porosity of medium and particularly large sized apples may therefore be due to changes in the structure of their peels, and probably the lenticels.

Smith (1940) reported apple varieties differed greatly in their cellular structure and that while the average cell size was related to the length of growing season, average fruit size was largely determined by the amount of cell-multiplication. Denne (1960) on the other hand found fruit size was determined by both the average cell size and cell number. Later Smith (1950) showed that either average cell size or average cell number or both together can be operative in determining fruit size. Within-tree variations in fruit size were found by Bain and Robertson (1951) to be mostly due to variations in cell number and only to a small extent to average cell size, and according to Martin et al (1964), the increase in susceptibility to disorders of such fruits was relatively small with increase in fruit size. On the other hand, Martin and Lewis (1952) reported between-tree variations in fruit size were primarily due to differences in the amount of cell expansion, fruits from light-crop trees having fewer cells and larger fruit diameters. Further, they found that in such cases the susceptibility to storage disorders increased greatly with increase in fruit size. This is a significant observation. Fruits with relatively fewer cells and larger fruit diameters will have a higher volume-weight ratio. Also, such fruits will very likely have high values for porosity and freer gas exchange.

In the light of the findings of others, and the positive correlations noted in the present study between fruit size and porosity, it would appear that in the studies made by Smith (1940, 1950), Martin and Lewis (1952), Martin et al (1954), as well as others, on average cell size and cell number in relation to fruit size, and susceptibility to physiogenic disorders little attention had been paid to the concurrent changes in the gas diffusion properties. This concept that increases in gas exchange are responsible for increases in the incidence of disorders could account for the conflicting findings reported by several workers (Letham, 1961; Sharples, 1967) on the relation of fruit size, cell number and cell size to disorder susceptibility.

There is an important role for porosity in gas exchange within the fruits. In the study of respiration rates of low and high porosity fruits it was demonstrated that at 30°C, gas exchange was probably not a limiting factor in the case of high porosity fruits, consequently, they respired at a slightly higher rate than low porosity fruits - a reversal of the situation observed for 20°C. Similarly, the effect of reduced fruit porosity by waxing to lower the respiration rates indicates that the peel porosity influences the rates of gas exchange in fruits.

In this connection, the findings of Alentoff (1954) are of interest. He not only observed a linear relationship between CO_2 fixation in McIntosh apples and concentration of external CO_2, but also found the fixation rates at harvest were concurrent with the respiratory climateric of the fruits. As mentioned earlier, porosity was found to increase significantly with delay in harvest. While with delay in harvest there is a rise in the respiratory climateric. It is therefore reasonable to suppose that the increased rates of CO_2 fixation reported, may be due, at least in part, to concurrent increases in fruit porosity. Studies of Chace (1959) showing increased recovery of ^{14}C from all $^{14}CO_2$ fruit tissues with increases in fruit diameter appear to confirm this influence of porosity as a factor affecting gas exchange.

When the effects of environmental factors and harvest maturity on CO_2 injury incidence were investigated, it was observed that the incidence of CO_2 injury ranged from 0 to 9% in fruits from three different harvest dates in as many as 64% of the trees under study. This small amount would suggest that the rise in the respiratory climateric with delay in harvest as noted for the orchards from which fruits were obtained, had no material influence on the development and incidence of CO_2 injury. Furthermore, fruits of tree 1 (Stover orchard) showed no CO_2 injury even when some increases in porosity were noted with each delay in fruit harvest; while fruits of tree 2, which in general were higher in porosity values than those of tree 1, recorded progressive increases in the amount of CO_2 injury. Therefore, this result and also the fact that low incidence recorded for CO_2 injury for 64% of the trees under study would seem to indicate that there is a threshold level of porosity influencing gas exchange in fruits, which if exceeded, injury occurs. It is also likely that in small fruits (105 grams weight and less) the critical threshold levels were not exceeded, but in fruits of about 130 grams weight and over, critical levels for porosity affecting gas exchange were exceeded so as to permit the development of CO_2 injury.

Storage studies with waxed and russetted apples have helped in further elucidating the possible role of gaseous exchange and the possible effects of CO_2 and O_2 on the development and incidence of both CO_2 injury and internal breakdown disorder. Internal breakdown, often referred to as a senescence disorder, was shown by Dewey et al (1957) to be associated with high levels of O_2 in the storage atmospheres. Its absence in this study in russetted Jonathans can be accounted for from the findings of Scott et al (1967) and Wilkinson (1965). Scott et al (1967) found that internal breakdown in Jonathans and Delicious apples decreased linearly with weight losses from moisture evaporation, while Wilkinson (1965) demonstrated that the permeability to air is seen to decline sharply in apple fruits stored under dry conditions due to concomitant shrinkage of both skin and flesh tissues. Since a decline in the permeability of the tissues to air would limit the oxygen supply, the absence of internal breakdown in the russetted Jonathans was a likely result of the reduced permeability of the tissues to O_2 gas with shrivelling. On the other hand, the fact that the incidence of total CO_2 injury in russetted Jonathans was slightly, though not significantly, higher - 62% as compared to 55% for the non-russetted fruits - would suggest that the shrinkage of the skin and flesh tissues due to moisture loss, had no great influence on the diffusion rates of CO_2 in the relatively high concentrations (18%) used in the storage atmosphere. Also, the resistance of apple fruit tissues to the diffusion of CO_2 do not change appreciably as they do for O_2 have been reported by Trout et al (1942), Hackney (1944, 1944a), Hall et al (1955), Marcellin (1956) and others.

The significantly low incidence of brown heart observed in russetted Jonathans may therefore be due to the reduced availability of O_2 gas in the core region, especially because of the low concentrations of O_2 in the storage atmosphere. This then would suggest that O_2, in some way, influences the development of CO_2 injury; if so, this finding is in agreement with those of Bogdanski (1960), Rasmussen (1961) and Eaves et al (1964) for apples; of Miyachi et al (1955) for green algae; and of Rhoads et al (1960) for the dark fixation of CO_2 by roots. Thus, Rasmussen (1961) and Eaves et al (1964) showed the incidence of CO_2 injury tends to increase when higher concentrations of O_2 were used in the CA atmospheres. Bogdanski (1960), on the other hand, considered the oxidation of ascorbic acid in the core tissues to be dependent on the supply of O_2, and demonstrated that the oxidation of ascorbic acid precedes the development of brown heart in apples.

Similarly, the significant reduction in the incidence and severity of internal breakdown in the waxed Jonathan of the present study, may be attributed to the reduced availability of O_2 to the internal tissues which resulted in a slower rate of tissue senescence or breakdown. Even so, waxing did not alter the incidence and severity of CO_2 injury (brown heart). It would therefore seem that the critical levels of O_2 under which CO_2 injury or internal breakdown develop are markedly different, being considerably lower for CO_2 injury than for the internal breakdown disorder. This conclusion gains added support when the data on the relationship of fruit size to the development of both CO_2 injury and internal breakdown (see Table 7, Figure 3) are examined.

That large fruits are much more susceptible to internal breakdown than small fruits is in agreement with Martin (1953, 1954, 1954a); Letham (1961) and numerous other workers.

There is the question as to whether fruits with high porosity receive a better supply of internal O_2 than fruits with low porosity. The work of Trout et al (1942) shows that internal O_2 levels in fruits increase in proportion to the amount of the epidermis that is either removed, ruptured or punctured. Hackney (1944) also found internal O_2 levels to be higher in fruits with open lenticels than in comparable fruits with closed lenticels. It is reasonable to suppose that high porosity apples will be able to maintain relatively higher levels of internal O_2 than fruits with low porosity. It is further suggested that the increases in the resistance of O_2 diffusion during storage as reported by Trout et al (1942), Hackney (1944, 1944a) and Hall et al (1955) was probably not critical for high porosity fruits since the incidence and severity of CO_2 injury were significantly higher for them than for fruits of low porosity.

It is suggested that these results be confirmed by storage tests employing fruits of varying sizes and harvest maturities under variable O_2 and constant CO_2 levels in the CA stmosphere. It would also be helpful to determine if CO_2 injury can be avoided, even under relatively high CO_2 levels, by controlling O_2 levels to a maximum of 2% from the start of the CA storage operation.

SUMMARY AND CONCLUSIONS

The purpose of these studies was to investigate the fruit factors that possibly affect gas exchange and therefore the development of CO_2 injury in Jonathan apples when stored in high CO_2 concentrations (about 18%) in controlled atmospheres.

Tests with CA stored apples showed that fruits which had developed the brown heart and internal breakdown disorders had significantly higher rates of O_2 diffusion than fruits of comparable size but free of disorders; that O_2 diffusion was significantly greater in large than in small fruits; and that there was a positive correlation between CO_2 injury and epidermal punctures which enhanced gas exchange.

Fruit porosity, as measured by gas flow through the apples, varied significantly due to fruit source (tree), but was positively correlated with harvest maturity and fruit size. When all fruit sizes were considered, the correlation coefficients (r values, non-linear terms) of porosity with the fruit physical characteristics of weight, volume, and volume-weight ratio were 0.71, 0.76, and 0.63, respectively. Determinations made for each fruit size, however, showed this relation was not true for Jonathan of small size (105 gram and less), while the porosity of medium and large sized Jonathan was correlated to fruit weight and fruit volume, but not to volume-weight ratios.

Fruit volume and total air space in fruit of the Jonathan variety were related linearly to fruit weight with r values above 0.96. Both values increased at slightly greater rates than fruit weight, thus, accounting for an increase in the volume-weight ratios with an increase in fruit weight. Similar increases in volume-weight ratios with increases in fruit weight were observed in fruits of Delicious, Rome Beauty and McIntosh varieties.

Respiration rates at 30°C for fruits of low and high porosity indicated that gas exchange was not a limiting factor to respiration in high porosity fruits, consequently, they had a slightly higher rate of O_2 uptake than the low porosity fruits. At 20°C, the high porosity apples had a slightly slower rate of respiration than low porosity fruits. Skin coating of wax reduced sharply the fruit porosity and also lowered the respiration rate as measured by O_2 uptake.

Fruits from light-crop trees and apples picked late in the season were susceptible to CO_2 injury. However, it is the fruit size which is an important factor influencing CO_2 injury. Jonathan fruits weighing 105 grams or less failed to develop CO_2 injury during CA storage; those weighing 110 grams had 1.4% injury; and injury increased steadily to about 8.5% in fruits of 121-125 grams weight. With larger size, the injury increased markedly to 45% for 126-130 gram fruits and further steady increases occurred with additional increases of fruit weight so that 100% of the fruits weighing 160 grams or more were affected. Severity of CO_2 injury was also related to fruit size with weights between 126 and 135 grams showing a sharp change between general injury, referred herein as 'simple' CO_2 injury, and typical brown heart symptoms. The incidence and severity of internal breakdown also increased rapidly at fruit weight categories of 125 grams and greater. The correlation coefficients (r values, non-linear terms) for the fruit characteristics of weight, volume, and volume-weitht ratio, respectively, were 0.78, 0.79, and 0.51 for CO_2 injury, and 0.60, 0.60 and 0.26 for internal breakdown. It is believed significant that the correlation coefficients of fruit size to porosity and of fruit size to CO_2 injury were of similar magnitudes since they indicate that freer gas exchange is an important factor in CO_2 injury. Up to 26% of the variations in CO_2 injury could be ascribed to the effects of the volume-weight ratio of the fruits, but for only 7% of the internal breakdown disorder variations. Fruits with volume-weight ratios of 1.28 and greater had a higher incidence of brown heart - a more severe form of CO_2 injury.

Although both disorders are normally associated with high rates of gas exchange in fruits, they are markedly different in etiology. CO_2 injury occurs in the core region while internal breakdown or senescence breakdown disorder occurs in the peripheral cortical tissues of the fruit. Waxing the fruit surface had no significant influence on the development of CO_2 injury, yet it reduced porosity and changed the internal breakdown incidence from 33% to about 4% in large sized Jonathans. Russetted Jonathans, which were somewhat shrivelled by end of the storage period, developed no internal breakdown and only a minor degree of brown heart, 2.2% as compared to 46% for the non-russetted Jonathans; therefore, the availability of O_2 was probably not a rate-limiting factor in the non-russetted fruits. The development and incidence of both CO_2 injury and internal breakdown increased with increased rates of gas exchange in the fruits.

The CO_2 injury of Jonathan apples appears to be determined by fruit porosity. Apples of an anatomical structure that permits a relatively free gas exchange are more likely to develop disorders than those in which gas movement is more limited. An adequate supply of O_2 at the core region seems essential for the development of brown heart and simple CO_2 injuries under controlled atmosphere conditions. The critical levels of oxygen have not been determined.

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