SOME ASPECTS OF THE PHYSIOLOGY AND DEVELOPMENT OF JONATHAN SPOT IN APPLE FRUITS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
Amos Edwin Richmond
1963

This is to certify that the

thesis entitled

Some aspects of the physiology and development of Jonathan spot in apple fruits.

presented by

Amos Edwin Richmond

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Horticulture

Major professor

Date August 7, 1963

ABSTRACT

SOME ASPECTS OF THE PHYSIOLOGY AND DEVELOPMENT
OF JONATHAN SPOT IN APPLE FRUITS

by Amos Edwin Richmond

Researches were conducted to determine the causal sequence involved in the development of Jonathan spot, a physiological disorder affecting ripe apple fruits of the Jonathan cultivar.

Morphological and anatomical observations revealed that the disorder commonly termed as Jonathan spot consists of two types. One occurs in association with an aperture in the fruit epidermis, and was named in this study "lenticel spot". The other, or "true" Jonathan spot, occurs without relation to lenticels or other apertures in the epidermis. Once the spot types were distinguished, the researches were confined to Jonathan spot.

Study of harvest and storage factors affecting Jonathan spot showed that early picking, storage in 60°F or above and as low as 0.7% CO_2 in the atmospheres surrounding the fruits reduced or inhibited spot formation. Relative humidity did not affect the disorder.

Various analyses of the spotted tissue were conducted. The pH of spotted tissue homogenates ranged between 4.4 and 5.2, which was 0.5 to 0.7 units higher than the pH of normal tissue. The buffering capacity of this homogenate all along the titration curve was approximately double that of normal.

Mineral accumulation in the spotted tissue was confirmed. To

ascertain a possible sequential relation between spot development and K accumulation, Cs^{137} was introduced into the fruits some 3 weeks before harvest. Autoradiographs showed the isotope to be uniformly distributed in the fruit peel immediately after harvest. The few spots that developed in early January were free of Cs^{137}; however, accumulation of the tracer was observed in several spots that developed later in the storage season.

Nonvolatile acid extraction and fractionation revealed an approximate two-fold increase in total acidity in the spotted tissue, as well as an increase in the percentage of citrate. The observed changes in cations and total acidity were shown to be related to the raised pH and the titrational characteristics of the spotted homogenate.

Jonathan spot characteristically appeared earlier and to a higher extent on the red areas of the apple skin. The anthocyanin destruction that accompanied spot development increased with aging of the spots. Thus, it was speculated that degradated anthocyanin served as a precursor for the synthesis of the brown pigment which is typical to the disorder. However, since mature green-colored apples eventually developed extensive spotting, anthocyanin would be only one of several possible precursors.

Respiratory measurements of peel discs in a Warburg respirometer showed that endogenous O_2 uptake was close to normal for newly spotted tissue, declining to one-half with the aging of the spots. O_2 uptake of normal and spotted peel discs upon the addition of catechol and ascorbic acid was identical, suggesting that the lower endogenous O_2 uptake of old spotted tissue was due to a lack of substrate. Addition of malate

revealed that spotted discs partially lost the capacity to decarboxylate malate.

A possible hypothesis for Jonathan spot development is that some cells in the periphery of the receptacle of the young fruit undergo modification early in the ontogeny of the fruit. With maturation, cations and phosphorus accumulate in the spot-susceptible cells. The cation influx is then responsible for the higher total acidity and higher pH observed for spotted tissue. The rise in pH is suggested as the initial causal factor promoting anthocyanin breakdown and the channeling of various carbonylic compounds and polyphenols into the synthesis of the brown pigment that gives the typical discoloration associated with Jonathan spot.

SOME ASPECTS OF THE PHYSIOLOGY AND DEVELOPMENT OF JONATHAN SPOT IN APPLE FRUITS

By

Amos Edwin Richmond

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1963

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to Dr. D. H. Dewey for his counsel and aid throughout this study and for his valuable guidance in the preparation of the manuscript.

The author is also greatly indebted to Drs. R. D. Dilley and M. J. Bukovac for their assistance in various aspects of this research and for reviewing the manuscript and serving as members of the guidance committee.

Acknowledgement is also accorded to Drs. I. Knobloch and N. E. Tolbert who reviewed the manuscript and served as members of the committee and to Mr. H. C. Dostal for his technical assistance.

CONTENTS

LIST OF TABLES

LIST OF TABLES CONT'D

LIST OF ILLUSTRATIONS

INTRODUCTION

Jonathan spot is a physiological disorder affecting ripe apple fruits. Although observed in a number of cultivars such as King David, Rome Beauty, Wealthy and others, it is observed most commonly in Jonathan. Apple breeding studies (Plagge et al., 1935) support the idea that Jonathan spot is an inherent disorder and that the susceptibility to it is a varietal trait.

Jonathan spot is manifested by dark spots in the skin, ranging in color from bluish black on the deep blush, to greenish brown on the less red colored areas of the fruit. The spots also vary greatly in size, from about 1 to 10 mm in diameter. Starting usually as small roundish spots, the affected areas continually increase in diameter, until in late stages, many spots become confluent, forming large irregular dark patches.

The importance of this disorder to the Jonathan apple industry may be evaluated from the fact that after a period of 5 months in storage at 36°F, the storage temperature recommended for Jonathan apples, nearly all the fruits will usually develop extensive spotting, thereby becoming commercially worthless.

Jonathan spot has been studied quite extensively in the U.S.A., Australia and recently also in Japan. So far, however, most of the research was concerned with factors associated with the incidence or the control of the disorder.

This study was oriented towards an attempt to gather data leading

to the postulation of a theory concerning the causal sequence involved in the formation of Jonathan spot that will meaningfully correlate the various observations regarding the development and inhibition of this disorder.

LITERATURE REVIEW

The first report found in the literature apparently concerning the Jonathan spot disorder was by Scott (1911), who described in the first volume of Phytopathology "a new fruit spot of apple." Observing that in most cases, no microorganisms could be isolated from the spots, Scott speculated that they were caused by lead arsanate, a newly introduced spray chemical at that time. Thus arose a controversy as to the causal agent involved in the formation of the spot. Three years later Cook and Martin (1914) reported that 90 per cent of the cultures of spotted tissue yielded "luxuriant and characteristic growth of the Alternaria." They therefore held that the disorder was pathological in nature. Scott and Roberts (1913) reported that the spots must result from physiological circumstances, a conclusion that Brooks and Cooley (1917) also reached. Studying lenticel spotting of apples in storage, Kidd and Beaumont (1925) confirmed these observations. They pointed out that Jonathan spot must have a functional origin, since microorganisms could not always be isolated at the early phases of the disorder.

But for all these reports, the fact that some fungi, and particularly Alternaria sp., could be frequently isolated from Jonathan spots has tended to add confusion to an understanding of its origin. Thus recently, Krapf (1961) still examined the possibility of a pathological causal agent and reported that "In general, Jonathan spot must be considered as a physiological, non-parasitic disorder," fungul infection being secondary.

The effects of various factors on the development of Jonathan spot have been investigated. These include the effects of temperature, humidity, cultural practices, nutrient levels, time of harvest, color and size of the apples, controlled atmosphere, coating of the fruit, growth regulators and seed number.

Brooks and Cooley (1917) reported that the occurrence of spots increased with a rise in temperature up to 68°F. Seven weeks following harvest, no spot development could be observed in fruit held at either 86° or 32°-40°. Tomana (1960) obtained similar results observing that spots developed more rapidly on fruit stored at 68° ± 9°, than on fruit stored at a constant temperature of 95° or 32°. Plagge and Maney (1924) found that after six months in storage, practically the same amount of total spotting developed at both 32° and 40°, but that the amount of badly spotted apples at 40° was twice that at 32°. Dewey et al. (1957) reported Jonathan spot to have become prevalent after 4 months of storage at 36° or 7 months at 32°.

Studying the effect of humidity in storage on Jonathan spot formation, Brooks and Cooley (1917) reported less spotting in open containers at 70% relative humidity than in similar lots stored in moist containers. Plagge and Maney (1924) described an experiment in which apples were wrapped in moist blotting paper and exposed to room temperature of 80°F. At the end of a 72 hour period, all the treated apples were badly spotted. Likewise, Tomana (1959) stated that regardless of storage temperature, spots developed more rapidly on fruits that were kept in high humidity treatments.

Early workers were concerned with the possible effects of various cultural practices on Jonathan spot formation. Brooks and Fisher (1918)

could find little consistent relation between irrigation levels and spotting. Nevertheless, they concluded that in general, heavy irrigation slightly favored the disorder. Plagge and Maney (1924), however, concluded that irrigation did not materially affect the degree of spot development. Also, having compared four different soil treatments - two types of sod, cover crop and clean cultivation - the same authors concluded that no direct effect of cultural methods could be observed on the incidence of the disorder. They did observe, however, that the more intensely colored portion of the apple skin is more likely to develop Jonathan spot. Indeed, Plagge et al. (1935) stated that "nitrogenous fertilizers have lessened the degree of spotting, largely, as they have decreased the amount of color in the fruit." Tomana (1959) also stated that there was no increased percentage of Jonathan spot as a result of nitrogen additions to the soil. Two years later, Tomana (1961) reported that spotted apples generally contained more nitrogen than less or non-spotted fruit. This was also the case with fruit harvested from a heavy nitrogen without phosphorus plot in a fertilizer experiment. Buneman (1959), however, surveying the nutrient levels of 16 orchards in Kent County, Michigan, concluded that Jonathan spot occurred without any consistent association to nutrient factors. Likewise Clerx (1960), comparing mineral content of spotted and non-spotted tissues could find no substantial difference in nitrogen content between these tissues.

Much work was conducted concerning the effect of fruit maturity on Jonathan spot development. Plagge and Maney (1924) experimented with "early" and "normal" picked fruit, the storage of which was either immediate or delayed for 2 weeks. In most cases, immediate storage gave the least amount of Jonathan spot, this being true for both late and

early picking, while late picking usually resulted in more Jonathan spot. Delay in storing the fruit was found to be more favourable for Jonathan spot formation than time of picking. In general, the longer the delay - up to 22 days - the higher was the incidence of spotting. Likewise, Trout et al. (1940) also reported a progressive increase in Jonathan spot development with increasingly longer delays between harvest and storage, as well as with late picking. Recently, Martin et al. (1961) stated that all workers agree that delay in cold storage and late picking increase the incidence of the disorder. Nevertheless, there is no general agreement regarding the effect of the date of harvest. Brooks and Cooley (1917), Brooks and Fisher (1918) and recently Tomana (1961), all reported the earlier picked fruit to be more susceptible to Jonathan spot.

The contradictory observations concerning time of harvest and storage would be difficult to reconcile unless it is speculated that the common name "Jonathan spot" has been used by some workers to describe different disorders. This possibility was suggested by Plagge and Maney (1924), when they observed that two general kinds of spots could be distinguished. One type centered and developed concentrically around lenticels; the other type occurred anywhere on the apple skin and was not necessarily confined to lenticels. The authors added, however, that the two types were considered to be the same disorder as they appeared to be caused by the same conditions.

An important contribution towards understanding the physiology of Jonathan spot was made by Pentzer (1925) when he observed that the spots of severely spotted apples treated overnight in saturated HCl atmosphere changed color to red. He found that the average pH value of

affected tissue was 4.7, while the normal tissue had a pH value of 3.8. The observed increase in pH often extended $\frac{1}{4}$ inch into the cortex. In another experiment, the pH of unaffected peel was 3.7, whereas after treatment with ammonia fumes the peel turned blue, its pH measuring 4.5. Pentzer concluded that the spots were formed as a result of decreased acidity in the spotted area which brought about an increase in the pH and the subsequent conversion of the color of anthocyanin from red to blue. Plagge and Gerhard (1930), however, have reached seemingly contradictory conclusions. Investigating changes in acidity of apples undergoing various storage treatments, they reported that there appeared to exist an inverse relationship between the percentage of total acid loss during storage and the susceptibility towards Jonathan spot, a low acid loss during storage usually producing a high percentage of spotting. Clerx (1960) also studied the acidity levels of skin and cortex of apples which underwent different storage treatments, but concluded it was doubtful that there was any causal relation of acid changes and spot development. To cite one case, spots did not develop at room temperature, yet the total titratable acidity dropped to similar or lower levels than those observed in highly spotted apples.

Clerx (1960) made a significant contribution towards a meaningful interpretation of the causal mechanism involved in Jonathan spot development, finding a marked accumulation of K, Ca, Mg and P to occur in the spotted tissue. Indeed, an important part of the present work had been centered around this finding.

The first report leading to what has become a method for the control of Jonathan spot was by Trout et al. (1940). Their storage studies included treatments of two temperatures, 32°F and 37 - 40°, as well as

three atmospheric gas combinations as follows: 2% CO_2, 19% O_2; 5% CO_2, 16% O_2 and 10% CO_2, 11% O_2. In all cases Jonathan spot was completely inhibited. Shortly afterwards, Plagge (1942) found that spot development was prevented with a CO_2 treatment ranging from 3% to 12%. Dewey et al. (1957) and Dewey (1962) reported practically no spot development in 3% O_2 and 5% CO_2.

Much work with skin coating of apples was carried out in Australia, (Trout et al. 1953. Hall et al. 1953). Many materials for skin coating were tried, one of the most successful combinations in reducing incidence of Jonathan spot being a 10 to 15% castor oil and shellac emulsion in alcohol. Hall et al. (1953) reported that castor oil - shellac coated Jonathan apples stored for 35 weeks at 40°F showed 3.7% spotting as compared to 5.6% of the control. Trout et al. (1953) were able to show that a sample of the internal atmosphere of coated apples contained 3.5% CO_2 and 16% O_2, while the uncoated controls contained only 2.5% CO_2 and 18% O_2. The positive effect of coating in reducing Jonathan spot development was explained as stemming from the increase in the internal CO_2 content.

Australian workers reported (CSIRO, 1958) that naphthalene acetic acid (NAA), adenine and kinetin applied as a spray in time of bloom increased fruit cell number significantly. Only kinetin, however, significantly increased the occurrence of Jonathan spot.

Recently, Martin et al. (1961), reported an interesting observation. Fruits showing the disorder contained a greater mean seed number than sound fruits. Within and between trees, the variables fruit weight, seed number and Jonathan spot were found to be positively interrelated, the partial correlation of Jonathan spot and seed number, holding fruit

size constant, remaining highly significant. Further evidence for the seed number - disorder relationship was obtained from thinning experiments. Within trees, when a large increase in fruit size was obtained without affecting seed number, no increase in Jonathan spot could be observed. Between trees, however, a treatment which produced populations of the same size but varying seed number yielded corresponding different levels of Jonathan spot. It was concluded that seed number affected the incidence of Jonathan spot, while the correlation between fruit size and the disorder resulted from the effect of seed number on fruit size.

The same authors also reported that seeds of fruits with Jonathan spot had a greater tendency to germinate within the fruit than seeds of sound fruits.

METHODS

Four general research methods were employed. The methods utilized are described accordingly under four sections which are also followed in reporting the results.

I. Morphology and Histology of Jonathan Spot

The morphology of various types of spots developing on Jonathan apples was observed in detail and photographed[1/] with a Hasselblad 1000F camera, using polarized light to control reflections. Close ups of X8 magnifications were taken with the same camera, employing a polarized ring.

For microscopic observations, skin segments measuring 6x6x3 mm with Jonathan and lenticel spots were cut from the fruit. These were killed and fixed in F.A.A. (Formalin-Aceticacid and-Alcohol), embedded in paraffin and sectioned with a rotary microtome. The sections were cut 12 μ thick and were stained with Safranin-Fast green, (Sass, 1961) mounted in Balsam and photomicrographed.

II. Harvest and Storage Factors Affecting Jonathan Spot

All the apples used in the experiment, unless otherwise stated, were harvested from the main block of the Horticulture Farm orchard at Michigan State University, East Lansing. Unless stated otherwise, the fruits were always randomized into treatment lots and placed in storage within 24 to 48 hours after harvest. Disinfection of the

---

[1/] Mr. P. Coleman, of the Information Service at Michigan State University did all the above mentioned photography.

fruit, whenever done, was carried out by dipping the apples into a 0.6% solution of sodium orthophenylphenate and rinsing the fruit with distilled water.

Testing the relationship of apple maturity and spot development, some 200 fruits were harvested on each day on the following days; September 6, September 19, October 6, October 22, and November 7, 1961. In all cases, the fruits were harvested at random from the south side of two trees. The third harvest (October 6) was the recommended commercial picking date for Jonathan at the Horticulture Farm.

Mature apples without red coloration were obtained by bagging fruits on the northeast side of the tree in size No. 3, brown kraft paper bags. This was done on Sept. 6, 1961, at which time most of the apples were either completely green or were showing first development of pink stripes. Fruits that grew adjacent to the bagged apples and approximately of the same size, were marked and served as controls.

Two experiments were conducted to evaluate the effect of humidity. Material for the first experiment was selected from apples of the 1960 crop held in controlled atmosphere storage at 32°F up to April, 1961, then transferred to air storage at the same temperature. On July 31, 1961, 24 non-spotted apples were selected and wrapped in Watman No. 1 filter paper and put on wire mesh over 1" of water in 5-gallon jars held at room temperature. The fruits were examined periodically. Material from the 1961 crop was used for the second experiment. It started on October 28, 1961 and included three temperature levels (41°, 63° and 86°) and two levels of humidity designated as "high" and "low". The temperatures were maintained in plywood cabinets measuring 2.5' x 2.5' x 6.0' in which the raised temperatures were obtained by

means of a heater and a fan arranged on the bottom of each cabinet. The desired temperature was automatically regulated by a thermoregulator (Cenco-Dekhetinsky, Chicago, Ill.). Thusly, 41° and 63°F were established in two separate cabinets that were placed in a refrigerated storage room maintained at 32°. A third cabinet, in which a temperature of 86° was established was left in the laboratory. Eighteen apples were placed and sealed in each of six 20 liter jars through which a stream of air at 100 cc per minute was passed. Two jars were placed in each cabinet, one jar kept in "high" and the other in "low" humidity. "Low" humidity was obtained by passing the incoming air through H_2SO_4 of 1.40 specific gravity, after which it was passed through Na_2CO_3 to absorb possible H_2SO_4 fumes. "High" humidity was achieved by bubbling the air through a 20 liter jar filled with water to a height of 10". Water condensation was clearly noticeable on the walls of the "high" humidity jars.

Three separate experiments were conducted to determine the effect of temperature on the incidence of Jonathan spot. For A, fruits picked on October 6, 1961 were kept in controlled atmosphere storage (3% O_2. 5% CO_2) at 32° until November 29, 1961, and then transferred to air storage. On December 12, the apples were sorted, and 30 sound fruits were randomly put into each of 12 6x16 inch perforated polyethelene bags which were subjected, in duplicate, to six temperature levels of 32°, 41°, 51°, 62°, 70° and 84°. The apples were examined on March 22 and were transferred to a 32° apple storage room for further observations.

Fruits of the same crop were used for Experiment B. They were kept in controlled atmosphere as mentioned above up to March 22, 1962, when

they were removed to air storage at 32° for one week. The fruits were then sorted and disinfected, and 17 apples were randomly put into each of 12, two-gallon plastic pails, and closed with a sheet metal cover. One-hundred cc of air per minute were passed through the pails, which were subjected, in triplicate, to temperatures of 34°, 46°, 58° and 70°.

Experiment C utilized fruit picked on September 25, 1962 from the Dwarf Block of the Horticulture farm orchard. Sound, well colored apples were disinfected and randomly put into the aforementioned plastic pails, 17 fruits in each. These were subjected, in triplicate, to the four temperatures of 32°, 36°, 40° and 44°.

Three experiments (D, E, and F) were conducted to evaluate the effects of controlled atmosphere and CO_2-enriched air. For test D, apples harvested on October 6, 1961, were kept in refrigerated storage at 32°F until October 23, 1961, when they were transferred into metal chambers measuring 15.0 x 17.5 x 40 inches. Each chamber enclosed 2 one-bushel crates, each containing ca.150 fruits, and was sealed with a plexiglass cover. Diaphragm pumps were used to circulate, at various rates, the atmospheres in the chambers through 10 liter glass jars containing 2 lbs. each of hydrated lime to absorb the excess CO_2. The CO_2 and O_2 levels in each chamber were determined once, or if necessary twice daily. With a few exceptions, the atmospheres were maintained ± .3% at the following levels: 0.0% CO_2 and 3% O_2; 0.7% CO_2 and 3.0% O_2; 1.5% CO_2 and 3.0% O_2; 2.2% CO_2 and 3% O_2; 3.0% CO_2 and 17% O_2; and 3.0% CO_2 and 3.0% O_2.

The apples for Experiment E were harvested on October 6, 1961 and were kept in controlled atmosphere storage as described above until May 5, 1962, after which the storage room was opened and ventilated.

The same drums as described for Experiment D were used, each holding 2 one-bushel crates containing ca. 150 apples per crate. The atmospheres were maintained with a close to the atmospheric amount of oxygen, but variable carbon dioxide contents of 0% (regular air), 1% and 2%, all in duplicate. Another chamber was maintained under regular air for "Near-spotted" apples.[1/] The level of CO_2 in the chambers was regulated by adjusting the width of an opening in the plexiglass cover. The per cent carbon dioxide was analyzed daily by an Orsat analyzer. Except for a few cases, the atmospheres were maintained within $\pm$ .2% of the planned carbon dioxide levels. In the control treatments, the covers were loosely attached to the chambers, preventing any gaseous accumulation.

Experiment F apples came from the 1962 harvest, picked on September 25, and maintained in a refrigerated apple storage at 32°F. Fruits were removed from storage on January 23, 1963, and 10 apples were randomly placed in 16, two-gallon plastic pails. These were sealed and exposed, in quadruples, to two temperatures; 34° and 70°, and two atmospheres, regular air from the laboratory air system and 1% CO_2-enriched air. The latter was obtained by mixing a stream of air with CO_2 from a cylinder, the flow rate of any of the gases being 75 cc/min. per pail.

III. Physiological Changes Associated with Jonathan Spot

The spotted tissue for the various analyses was prepared as follows: sections of epidermal tissue with some adhering cortex measuring 1.0 to 1.5 mm in thickness were obtained by peeling spotted apples with a household potato paring knife. The spotted tissue was removed with a No. 1 or 2 stainless steel cork borer. For comparison, a control was

---

[1/] See H in Fig. 1.

similarly secured by sampling adjacent normal tissue. Thus for each spotted disc, an adjacent normal disc was obtained. In some cases, the cortex adhering to the peel sections was scraped off with a porcelain spatula ("scraped peel tissue.") According to Neal and Hulme (1958) and Flood et al. (1960), such treatment yielded peel tissue ca. 10 cells in depth, consisting mainly of cuticle, epidermis and subepidermal chlorenchyma.

The anthocyanin content of affected and normal peel tissue was determined from "scraped peel tissue". Three types of spots were analyzed: dark blue, developing early in storage on highly red colored apples; dark brown, showing usually on less colored areas in the fruit, and large brown spots, typically showing on apples after a long storage period. The first two types were obtained from apples of the new crop (1962), the latter type taken from apples of the previous year (18 months in storage). The samples were weighed and boiled for 10 minutes in 5 ml of 1% HCl in methanol, cooled, filtered through Whatman No. 1 filter paper, brought up to a specific volume in citrate buffer of either pH 2.0 or 7.0 and read in a Bausch and Lomb spectrophotometer at 530 μm (Siegelman & Hendricks, 1958). The amount of anthocyanin per 1 mg dry weight was calculated by subtracting the reading at pH 7.0 from that at pH 2.0, and using a molar extinction coefficient 3.43×10^{-4}, found by Siegelman & Hendricks, (1958) for idaein, the anthocyanin isolated from Jonathan apples. (Sando, 1937.)

For a chromatographic comparison, samples of normal and spotted peel tissue were ground with a mortar in a pestle in 1% (v/v) hydrochloric acid in ethanol. The homogenate was strained through 4 layers of wetted cheese cloth and was evaporated to near dryness. The

remaining sample was then applied on Whatman No. 3 paper and developed ascendingly for 12 hours in the upper phase of n-butanol, acetic acid and water, 4:1:5, (v/v) (Harbone, 1959).

For dry weight determinations, samples were obtained in duplicates, quickly weighed, dried in a 100°F oven for 3 days and reweighed.

Material for titration curves was prepared in a 32° cold room. Two g of spotted and normal tissue were placed in 95% ethanol and ground for 3 minutes with a mortar and pestle. The homogenate was slowly evaporated on a hot plate, taken up in 20 cc water, strained through 3 layers of wet cheese cloth, centrifuged for 5 minutes in an International Clinical Centrifuge and the pH determined with a pH meter (Type PHM 4c, Radiometer Copenhagen). The homogenate was then divided into 2 equal parts. Titration with .0364N HCl was carried out to pH 2.5 on one-half of the homogenate, reading the pH after each increment of 1 ml acid. Total titratable acidity was determined with the remaining half of the original homogenate, using 0.0280N NaOH. For comparison, two acid preparations were made. One preparation, containing the equivalent acids found in 1 g spotted tissue, included 0.5 meq malate and 0.2 meq citrate. The other contained the equivalent acidity observed in 1 g normal tissue, namely 0.35 meq malate. The pH of these samples was brought up with NaOH to 5.2 and 4.7, respectively, (the pH observed in the respective tissue homogenates.)

Nonvolatile acids present in spotted and normal tissues were determined as outlined by Markakis et al. (1963), with modifications in the eluting solutions as follows, listed in order of application: 50 ml 1.5 N; 50 ml 3.0 N; 75 ml 4.5 N and 50 ml 6.0 N acetic acid; 50 ml 6.0 N and 125 ml 8.0 N formic acid. Paper chromatography was used to

tentatively establish the identity of the acids found.

For mineral analysis, scraped and non-scraped spotted and normal peel tissues were dried at 120°F for several days, and then ground in a Wiley mill through a 20 mesh screen. Samples were analyzed by a photoelectric spectrometer (Applied Research Laboratory, Glendale, California) for P, Ca, Mg, Na, Fe, Zn, Al, Mo, and B. Potassium was analyzed with a Beckman Model B flame spectrophotometer and nitrogen by the Kjeldahl-Grunning method.

A stock solution of 10 µc/cc of Cs^{137} ($\frac{1}{2}$ life of 2 years) was prepared, and on August 10, 1962, one drop of this solution, containing ca. 0.6 µc was applied on the dorsal side, along the midrib of each of 4 or 5 large spur leaves growing near the fruit. Some 160 leaves adjacent to 40 fruits growing on a 9 year old tree, Blackjon cultivar were so treated, the same dosage being reapplied to the same leaves 2 days later. The fruit, designated as "lot A," was harvested on September 20, 1962, put in 70° storage room for one week and transferred to a 34° apple storage room.

Using another approach, the tracer was introduced through the twigs. Branches ca. $\frac{1}{2}$" in diameter, each carrying between 8 to 12 apples were cut off from 8 year old Jonathan trees on September 3 and the leaf number reduced to ca. 5 leaves per fruit. The twigs were quickly transferred to the greenhouse and put in paper cups filled with 150 cc of Cs^{137} solution containing 2.2 to 3.2 µc, or approximately 0.25 µc per fruit. After one or two days, the paper cups became nearly empty. The lower $\frac{1}{2}$" of the twigs was then cut off and the twigs placed in 5-gallon crocks, to which tap water was added daily. The apples were harvested into perforated polyethelene bags on September 14, 1962,

and placed in a 70°F storage room for one week. They were then transferred to a refrigerated (34°) apple storage room and were designated as "lot B".

At different intervals following harvest, fruits were pared and the peel was prepared for autoradiography: peel sections were pressed between sheets of blotting paper and placed in a 70° oven for one week. The dried sections were then carefully transferred to clean 8 x 10 inch paper sheets over which saran film was stretched. Kodak x-ray "Blue Brand" photographic film was placed in contact with the material for 15 to 30 days, developed and printed. A modification was introduced in the preparation of the last two autoradiographs (3 and 4 below): In order to better correlate the location of the spot on the peel with the possible spot on the autoradiograph, the fresh peel sections were placed in acetate folders and photographed, maintaining the same section arrangement when transferring the sections to the blotting paper and later on to the clean sheets as described above. This procedure was employed because the spots on the poorly colored peel tended to fade away after the peel was dried.

Autoradiographs were prepared as listed.

(1) About 2 weeks after harvest, (October 4, 1962) of 5 randomly chosen apples from "lot B" (water cultured branches).

(2) On January 3, 1963; of 5 apples from "lot B" which were the only apples in the lot on which a few Jonathan spots were observed. At that date, no spots were found in apples of "lot A".

(3) On March 26, 1963; of 8 spotted apples from "lot A". At that time, many fruits in "lot A" showed Jonathan spots.

(4) On May 20, 1963; of 5 spotted apples each in lots "B" and "A".

The rest of the fruit in lot "B" did not exhibit any spotting, while most of the fruits in lot "A" were severely spotted.

IV. Studies of Basic Respirations - CO_2 Output and O_2 Uptake

Spotted and normal scraped peel discs were prepared as already described and rinsed in tap water. All work was done in a 32°F room, where the discs were left overnight in a petri dish lined with wet Whatman No. 1 filter paper. 300 or 400 mg of such material suspended in 2.5 ml of 0.1M phosphate or citrate-phosphate buffer pH 4.0 were used in each Warburg flask. The direct method of Warburg was employed and the experiments were carried out always in duplicate. The gas phase was air and the bath temperature was 86°. The readings were taken at 15 minutes intervals.

For malate effect, the capacity of 300 mg spotted peel discs to decarboxylate malic acid (malate effect - Neal & Hulme, 1958) was measured by dipping 0.5 cc of 0.1M L malate after 60 minutes of endogenous respiration.

Oxygen uptake in various substrates was obtained using 0.1M citrate - phosphate buffer. Substrates tried were 0.5 ml each of 0.1M ascorbic, glycolic, catechol and glutathione. The substrates were dipped after 60 minutes of endogenous respiration. For estimation of autocatalysis, peel discs that were boiled for 3 minutes in water and rinsed several times in distilled water served as controls.

RESULTS

I. Morphology and Histology of Jonathan Spot

Morphological observations revealed that various kinds of spots develop on Jonathan apples. In general, two types of spots may be readily distinguished. In one type, which is called by some workers "lenticel spot" (E, F, and G in Figure 1), the affected areas are clearly centered around a lenticel. The other type, shown in A through D in Figure 1, is typical Jonathan spot in that it is formed regardless to the location of the lenticel.

Confluency of spots, typical to spots that had been developing for a long time ("old" spot), is shown in C. E and F are examples of what may be called "small" lenticel spot. Such spots may develop excessively on certain lots of Jonathan apples when exposed to 70 to 85°F for a few days.

Another type of lenticel spot is shown in G. The cortex immediately underlying the affected peel is quite often necrotic, in which case the spotted area becomes sunken. This type of spot, in preliminary culture experiments, yielded a prolific growth of Alternaria sp.

"Near spot" (Bünemann, 1957) is shown in H of Figure 1. These are streaks of dark blue skin discolorations that nearly without exception are formed on deep colored fruits.

Susceptibility of the fruit to lenticel spot seems to vary extremely from year to year and from one orchard to another. Also, fruits from young and from dwarf trees seem more susceptible. Unlike Jonathan

Figure 1. Jonathan apples showing various types and developmental stages of spot disorders.

A. Newly formed Jonathan spots of various sizes.

B. X8 magnification of a spotted area of the epidermis; the white dots are lenticels.

C. "Old" Jonathan spots, on apples held 14 months in cold storage.

D. "Old" spots on the green-colored area of the apple.

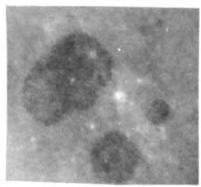
E. "Small" lenticel spots.

F. X8 magnification of a spotted area in E.

G. Lenticel spots with the affected tissue slightly sunken.

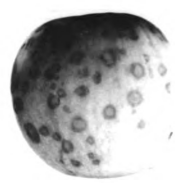
H. "Near spots".

A



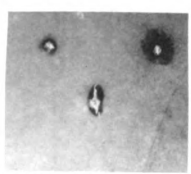
B

C



D

E



F

G

H

spot, all the other spot-types may develop on the tree before harvest.

Histological observations revealed two aspects in which the anatomy of spotted tissue (Figure 2, middle) differed from normal tissue (Figure 2, top). In the spotted tissue, the epidermis and adjacent 3 to 5 layers of collenchyma cells contained a deposition of dark pigment as compared to 2 layers of anthocyanin bearing collenchyma cells in the normal tissue. Another modification in the spotted tissue concerned the size and degree of compactness of the collenchyma cells. Thus there were 5-7 tiers of cells in the spotted tissue from the cuticle down to the arbitrary line drawn on the micrograph, as compared to 4-6 layers of cells in the normal tissue. Clearly, the cells in the spotted tissue were more compressed and radially elongated than were the cells in the normal tissue.

As shown in Figure 2, bottom, a "small" lenticel spot involved a significant gap in the collenchyma. The deposition of dark pigment was not as obvious as in Jonathan spot, probably indicating that fewer cells were involved or that the pigment concentration was lower.

II. Harvest and Storage Factors Affecting Jonathan Spot

Date of Harvest

Delaying the date of harvest hastened the appearance of Jonathan spot during cold storage (Table 1). On February 18, when the fruits were first examined, no spots were observed on the earliest picked fruits, while the latest picked apples were nearly totally spotted.

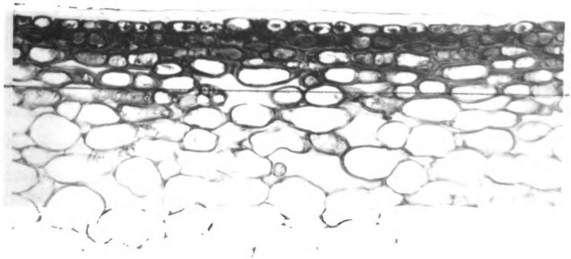
The apparent inhibitory effect of early harvest on the incidence of Jonathan spot was only temporary, since some four months later, on June 17, there was only a 11.4 difference in the percentages of spotted fruits between the two extreme harvest dates.

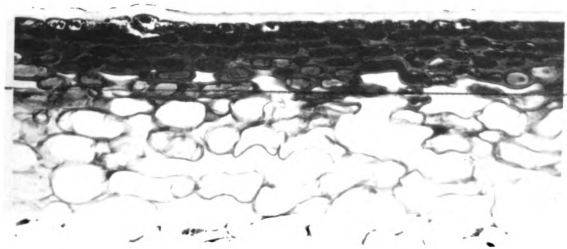
Figure 2. Cross sections of peel tissue with the adjacent cortex from Jonathan apple fruits.

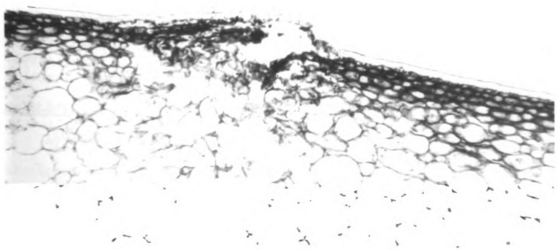
Top: Peel without spot disorder, X 250.

Middle: Jonathan spot, adjacent to the normal section, X 250.

Bottom: "Small" lenticel spot, X 125.







Mature Apples Without Red Coloration

This experiment, in which the apples were covered with paper bags before harvest, was conducted in order to ascertain if anthocyanin was essential for spot formation. The fruits were examined for spot incidence on three occasions during the cold storage period. On February 15, 1962, no spots were observed in either the bagged or control fruits. On March 29, 59% of the control apples were spotted, while most of the green apples showed superficial and irregular brown areas which were not identified as spots. By June 17, the bagged fruit developed definite Jonathan spots; 78% as compared to 100% spotting in the control.

TABLE I. The Effect of Time of Harvest on Jonathan Spot Development During Storage in Air at 34°F

Harvest Date	No. of Apples	Per cent Spotted Fruit 2-18-62	6-17-62
9- 6-61	253	0.0	88.6
9-19-61	235	6.0	90.0
10- 6-61	219	41.8	98.6
10-22-61	216	68.0	100.0
11- 7-61	15[a]	93.4	100.0

[a] Tree picked early, these 15 apples remained at the top of the tree.

Relative Humidity During Storage

As shown in Table II, apples stored under high or low humidities developed similar amounts of Jonathan spot at 40°F, and no spots developed at higher temperatures. Also, wrapping apples in wet Whatman No. 1 paper for 2 weeks, a method which according to Plagge and Maney (1924) induced Jonathan spot, was of no effect on spot development.

TABLE II. The Effect of Three Temperatures and Two Humidity Levels on the Incidence of Jonathan Spot in Apples Stored in Air from Oct. 28, 1961 to Feb. 8, 1962.

Treatment	Per Cent Spotted Fruit		
	Temperature		
Humidity	40°F	63°F	86°F
"Low"	50.0	0.0	0.0
"High"	44.4	0.0	0.0

Effect of Storage Temperature

All temperature experiments conducted in 1961-62 showed that Jonathan spot development was favoured by low temperature and retarded by high temperature. As may be seen in Table II, no spots developed at 63° or 86°F. The optimal temperature for spot development in experiment A reported in Table III was seemingly below 41°. On the first examination on March 22, after 3 months in storage, no spots were observed on apples at 51° and above. At this time, all the apples were transferred to a 32° storage room for further observation. Some 5 months later, on August 30, the fruit that was initially at 61° and 70° exhibited only a limited degree of spotting. Spot development was also delayed by storage at 51°, as shown by the value (25.4%) on June 17.

The results of experiment B (with apples of the 1961-crop) are summarized in Table IV. The highest incidence of Jonathan spot was again at the lowest temperature level. Lenticel spot (type E of Figure 1) was prevalent on fruit exposed to 70° and 58°, and absent on fruit at 34°.

TABLE III. The Effects of Temperature on Jonathan Spot Development for 1961-Crop Apples Stored for Approximately One Year in Air

Temp. (°F)	No. of Fruits	Percentage Spotted Apples On March 22[a]	June 17	August 30
32	59	42.4	94.8	96.5
41	60	26.7	64.7	85.2
51	60	0.0	25.4	79.2
62	58	0.0	0.0	22.8
70	58	0.0	0.0	22.3

[a] After this date, fruits were transferred to 32° apple storage room.

TABLE IV. The Effect of Temperature on the Occurrence of Jonathan Spot and Lenticel Spot on Apples Removed from CA Storage and Treated From March 28 to July 16, 1962.

Temperature	Average Jonathan Spot	Average Lenticel Spot	Total Spot
(°F)	(%)	(%)	(%)
34	77.8	0.0	77.8
46	9.3	9.6	18.9
58	2.0	52.0	54.0
70	0.0	34.5	34.5

If no distinction is made between Jonathan spot and lenticel spot, the total spot occurrence showed no consistent relationship to temperature level ("Total spot" column in Table IV above).

The critical temperature for spot development could not be

substantiated in 1962-63 (experiment C). As shown in Table V, Jonathan spot in this season developed to very much the same extent at all temperatures, from 32° to 44°F.

TABLE V. Effects of Temperature on the Occurrence of Jonathan Spot for Fruit of the 1962 Crop

Temperature (°F)	Per Cent Spotting			
	Lot 1	Lot 2	Lot 3	Average
32	41.1	47.0	47.0	45.0
36	64.7	53.0	76.4	64.7
40	64.7	47.0	58.2	56.6
44	58.2	64.7	29.4	50.7

Controlled Atmospheres

In experiment D, the lowest level of CO_2 employed, 0.7%, was found to materially reduce the incidence of Jonathan spot. A high degree of spotting occurred at 0% CO_2 with limited O_2 (treatment No. 1 in Table VI); whereas, practically complete control of this disorder was achieved in the presence of CO_2, even with O_2 tension as high as 17% (treatment No. 6). An effect of the O_2 level on spot formation is suggested by comparing treatments 5 and 6 of Table VI. The only gas combination in which the disorder did not develop was 3.0% CO_2 and 3.0% O_2 (treatment 5). "Near spot" developed to a very similar extent in all the gas combinations tried.

TABLE VI. Effects of Various Combinations of CO_2 and O_2 in the Atmosphere Surrounding Apple Fruits on the Incidence of Jonathan Spot and "Near Spot".

Treatment No.	Storage Atmosphere O_2 (%)	CO_2 (%)	No. of Fruits	Jonathan Spot Avg. (%)	Near Spot Avg. (%)
1	3.0	0.0	311	54.6	22
2	3.0	0.7	307	4.9	26
3	3.0	1.5	302	2.3	14
4	3.0	2.2	306	0.9	13
5	3.0	3.0	259	2.3	19
6	17.0	3.0	316	0.0	18

The results of experiment E in the series of controlled atmosphere studies are shown in Table VII.

TABLE VII. The Effects of Storage in Air and in CO_2 - enriched Air from May 10 to Sept. 7, 1962, on the Incidence of Jonathan Spot in Apples Previously Stored in Controlled Atmosphere of 3% O_2 and 5% CO_2

Treatment	Chamber No.	No. of Apples	Spotted Apples (%)	Average Spotting (%)
1 per cent CO_2 - enriched air	1	222	1.4	0.9
	2	226	0.4	
2 per cent CO_2 - enriched air	1	197	0.0	0.0
	2	218	0.0	
Control, (air)	1	227	33.0	32.3
	2	226	31.5	
Control, "near spotted" fruit	1	208	65.2	65.2

The low incidence of spots in fruit stored in 1% CO_2 with atmospheric air agrees with the results obtained in Treatment 6 Table VI. The incidence of spotted apples was twice as high in apples affected with "near spot" at the beginning of the test as compared with normal apples.

Good control of Jonathan spot with 1% CO_2 enriched air was not obtained in Experiment F, summarized in Table VIII. All lots held at 34°F showed essentially the same degree of spotting regardless of the gas mixture to which they were exposed. Consistently, as before, Jonathan spots did not develop on fruits at 70°F. Lenticel spotting exhibited an opposite response than Jonathan spot in that it developed in the high temperature only.

TABLE VIII. The Effects of Storage in CO_2-enriched Air at Two Temperatures, from Jan. 23 to April 17, on the Incidence of Jonathan and Lenticel Spots in Apples Previously Held in Air Storage at 32°

Temperature °F	Lot	Air & 1% CO_2		Air	
		Jonathan Spot (%)	Lenticel Spot (%)	Jonathan Spot (%)	Lenticel Spot (%)
34	1	60	0	80	0
	2	50	0	50	0
	3	50	0	70	0
	4	60	0	70	0
	Avg	55	0	67	0
70	1	0	60	0	50
	2	0	70	0	60
	3	0	80	0	50
	4	0	50	0	60
	Avg	0	65	0	55

III. Physiological Changes Associated With Jonathan Spot

Anthocyanin Content

Extraction of the anthocyanin present in scraped, spotted and normal peel tissue with 1% HCl in methanol revealed that a substantial loss of anthocyanin occurred in the spotted tissue, this loss becoming larger with time. Thus the anthocyanin content of new and old spotted peel was about 60 and 30 per cent of normal, respectively (Table IX). There was no difference in the amount of anthocyanin present in brown-spotted and blue-spotted peel tissues.

Identical Rf values (0.27) for paper chromatographed anthocyanin were obtained for both spotted and normal peel tissues.

TABLE IX. Anthocyanin Content of Spotted and Normal Peel Tissues

Fruit Tissue	Time Fruit Held in Storage	Anthocyanin per mg Dry Wt of Peel	Anthocyanin Content of Spotted Peel as Per Cent of Normal Peel
	(months)	(μ mole)	(%)
Old spotted peel	18	2.1×10^{-2}	32
normal peel	18	5.5 "	-
Brown spotted peel	6	2.8 "	60
normal peel	6	4.0 "	-
Blue spotted peel	6	5.7 "	64
normal peel	6	7.4 "	-

Dry Weight

Dry weight analyses are summarized in Table X. Spotted tissues had a greater dry weight than non-spotted tissues, evident only for

spotted tissue which had been scraped in preparation, and amounted to 9.6% for new and 18.2% for old spots. In the non-scraped spotted peel the presence of cortex evidently masked these changes in dry weight in the epidermal and hypodermal tissues.

TABLE X. Per cent Dry Weight of Spotted and Normal Epidermal Tissues

		Non-Scraped Peel	Scraped Peel	
			New	Old
Spot	a	17.8		
	b	17.9	28.42	27.96
	c	18.1	30.23	24.60
	Avg	17.9	29.33	26.28
Control	a	17.6		
	b	16.4	27.09	22.24
	c	18.3	26.41	22.19
	Avg	17.4	26.75	22.22
Mean difference		0.5	2.58	4.06
Increase in dry wt of spotted peel (as % of control)		2.4	9.6	18.2

Titration Curves

Titration curves of normal and spotted tissue homogenates and of preparations containing their respective acid equivalents are shown in Figure 3. The titration curve of the preparation containing the equivalent acidity of the spotted tissue homogenate followed very closely the curve of the actual homogenate. The titration curve of the normal acid preparation however, did not follow the curve of the normal tissue homogenate as closely.

There was an approximate two-fold increase in the buffering capacity of the spotted homogenate throughout the pH range employed.

This is evident when comparing the milliliters of acid needed to change the pH of the spotted and normal tissue homogenates (Table XI).

The joining of curves 1 and 3 in Figure 3 slightly above pH 7 indicates that the normal and spotted homogenates had a similar total titratable acidity.

TABLE XI. Millilitres Acid or Base Needed to Affect a Change in pH of Spotted and Normal Homogenates[a/]

Homogenate	pH			
	2.5 to 3.5	3.5 to 4.5	4.5 to 5.5	5.5 to 8.0
Spotted	13.5	10.5	10.0	8.0
Normal	7.0	5.5	5.0	4.5

[a/]Corrected for water blank. (Neiland and Stumpf, 1958).

Nonvolatile Acid Fractionation

Fractionation of the nonvolatile acids revealed malic acid to be the predominant acid in both normal and spotted tissue. In the latter, however, citric acid content was higher, being 27 per cent of total acid in the July 12 analysis, (Figure 4). The per cent of phosphoric acid was also higher in the spotted tissue, but not to the extent of citric acid. The percentages of shikimic and quinic acids were similar in normal and spotted tissues. Fractionation of the acidity of two runs, as percentage of the total acid content, is shown in Figure 4. In all the runs, total acid in the spotted tissue was over twice as high as in the normal tissue.

Mineral Analysis

Mineral analysis on dry wt basis of spotted and normal tissues is

Figure 3. Titration curves of spotted and normal tissue homogenates and of acid preparations containing the respective equivalent acidities.

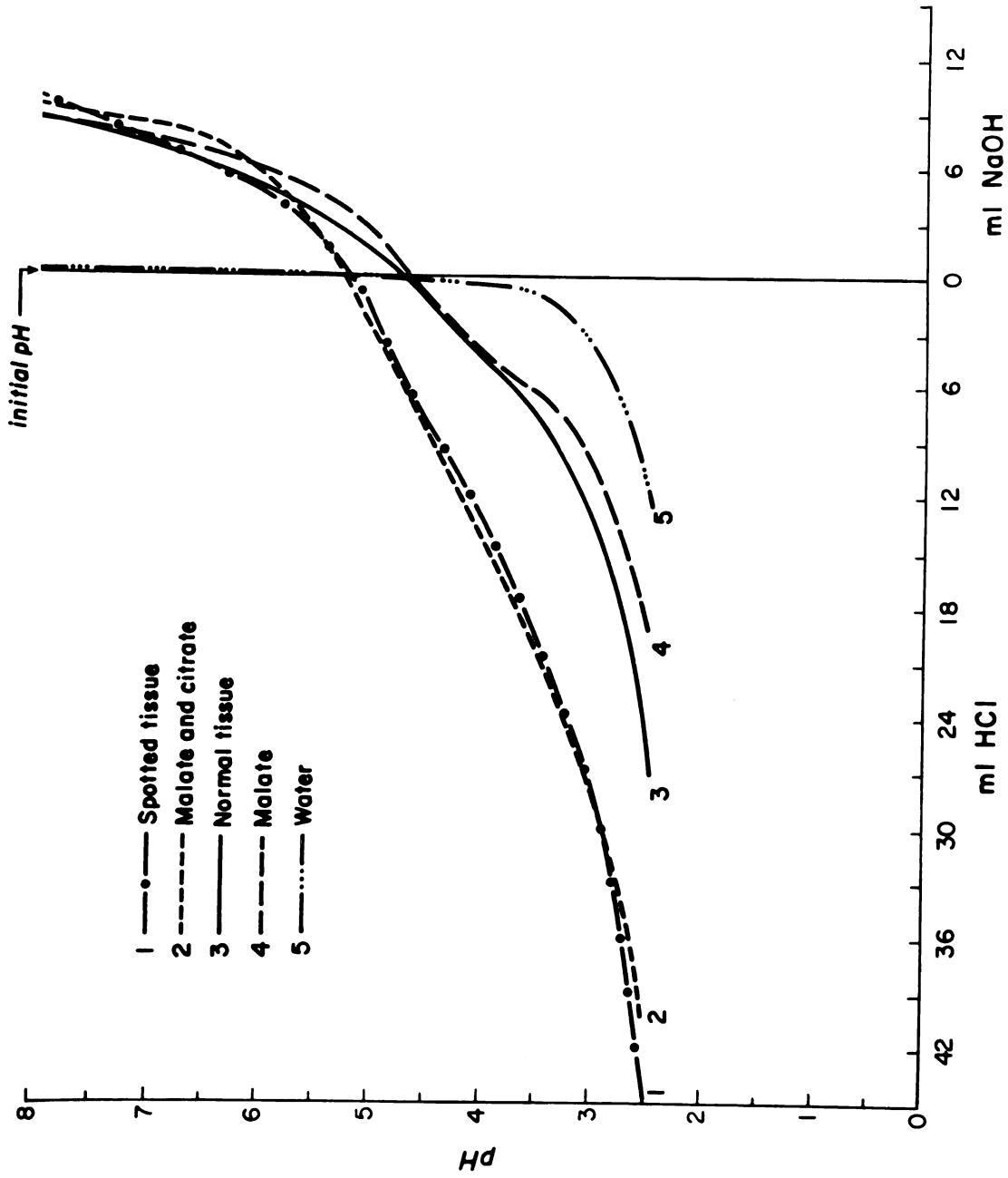
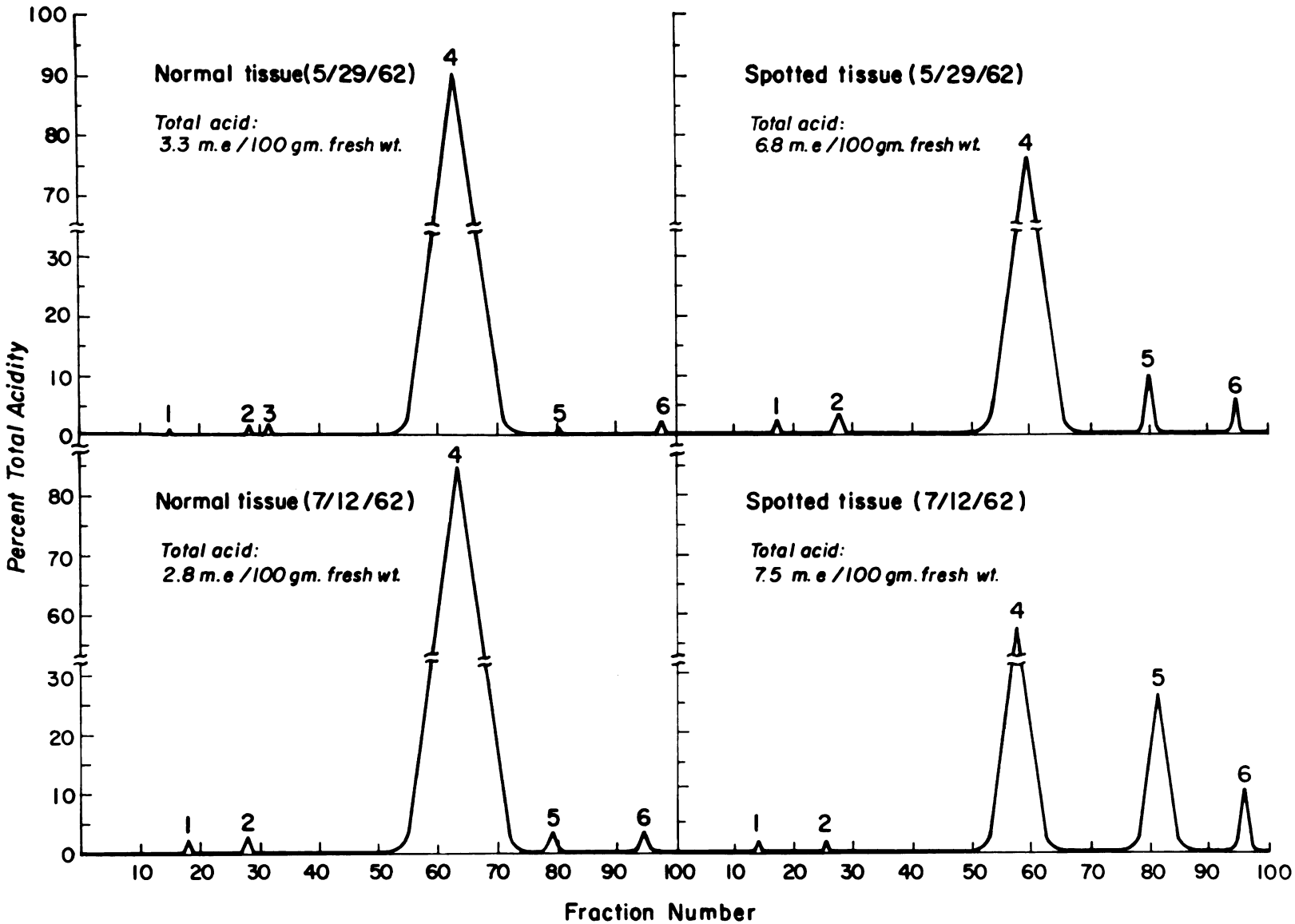

initial pH
1 — Spotted tissue
2 — Malate and citrate
3 — Normal tissue
4 — Malate
5 — Water
pH
ml HCl
ml NaOH

Figure 4. The percentage total acidity of column chromatographic fractions of acids from spotted and normal tissues.

Tenative identity of the acids: 1. shikimic; 2. quinic; 3. unidentified; 4. malic; 5. citric; 6. phosphoric.


Normal tissue(5/29/62)
Total acid:
3.3 m.e /100 gm. fresh wt.
Spotted tissue (5/29/62)
Total acid:
6.8 m.e /100gm. fresh wt.
Normal tissue (7/12/62)
Total acid:
2.8 m.e /100 gm. fresh wt.
Spotted tissue (7/12/62)
Total acid:
7.5 m.e /100 gm. fresh wt.
Percent Total Acidity
Fraction Number

recorded in Table XII. As found by Clerx (1960), there was a marked accumulation in the spotted tissue of K, Mg, Ca and P. Cu and B contents of the spotted tissue was also higher.

Mineral analysis of peel tissues obtained from apples that were exposed to 34° and 46° is shown in Table XIII. As already reported, most of the apples exposed to 34° were fully spotted whereas most apples exposed to 46° were non-spotted. Since originally all these apples came from one randomized lot, their original mineral content is assumed to have been similar. Clearly, the extent of the K, Mg, P and Cu increase in peel sections containing Jonathan spots was modified when compared to the increase of these elements in Jonathan spots alone. (Table XII). It is noteworthy that the Ca and Mn content of peel sections with Jonathan spots and of sections not containing spots was very similar.

The observation that red fruit was more susceptible to Jonathan spot led to the analyses in Table XIV. No difference in mineral content could be observed between peel of red and green apples and between the green and red in color peel tissues of the fruit.

Some quantitative relationships of K, Mg, Ca, and P in spotted and normal peel tissues are shown in Table XV. K was the predominant cation, comprising 74.1% and 60.8% of the total cation equivalents in the normal and spotted peel tissues, respectively. The decrease in the percentage of K in the spotted tissue was followed by an increase in the percentage of Mg. Altogether, in the spotted tissue, there was a close to three-fold increase in total cation and total mineral equivalents.

TABLE XII. Mineral Analysis of Spotted and Normal Peel Tissue, on Dry Weight Basis

Analysis No.	Date[a] Prepared	Tissue	Per cent N	K	Mg	Ca	P	PPM Na	Mn	Fe	Cu	Zn	Mo	Al	B
I	August 1961	Jonathan Spots	[b]	1.28	.15	.10	.159	17	8	465	19	7	.6	12	37
		Control		.56	.02	.02	.063	15	1	442	11	5	.9	10	27
II	February 1962	Jonathan Spots		1.20	.18	.10	.175	24	12	325	16	0	1.0	12	42
		Control		.50	.02	.02	.057	12	0	344	7	0	.5	5	28
III	August 1962	Jonathan Spots		1.28	.22	.16	.168	39	12	332	11	0	.4	12	39
		Control		.56	.06	.05	.057	33	1	262	5	0	.5	10	26

TABLE XIII. Mineral Analysis on Dry Weight Basis of Peel Sections Containing Jonathan Spots Obtained from Apples Stored at 34°F and of Peel Sections Without Spots, Obtained from Apples Stored at 46°

Date Prepared	Tissue	Per cent N	K	Mg	Ca	P	PPM Na	Mn	Fe	Cu	Zn	Mo	Al	B
December 1962	Peel Sections Containing Jonathan Spots	.48	1.16	.23	.22	.136	67	19	78	10	9	1.0	40	34
		.56	1.00	.23	.24	.128	60	22	85	20	17	1.2	40	26
		.50	1.08	.25	.24	.128	57	16	26	10	7	1.0	30	42
	Peel Sections Without Jonathan Spots	.52	.62	.12	.24	.076	50	19	85	8	11	1.2	30	32
		.28	.44	.11	.27	.063	52	16	75	6	9	1.2	30	32
		.44	.62	.11	.32	.084	42	22	72	10	13	1.4	26	23

TABLE XIV. Mineral Analysis on Dry Weight Basis of Peel Tissue Secured from Mature Green and Red in Color Jonathan Apples, and of Peel Tissue Obtained from the Red and the Green in Color Areas of Jonathan Apples

	Per cent N	K	Mg	Ca	P	PPM Na	Mn	Fe	Cu	Zn	Mo	Al	B
Peel tissue of Mature Green Apples (Bagged Fruits)	.42	.88	.16	.13	.084	70	8	37	6.8	5	.5	30	39
Peel tissue of Red Apples (Control)	.38	.88	.18	.13	.099	57	4	31	4.3	0	.5	30	40
Green Peel Tissue	[b]	.34	.11	.16	.076	[b]	16	143	7.2	4.1	1.6	58	47
Red Peel Tissue		.36	.13	.22	.069		16	95	10.1	5.4	1.4	30	36

[a] Date tissue prepared for analysis.

[b] Not analyzed.

TABLE XV. The Average K, Mg, Ca, and P Content of Spotted and Normal Peel Tissues

	Average[a] Per cent on Dry Weight Basis		Average[b] Per cent on Fresh Weight Basis		meq per 100 gm Fresh Weight		Per cent of Total Cation Equivalents		Fold Increase in Spotted Tissue
	Normal	Spot	Normal	Spot	Normal	Spot	Normal	Spot	
K	.54	1.26	.0940	.2260	2.41	5.80	74.1	60.8	2.4
Mg	.04	.18	.0069	.0320	.58	2.68	17.8	28.1	4.6
Ca	.03	.12	.0052	.0210	.26	1.05	8.0	111.0	4.0
P	.059	.167	.0103	.0299	1.00	2.91	—	—	2.9
Total cations					3.25	9.53			2.9
Total minerals					4.25	12.44			2.9

[a] Average based on analyses I, II, and III in Table XII.

[b] Computed on the basis of 17.4 and 17.9 per cent dry weight for normal and spotted peel tissues, respectively.

Autoradiography

Autoradiographs of peel sections that were obtained from apples in "Lot B" some two weeks after the apples were harvested showed that Cs^{137} was evenly distributed in the skin. The different shade intensities of the autoradiograph reflect the variable thicknesses of the sections. Thus the darkest area of one section measured 17 units (B, Figure 5 left), whereas the lightest sections (C and D in Figure 5 left), measured only 5 units of thickness each.

Autoradiographs that were prepared on Jan. 2, 1963, when a few Jonathan spots were first observed in the Cs-treated apples showed that the tracer moved out of the spotted tissue. (Right, top and bottom in

Figure 5).

Autoradiographs of spotted peel sections obtained on March 26 from apples of Lot "A" were very light and showed no conclusive evidence as to the distribution of Cs^{137}.

Figure 6 shows autoradiographs of peel sections prepared on May 20, 1963 from apples in lots "A" and "B". As reflected in these autoradiographs, the distribution of Cs^{137} in the sections was extremely variable: Corresponding to the spots in the upper and bottom sections shown in photograph A, there appear white areas in the respective autoradiograph B, pointing to the loss of Cs^{137} from the spotted tissue. The opposite may be observed for some spots (marked with arrows) shown in photograph C, corresponding to which there are dark areas in autoradiograph D, pointing to an accumulation of Cs in the spots. Similarly, corresponding to the middle spot in the upper section of photograph E there is a dark spot in autoradiograph F, and corresponding to the spots marked with arrows on the lower section in E, there are dark spots in F. The situation is obscure in the middle section in F, where only very faint shades correspond to the spots shown in E. No relationship seems to exist between the spots photographed in G and the uneven Cs^{137} distribution portrayed in autoradiograph H.

IV. Studies of Basic Respirations - CO_2 Output and O_2 Uptake

The introduction of malic acid into Warburg reaction flasks containing peel discs obtained from the new crop apples accelerated a marked CO_2 evolution. This malate effect, however, was more pronounced with normal peel discs than with spotted peel discs. With peel tissues obtained from 18-month-old-apples (Figure 7, II), the quantitative

Figure 5. Autoradiographs and photographs of peel sections prepared from Cs^{137}-treated Jonathan apples.

Left: Autoradiographs of peel section taken on October 4, 1962 shortly after fruits were harvested. Arrows indicate position where the thickness of the section was measured in a calibrated microscopic field.

A=6, B=17, C=13, D=5, E=5, units of relative thickness.

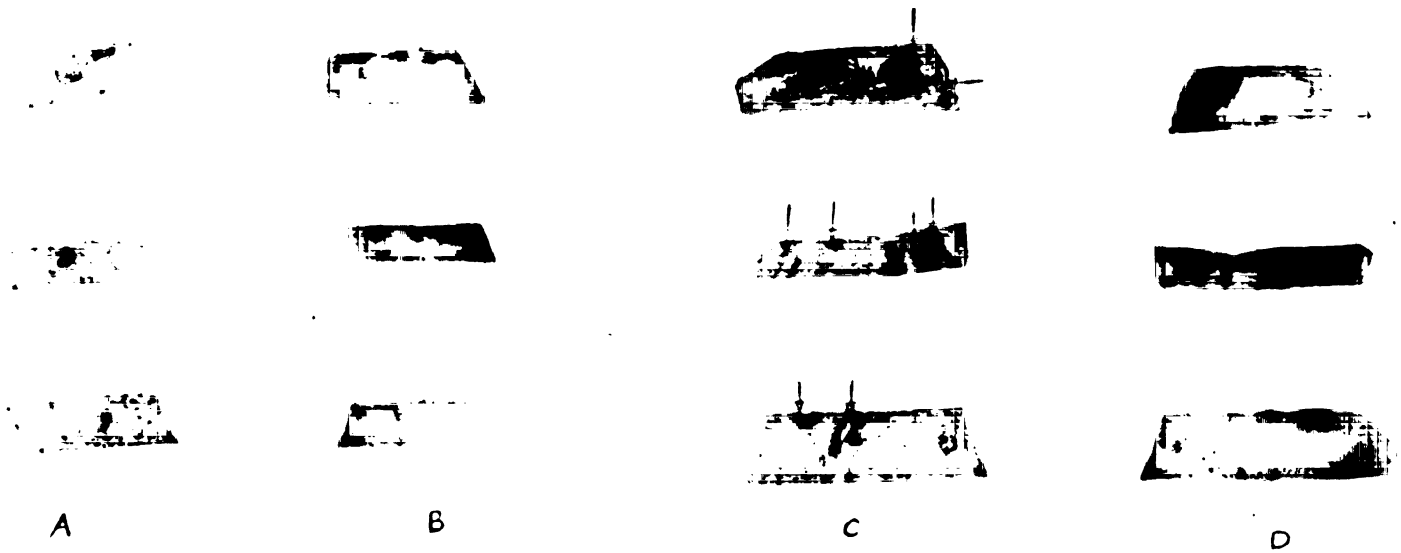
Middle, top and bottom: photographs of peel sections secured when spots initially appeared on January 3, 1963.

Right, top and bottom: the respective autoradiographs.

Arrows in the middle, lower photo indicate the location of spots which become barely visible when the sections were dried.

Figure 6. Photographs and the corresponding autoradiographs of spotted peel sections obtained from apples in Lots "A" and "B" on May 20, 1963.

A, C, E, and G are photographs of the peel sections; B, D, F, and H are the respective autoradiographs.


A
B
C
D

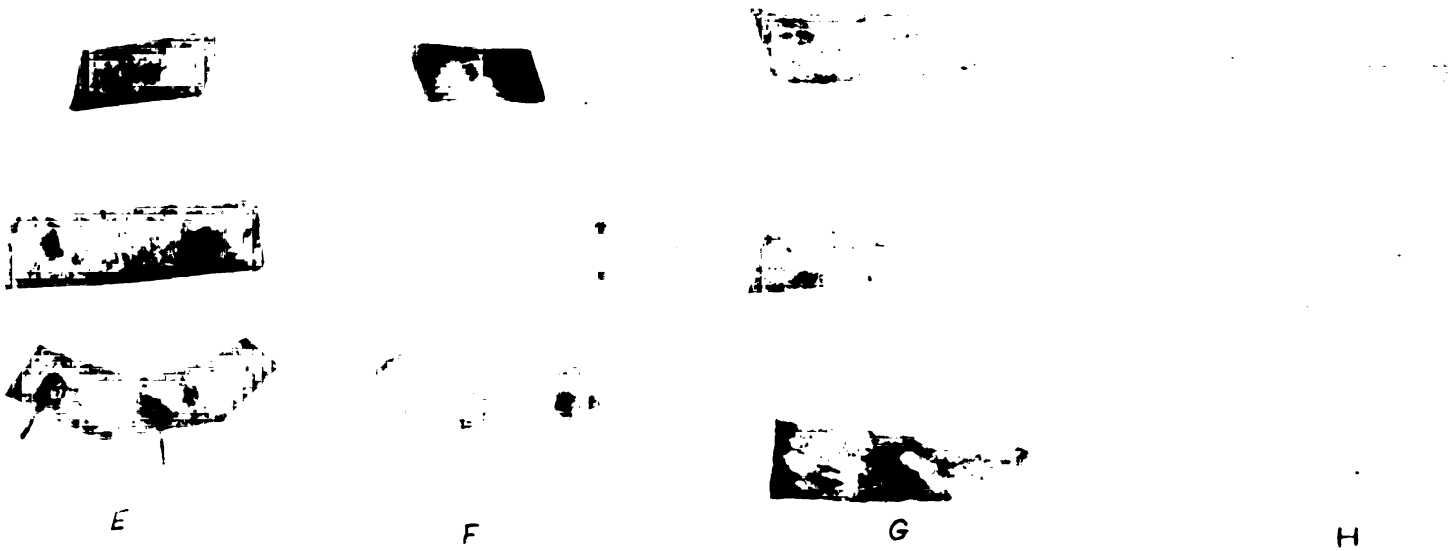
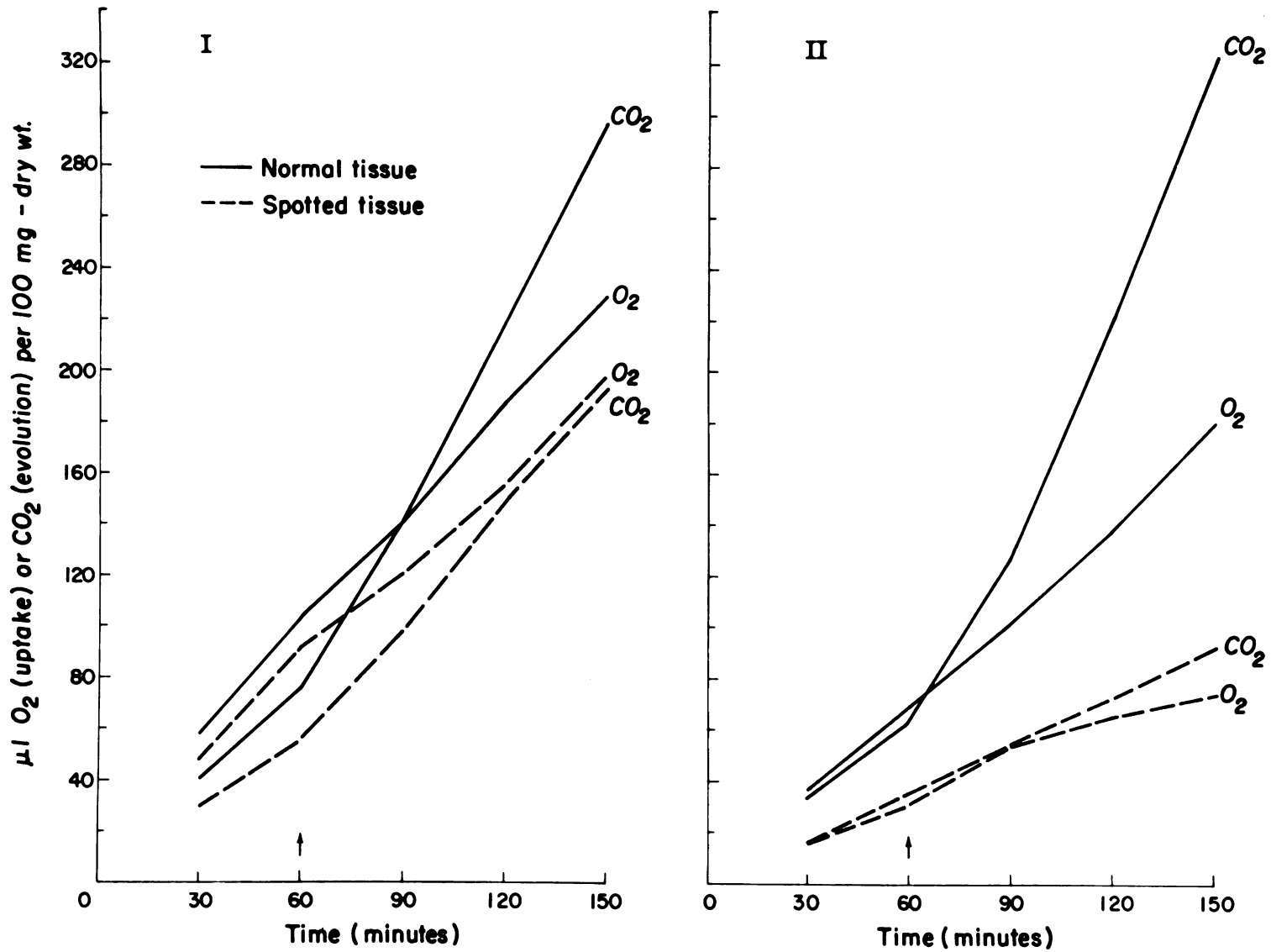

E
F
G
H

Figure 7. Endogenous respiration and malate decarboxylation of spotted (dashed curve) and normal (full curve) tissues.

I. Apples held 6 months in storage, spots ca. 2 months old.

II. Apples held 18 months in storage, spots ca. 14 months old.

0.5 ml of 0.1M malate dipped at 60 minutes (shown by arrow).

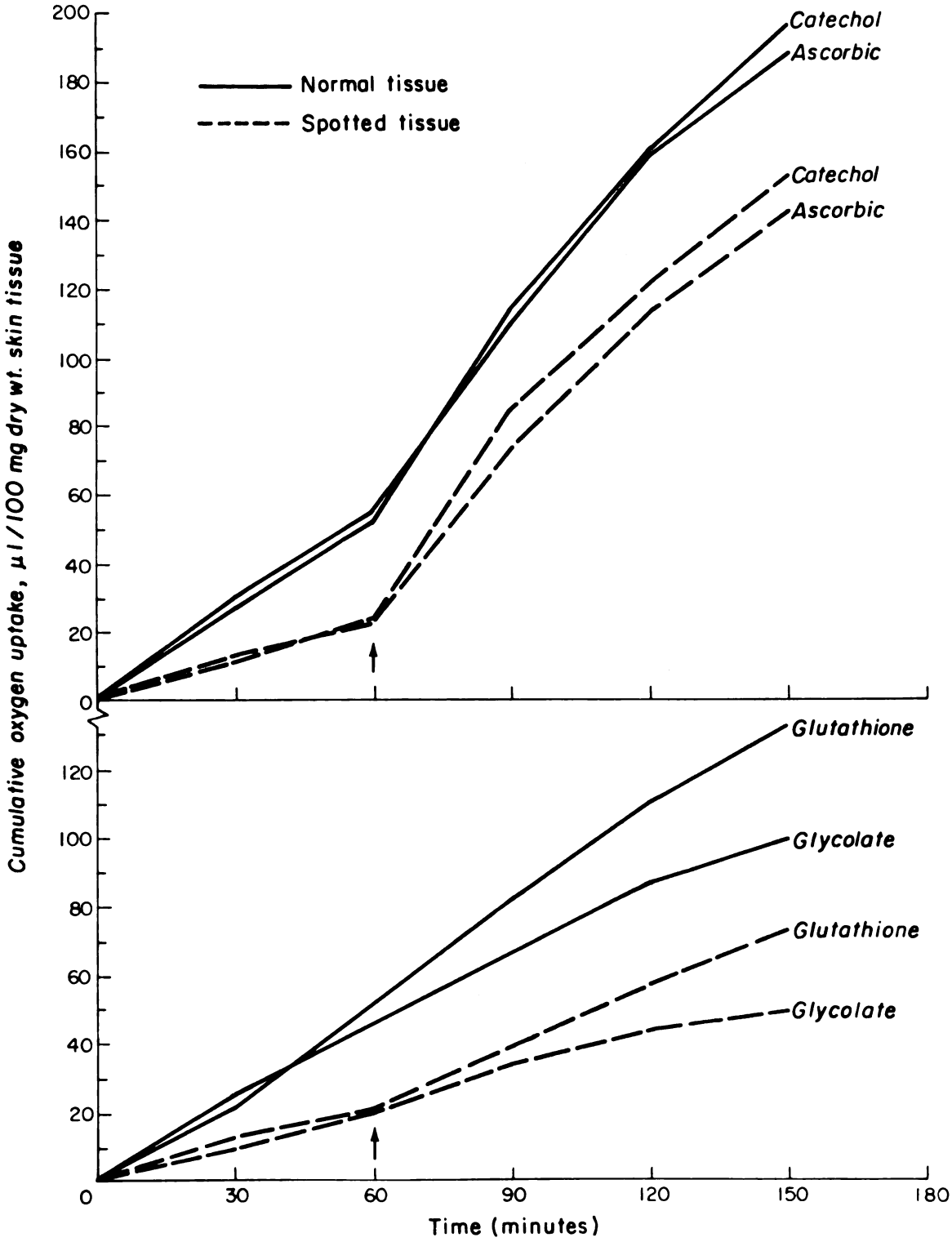

I
II
Normal tissue
Spotted tissue
CO_2
O_2
O_2
CO_2
CO_2
O_2
CO_2
O_2
μl O_2 (uptake) or CO_2 (evolution) per 100 mg - dry wt.
320
280
240
200
160
120
80
40
0
30
60
90
120
150
Time (minutes)
Time (minutes)

differences in CO_2 evolution between normal and spotted peel discs were greater, spotted discs exhibited a nearly complete loss of capacity to decarboxylate malate, while normal tissue showed an increase in this capacity. Thusly, total CO_2 evolution by old normal tissue (Figure 7, II) with the introduction of malate was 210 µl/100 mg dry wt/hour, while the evolution by old spotted tissue was 60 µl. The straight line that describes the course of evolution of CO_2 by old spotted tissue (Figure 7, II) indicates little malate effect. Endogenous oxygen uptake (up to the introduction of malate), was also greatly diminished in the old spotted tissue, becoming ca. 50% of the old normal tissue. Whereas a slight increase in O_2 uptake accompanied the introduction of malate in the old normal tissue, a slight decrease in O_2 uptake occurred in the old spotted tissue.

The O_2 uptake of spotted and normal discs increased remarkedly and to the same extent upon the addition of 0.5 cc of either 0.1M ascorbic acid or catechol (Figure 8, C and D). Endogenous O_2 uptake of spotted tissue was ca. 50% of the adjacent normal tissue.

Figure 8. Cumulative oxygen uptake of spotted and normal tissues.

0.5 cc of 0.1M substrates, forming a final substrate conen of .016M, were added at 60 minutes as indicated by arrow.


Normal tissue
Spotted tissue
Catechol
Ascorbic
Catechol
Ascorbic
Glutathione
Glycolate
Glutathione
Glycolate
Cumulative oxygen uptake, µl/100 mg dry wt. skin tissue
Time (minutes)
0
20
40
60
80
100
120
140
160
180
200
30
60
90
120
150
180

DISCUSSION

Early in this investigation it became apparent that the disorder must be delineated in respect to the terminology employed in the literature and of symptoms describing the disorder and the relation of these to previous researches concerning Jonathan spot. Much of the evidence in the literature seems controversial and could not be verified. For example; Tomana's report (1959) that spotting was more prevalent in the early harvest and the observation by Plagge and Maney (1924) that short exposures to high humidity caused an immediate high incidence of spotting. Humidity was found to have no effect on spot formation, and late harvest rather than early harvest highly accelerated the degree and time of appearance of the disorder. Also, this study showed that a spot centered around a lenticel could be quickly developed in certain apple lots at 70°F, at which temperature Jonathan spots did not form. Plagge and Maney's (1924) suggestion that the two types of spots they recognized were identical, being caused by the same conditions, therefore seems unfounded. The photomicrograph of a type of lenticel spot which rapidly develops in room temperature and high humidity (C in Fig. 3) clearly portrays the break in the sub-epidermal layer associated with an open lenticel (Clements, 1935). No such breaks were found in epidermal tissue affected by Jonathan spot. A lenticel spot may be superficially similar to Jonathan spot but it has dissimilar anatomical features and may form rapidly under very different temperature conditions. There can be little doubt that Tomana, Plagge and Maney, Smock (1950) and Krapf

(1961), among possible others, have been including more than one or altogether different, distinct physiological disorders under the name "Jonathan spot."

Many factors could account for the acceleration of spotting of fruits from late and delayed harvests. Starting with the time of bloom, the respiration rate curve of the attached Jonathan apples reaches its low point sometime around the commercial harvest period. It rises again after that date, reaching its post harvest peak in late October or early November (Ezell and Gerhardt 1942). Concomitantly, the protein content rises (Hulme 1958) in association with the climacteric rise. The buildup in protein may, to a certain extent, be qualitative, providing the necessary catalyst for some possible limiting stages in the biogenesis of the spot. Likewise, a limiting substrate may be formed or some reaction rates changed to favor spot formation.

In no case was the disorder observed immediately after harvest and thus there can be little doubt that a certain preparatory period must elapse before Jonathan spots appear. The accelerated development of spots after delayed harvest or storage as well as after being placed in non-refrigerated storage where the temperature declines only with the advent of winter, all point to a certain high temperature optimum in this preparatory period. Since spots did not form at a temperature above 60°F and in one year (1962) did not form above 50°, it is suggested that the sequence of Jonathan spot development includes at least two temperature phases, with the optimal temperature of the second phase being lower than that of the first phase. The failure in 1963 to bracket the optimal temperature of the second phase indicates, in the author's opinion, that this optimal may vary from season to season and

possibly from one lot of fruit to another. This possibility shall be elaborated shortly.

The data obtained in the temperature experiments of 1962 indicate that Jonathan spot may be classed as a "chilling disorder", being possibly a modified case in that optimal development favoured an initial exposure to a certain high temperature. Here it becomes clear that unless a distinction is made between Jonathan and lenticel spots, the temperature effect on Jonathan spot formation becomes rather obscure, especially in fruits having a pronounced tendency to develop lenticel spot.

Oxygen tension, at least in the range of 3 to 17%, seemed unimportant as a causal factor in Jonathan spot formation, since little difference in spotting was observed between a treatment of 3% CO_2; 3% O_2, and 3% CO_2 and 17% O_2. It is likely, then, that the complete control of Jonathan spot obtained in controlled atmosphere storage is not a result of the lowered oxygen tension and decreased respiration rate, but is probably a direct affect of the increased CO_2 level. Surprisingly, a concentration as low as 0.7% CO_2 effectively inhibited spot development, proving whatever factor is involved to be extremely sensitive to an increase in the CO_2 tension.

Noteworthy, however, the reported effect of CO_2 on spot formation in this study as well as in the literature is in itself only an assumption. Other apple volatiles are also very probably present in higher concentrations in controlled atmosphere chambers and may be, like CO_2, absorbed by hydrated lime. the material used to scrub the CO_2 in the chamber down to the desired level. In view of this possibility, the effect of CO_2 was observed in the 1963 experiment by passing a

commercially prepared gas mixture over the fruit, preventing the accumulation of any apple volatiles. The results of this experiment (Table IX), showing no difference between the treatments and the control, may be interpreted as an indication that CO_2 is not the factor which inhibited spotting. However, the experiment was started late in January, when some apples had already developed Jonathan spot. According to Clerx (1960), who experimented on the effects of various combinations of storage periods of controlled atmosphere and regular cold storage on Jonathan spot development, once the fruit had attained a certain stage of development in ventilated storage, spotting occurred even when the fruit was subsequently transferred to C.A. storage. Therefore no conclusion could be drawn from this last experiment and the question whether it was CO_2 or some other apple volatile that retarded spot development remains open. Whatever the case may be, the possible practical application of this true or pseudo CO_2 effect is quite obvious: it should be rather technically simple to increase the CO_2 level (and concomitantly the concentration of other volatiles) in regular or common storages to a level of, or associated with, approximately 1% CO_2. One possible method would be plastic lining of bulk containers (Dewey *et al.* 1959). Using a suitable material, establishment of the slight atmospheric modification needed to control Jonathan spot should present no difficult problem. Such a modification may be also established with a CO_2 generator.

Another finding that carries practical ramifications is the relatively low amount of spotting - 32.2% - that was observed in the control fruit examined as late as September 7 (Table VIII), almost a year following harvest. This may reflect a residual effect of controlled atmosphere storage, where the apples were held since harvest, prior to the beginning

of the experiment on May 10.

The sensitivity of a factor in the pathway of Jonathan spot formation to CO_2 or some apple volatile may offer a clue to the absence of spot development in certain high temperatures. The higher the temperature, the higher the rate of respiration and other metabolic processes which result in an increased gaseous production by the fruit, modifying the internal atmosphere. Apple fruits, however, are known to greatly vary in their degree of permeability, this degree being a function of the skin anatomy, number and size of open lenticels and other factors. If high temperature inhibited spot formation by altering the internal atmosphere, then the less permeable the fruit, the lower must be the inhibiting temperature and vice versa. This is suggested as a possible explanation for the experimentally observed shift in optimal temperature for spotting that was mentioned previously.

An important aspect of this study was the insight it provided into the buffering mechanism of plant cells. Jacobson and Ordin (1954), investigating organic acid metabolism associated with ion absorbtion in excised wheat roots concluded that "when excess cation absorbtion occurs, exchange for previously absorbed cations and the production of malate are the most important means of ion compensation." Ulrich (1941) from similar studies summarized that when excised roots absorbed cations in excess of anions, and provided that an ample supply of sugar was available in the cells, organic acids were formed as a response to the tendency towards an increase in the pH of the root sap. Significantly, the results reported here for Jonathan spot, a disorder in which the affected cells are apparently stimulated to maintain ionic balance, support the conclusions derived from studies that were conducted with excised plant organs.

The doubling of buffering capacity seen in the titration curves of the spotted tissue (Fig. 3) is understandable in view of the organic acid and cation content of this tissue. The two-fold increase in organic acid and three-fold increase in cation equivalents would both double the buffering capacity and raise the pH.

Assuming that the negative charges of the phosphorous compounds in the cell approximately equal the positive charges contributed by the calcium and magnesium present, the pH of the tissue homogenate can be explained stoichiometrically with the analytical data obtained for potassium and total acid. Employing the Henderson-Hasselbalch equation, $pH \cong pK + \log ([Salt] / [acid])$, and considering 7.0 and 3.5 meq/100g fresh wt of total acid (as malate) in the spotted and normal tissues, respectively (Fig. 4); and 2.4 and 5.8 meq of K (Table XV) respectively, the following approximations hold: In the normal homogenate, there are 1.7 meq of each carboxyl of malic (3.5/2). At pH of 4.7 (Fig. 3) the first carboxyl of the acid (pK = 3.4) is close to being completely dissociated, in equilibrium with ~1.7 meq of K. Thus 0.7 meq of K, (2.4 - 1.7) are left for the titration of the second carboxyl ($pK \cong 5$). With 0.7 meq of this carboxyl dissociated, the pH becomes:

$$pH \cong 5 + \log \frac{0.7}{1.0} \text{ (total malic and malate}^- \cong 1.7 \text{ meq).}$$

$pH \cong 4.85$; high by 0.15 pH unit from the observed pH of 4.70.

At pH 5.2 of the spotted tissue, (Fig. 3) again the first carboxyl is fully dissociated, leaving ~3.5 meq (7.0 - 3.5) to react with 2.3 meq of K (5.8 - 3.5). The pH becomes:

$$pH \cong 5 + \log \frac{2.3}{1.2} \text{ (total malate}^- \text{ and malate}^= \cong 3.5 \text{ meq).}$$

pH $\cong$ 5.28; high by 0.08 pH units from the observed pH.

However, such a good stoichiometric agreement did not always exist. The pH of apple tissue homogenates varies greatly after storage, being affected by such factors as the date of harvest, temperature in the storage, and the length of the storage period (Clerx, 1960). Thus the pH analysis in 1962 showed the usual pH of the normal and spotted tissue homogenates to be 3.7 to 3.9 and 4.5 to 4.7, respectively. Such pH values can not be explained as satisfactorily as above on the basis of the analytical data, pointing to a basic difficulty encountered throughout this study that stemmed from the extreme variability and complexity of the material. Nevertheless, even with the above frequently observed low pH, certain analytical relations hold: Using the former assumptions and formula and starting with the acid concentration that the Henderson-Hasselbalch equation would demand for the observed pH and potassium content found in the normal tissue, the rise in pH of the spotted tissue can be explained as follows:

Initially, in the normal tissue: pH of 3.7 = 3.4 + log X; X = 2
Ratio of salt/acid is thus 2/1 and total malic and malate$^-$ = 3 units.

In the spotted tissue, where the total acidity is twice as high, total malic and malate = 6 units (3 x 2). If K content would have only doubled, the same ratio of salt to acid as in the normal homogenate would have been maintained, doubling the buffering capacity only (as observed, Fig. 3) but leaving the pH unchanged. Potassium, however, had more than doubled in the spotted homogenate (Table XV). The pH thus rises, (usually by 0.7 units), and becomes 4.4, (3.7 + .7).

Thus:

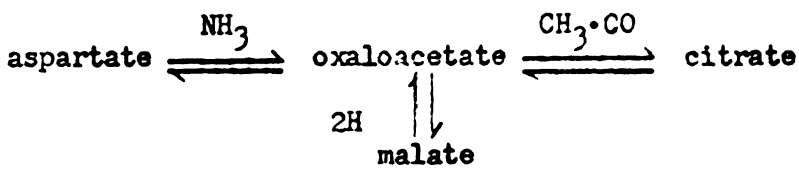
$$4.4 \cong 3.4 + \log X; \quad X = 10$$

Ratio of salt/acid became ca. 5.45/.55, which involved a 2.7 fold increase in K (from 2.0 to 5.45), which is not very far from the 2.4 fold increase reported in Table XV.

However, at pH 4.4, some 25% of the second carboxyl ($pK_2 \cong 5.0$) is also dissociated. Also, ca. 25% of the total acidity in the spotted tissue is comprised of citric acid, which at pH 4.4 is nearly fully dissociated at one carboxyl ($pK_a = 3.1$) and some 35% dissociated at another carboxyl ($pK_a = 4.75$). These factors would further increase the demand on K needed to bring the pH to the observed level. Therefore, in the spotted tissue, K can not be assumed to be the only important cation associated with the buffering mechanism of the cell. The four-fold increase in Ca and Mg found in the spotted tissue must be considered here. Very probably, a certain part of these cations react with malic acid forming salts or complexes of low solubility products which do not participate in the buffering mechanism of the cell. This would decrease the concentration of free acid in the cell sap and thus decrease the amount of K needed to bring the pH of the spotted homogenate to that observed, thus diminishing the above mentioned discrepancy between the empirically found and theoretically needed amounts of K for the establishment of the observed pH.

The fact that the acid profile in the spotted tissue changed to include a higher percentage of citrate deserves special attention. Accepting Ulrich's interpretation of acid synthesis accompanying cation uptake as a mechanism to preserve the pH in the cell, a tricarboxylic acid such as citrate will be more effective than malic for this purpose. Normal tissue also contains some citric acid, thus the rising pH could be thought as merely changing the balance in the synthesis of malate and

citrate. The following mechanism, suggested by Biale (1960) for lemon fruits, may be considered:



Biale stated that the enzymes required for these reactions - malic dehydrogenase, transaminase and condensing enzyme were widely encountered in organisms. These may be present in apple fruits also. Tentatively adapting such or similar mechanism for apple peel, it is possible to envisage a change in pH bringing about a modification in the equilibrium involving the synthesis of the acids.

The observed increase in cation equivalents was one of the major subjects investigated. One question that was posed concerned the origin of the transported elements. The fact that the extent of the increase in K, Mg, P and Cu was modified when whole peel sections containing spots were analyzed while Mn and Ca did not show an increase in such material may be significant. It may suggest that the latter two elements reach the spotted tissue from the peel itself, causing the overall Mn and Ca content of the peel to remain the same. The former 4 elements may be translocated to the spotted tissue from the cortex, thus showing an increase, even though modified, in whole spotted peel sections.

Another question concerned the cation accumulation itself. According to the above discussion, cation accumulation is suggested to be responsible for the rise in pH which presumeably triggers the eventual formation of the spot. To provide evidence for this proposed sequential relation between cation accumulation and spot development, Cs^{137} was introduced into the fruit as an analog of K. The initial migration of the label

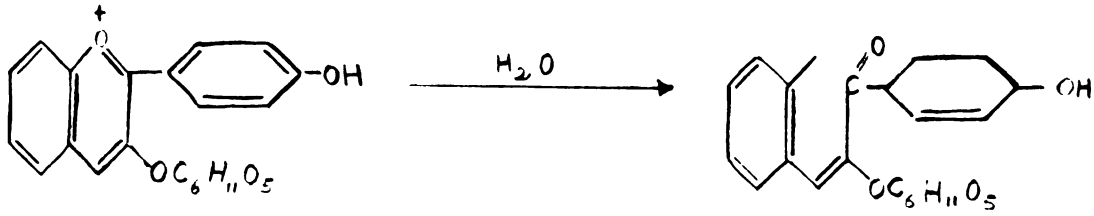
from the spotted tissue was not anticipated, but it may be explained from the afore mentioned conclusions of Jacobson and Ordin that cation uptake involved an exchange of Mg and Ca for absorbed K. Accordingly, Cs may be thought as being initially excreted from the spotted tissue as a compensatory measure for the cation influx that must occur in that tissue. However, acceptance of this explanation for the migration of Cs from the spotted tissue carries the conclusion that Cs cannot be regarded as an analog of K without some reservations, contrary to the current idea adapted in cation uptake studies.

Since later autoradiographs (Fig. 6) indicated in many cases a Cs accumulation in the spotted peel tissue, there may be a reabsorbtion of Cs in later stages of spot development. If so, Cs is reabsorbed following the assumed continuous cation influx into the affected cells. Thus, autoradiographs taken later, after spots had developed for some time, should show three basic patterns of Cs distribution, namely: White areas, corresponding to young spots; dark areas corresponding to old spots; and no definite pattern corresponding to the in-between stages of spot development. All these patterns may be observed in Fig. 6, where the autoradiographs were prepared when spotting of the peel was well established.

Accepting that the first stage in cation influx is accompanied by an outward migration of Cs, two possibilities arise. One possibility is that cation accumulation takes place after the initial formation of the spots and thus the white areas on the autoradiograph must correspond only to a fully recognizable spots. This did not occur. The second possibility is that the accumulation of cations preceeds the formation of the brown pigment. Then in some very early stages of spot development, Cs would have already migrated from the susceptible to spotting

areas before the spot can be observed. This offers an explanation for some white areas which may be seen in the autoradiographs presented in Fig. 8 which show no correspondence to observable spots in the photos.

The anthocyanin content of the spotted peel was determined to offer a possible clue to the origin of the black-brown color of the spot. Since the anthocyanin content of spotted peel was between 30 to 60% of normal, some anthocyanin destruction must have occurred. There is evidence that degradated anthocyanin may serve as a starting material for the observed brown pigment which typifies Jonathan spots. According to Joslyn and Ponting (1951), Nagai (1921) reported that certain anthocyanins were completely decolorized by the action of oxidizing enzymes. He showed that purified preparations of many plant pigments yielded brownish colors by the action of freshly prepared plant juices containing oxidizing enzymes. Markakis et al. (1957) proposed a type of anthocyanin hydrolysis that involved the opening of the pyrilium ring at position 1-2, with formation of a ketone:



Markakis et al. also stated that "further degradation of this ketone would eventually lead to the brown precipitation which has constantly been observed as an end product of the degradation of the pigment." They further reported that when pelargonidin-3-monoglucoside C^{14} was stored in citrate buffer, 85% of the original radioactivity of the pigment was recovered in the brown precipitate.

There are many additional reports concerning brown pigment synthesis. Hodge (1953) stated that naturally occurring compounds from which browning originates contain usually a multiplicity of potential carbonyl groups such as catechols, which are easily oxidized to quinones. Joslyn and Ponting (1951) concluded that some of the reactions of quinones may conceivably be enzyme-catalyzed but that nonenzymatic browning is believed to predominate. It is therefore apparent from the literature that anthocyanin may be degradated enzymatically and that the reactions leading to the polymerization of the brown pigment are usually catalyzed nonenzymatically.

Although Jonathan spots develop earlier in the red in color areas of the apple skin, the eventual occurrence of spots on the non-red colored areas indicates that anthocyanin is not essential for the formation of this disorder. Considering the multiple substrates from which the brown pigment may be formed, it would have been surprising if degradated anthocyanin was to serve a sole possible substrate for the various reactions involved in brown pigment synthesis. Thus Siegelman (1955) isolated l-epicatechol from peel tissues of Grimes Golden and Golden Delicious apples and found it to be the principal browning substrate for polyphenoloxidase in apple peel tissue. Likewise, it is possible that quinic and shikimic acids, which occur in relative abundance in apple peel tissue (Fig. 4), are instrumental in the biogenesis of the brown pigment. Davis (1951) identified shikimic acid as a precursor of phenylalanine and tyrosine in certain strains of bacteria and fungi. Oxidative metabolism of tyrosine may then result in the formation of melanin, a brown pigment which is believed to be a polymer of indol-5,6-quinone (Cromartie et al., 1957). A summary of these pathways

is given by Fruton and Simmonds (1958). Accordingly, tyrosine may be oxidized to phenylalanine-3,4-quinone, subsequently forming a dihydroxyindol, which upon oxidation becomes a dihydroindol quinone. An alternate pathway is proposed for this compound: At pH 1.3 to 2.0, it is converted to a carboxy dihydroindole, whereas at pH 5.6 to 6.8, it eventually becomes indol-5,6-quinone, the polymerization of which yielding melanin.

According to the above proposed pathway, the pH of the system governs the eventual formation of melanin. Noteworthy, the pH of scraped spotted peel was found in one analysis to be as high as 5.45. It is certainly possible that the pH of the 4-5 outer layers of cells, where the brown pigment was observed, is 5.6 or even higher. The actual pH of these cells was impossible to obtain with the methods employed here.

There are still other possible pathways for the genesis of the brown pigment. Haas and Stadtman (1949) reported that quinones, among other compounds, will undergo browning in the absence of enzymatic catalysis, and that in most foods, these reactions occur in the presence of accelerators such as carboxylic acids and their salts, phosphates and metallic ions. As already discussed, all these accelerators increased by two or three-fold in the spotted tissue.

The malate decarboxylation studies were conducted to determine if accelerated decarboxylation of malic acid in the spotted tissue would account for the rise in pH accompanying the development of the spots. The observed decrease in malate decarboxylation in that tissue does not lend support to such a theory.

The very similar endogenous O_2 uptake that newly spotted and normal discs exhibited in early experiments (1962) was not substantiated later

(1963), when a 50% decrease in endogenous O_2 was observed in the spotted discs. Possibly, this discrepancy occurred because the 1963 experiments were conducted some two months later than in the previous year or because citrate-phosphate instead of phosphate buffer was used.

Since an identical O_2 uptake resulted from the introduction of ascorbic acid and catechol to spotted and normal discs, the amounts of ascorbic acid and polyphenol oxidases per unit dry wt of these tissues is similar. Polyphenol oxidase is known to be an important terminal oxidase in mature apple fruits (Rubin et al., 1952), and thus the reduction in endogenous O_2 uptake that the spotted tissue exhibited probably reflected a decrease in substrate in that tissue.

The phase in the ontogeny of the fruit at which Jonathan spot is initiated is not known. Some findings suggest that inception occurs prior to harvest of the fruit. The observations on the anatomy of the skin indicate a higher cell number per unit area in the spotted skin. Early inception is therefore indicated since cell division is apparently completed within the first 25 days after full bloom (Tukey and Young, 1942). This is substantiated by reports concerning the positive effect of kinetin applied at bloom and relating the seed number to the incidence of Jonathan spot (C.S.I.R.O. 1958, Martin et al., 1961). Kinetin is known as a regulator of cell division (Das et al., 1956) and apple ovules are known to excrete indolaceticacid (IAA) shortly after bloom (Luckwill, 1957). It is possible that some factor that is excreted more abundantly by the ovules of Jonathan and related cultivars affects certain cells at the periphery of the fruit at some early stage of cell division. This could result in certain areas in the hypodermis undergoing different rates of cell division and possibly some permanent

modifications in the Donan free space. In time, after harvest, when the fruits would be exposed to suitable external conditions, these predisposed areas of the skin may become spotted.

On the basis of various findings and pertinent literature that were reported in this thesis, the following hypothesis is suggested:

1. Some factor, perhaps associated with the seeds, brings about a certain modification in some cells in the peripherial tissue of the young fruit.
2. After the harvest, there exists a preparatory stage in the path of spot formation which is biphasic in regard to temperature, the second phase having a lower optimal temperature than the first one.
3. During this preparatory stage cations and phosphorus start to accumulate in the spot susceptible cells.
4. The cation influx into these cells triggers a higher rate of acid production or affects a lower rate of acid destruction.
5. As a result of this cation influx, the pH of these cells rises.
6. The rise in pH is suggested as the initial causal factor promoting anthocyanin breakdown and possibly channeling carbonylic compounds such as indolquinone and polyphenols into the synthesis of the brown pigment that visually characterizes Jonathan spot.

CONCLUSIONS AND SUMMARY

The genesis and physiology of a spot disorder affecting the epidermal tissue of matured Jonathan apple fruit was investigated in an attempt to postulate a theory concerning the causal sequence involved in its formation.

It was initially determined from morphological observations that the disorder commonly termed as Jonathan spot consists of two types, one of which occurs in association with an aperture in the epidermis, particularly the lenticels, and has therefore been referred to in this study as lenticel spot. The other, or "true" Jonathan spot occurs without relation to lenticels or other breaks or openings in the epidermis.

Differing anatomical modifications of the skin tissue were observed for lenticel and Jonathan spots. Lenticel spot occurs in conjunction with a break in the epidermal tissue, whereas Jonathan spot occurs without relation to any apertures and is characterized by a higher number of compressed cells per unit cross sectional area of epidermal tissue.

Temperature had a dissimilar effect upon the development of the two spot types. Lenticel spot was often accelerated by high temperature ($70^{\circ}F$), whereas Jonathan spot was inhibited by high temperature. The optimal temperature for Jonathan spot development was below 60°.

It is evident that much of the confusing and contradictory data of the literature is due to a lack of a distinction between the two disorders. Having once resolved that there was a definite distinction between these two spot types, this study was devoted exclusively to the

genesis and physiology of Jonathan spot.

Jonathan spot seemed biphasic in respect to the influence of temperature in that its incidence was accelerated when fruits were initially exposed to a high temperature after harvest, but lower temperature was required for the formation of the visible symptoms that characterize the spots. It is thus possible that Jonathan spot should be classified as a low temperature disorder. Noteworthy, prolonged exposures of apples to temperatures near 60°F substantially decreased the disorder.

Relative humidity was an irrelevant factor in Jonathan spot development. This finding would seem contrary to observations made by other workers, who were probably concerned with lenticel spot rather than with Jonathan spot in their studies.

Above normal levels of CO_2 surrounding the fruit, as low as 0.7% inhibited Jonathan spot. The possibilities that other fruit volatiles were involved with this apparent CO_2 effect, however, were not resolved. Similar to the effects of high temperature, prolonged storage in controlled atmospheres (3% O_2, 5% CO_2) tended to delay and to reduce the incidence of Jonathan spot.

Various analytical determinations were made in an attempt to explain the rise in pH of the spotted tissue. Although the pH of the spotted tissue homogenate varied considerably from 4.4 to 5.2, it was consistently 0.5 to 0.7 pH units higher than the pH of normal tissue but had an approximately double the normal buffering capacity all along the titration curve. Therefore, total titratable acidity of spotted and normal tissues was very similar.

Mineral accumulation in spotted tissue was confirmed, amounting to a three-fold increase in the major cations (K, Ca, Mg) and P.

Cs^{137} was employed in an attempt to ascertain a possible sequential relation between the actual formation of the spots and the accumulation of K. It was applied to apple fruits about 3 weeks before harvest through the leaves or twigs. Autoradiographs showed that the label was uniformly distributed in the peel right after harvest of the fruits, challenging the idea that an early, pre-harvest accumulation of cations is responsible for Jonathan spot. Autoradiographs taken when spots were first observed indicated the absence of the tracer at the spots. Other autoradiographs taken later in the season were less conclusive, but revealed in several cases an accumulation of Cs^{137} in the spots. Thus Cs^{137} proved of little value in ascertaining if cation accumulation occurred prior, concomitantly or after the actual appearance of Jonathan spots. Nevertheless, one possible interpretation of the autoradiographs is that cation accumulation occurred prior to the visual symptoms of Jonathan spots.

Acid extraction and fractionation showed an approximate two-fold increase in total acidity in the spotted tissue, as well as an increase in the percentage of citrate.

It is postulated that the changes in cations and acidity were reflected in the raised pH and in the titrational characteristics of the spotted homogenate.

Jonathan spot characteristically appeared earlier and to a higher extent on the red in color areas of the apple skin. Newly spotted tissue showed a marked loss of anthocyanin, which became greater with time. Although Jonathan spot has long been considered a disorder affecting anthocyanin-bearing cells, anthocyanin in itself, however, can not be considered as a mandatory precursor for the formation of Jonathan

spot since mature green apples eventually developed extensive spotting. It is speculated that degradated anthocyanin served as a precursor for the synthesis of the brown pigment which is typical to the disorder, but that it was only one possible precursor for this pigment, others being quinic and shikimic acids and l-epicatechol that were found in relative abundance in apple peel.

The respiratory measurements of spotted peel discs in a Warburg respirometer revealed that endogenous respiration of spotted peel discs varied greatly. In 1962, newly spotted discs suspended in phosphate buffer showed a similar to normal endogenous O_2 uptake, refuting the idea that Jonathan spot involved an immediate breakdown of the affected tissue. Old spotted tissue, however, exhibited a 50% decrease in O_2 uptake. In 1963, when respiration measurements were conducted some two months later than in the previous year and citrate-phosphate buffer was used, the discs exhibited a 50% decrease of the normal endogenous O_2 uptake.

Addition of malate showed that the spotted discs partially lost the capacity to decarboxylate malate, weakening a tenative assumption that Jonathan spot resulted from an accelerated decarboxylation of malic acid. Addition of catechol and ascorbic acid resulted in identical O_2 uptake for both the spotted and normal discs, indicating a similar content of ascorbic acid and polyphenol oxidases per unit dry wt. It was suggested that the reduction in endogenous O_2 uptake that the spotted tissue exhibited may be due to a lack of substrate in that tissue.

A hypothesis of the causal sequence involved in Jonathan spot development was proposed. It included two major assumptions, namely, (1) that some cells in the peripherial tissues of the receptacle undergo

a certain modification early in the ontogeny of the fruit, and (2), that cation and phosphorus accumulation which later occurs in that tissue, triggers the eventual formation of Jonathan spot.

It is evident that a satisfactory comprehension of the genesis and development of Jonathan spot can be accomplished only with a better basic understnading of the gross effects of the seeds in young fruits and of the processes involved in mineral transport through cellular membranes.

LITERATURE CITED

Australia, Commonwealth Sci. Ind. Research Organization. 1958. Fruit investigations. Tenth Annual Report: 20-29.

Biale, J. B. 1960. The post harvest biochemistry of tropical and subtropical fruits. Advances in Food Research 10:293-354.

Brooks, C., and J. S. Cooley. 1917. Effects of temperature aeration and humidity on Jonathan Spot and scald of apples in storage. J. Agr. Research 9:287-318.

_____, and D. F. Fisher. 1918. Irrigation experiments on apple spot diseases. J. Agr. Research 12:109-137.

Bunemann, G. 1958. The relation of the nutrient element content in the leaves and fruits to the storage quality of Jonathan apples in regular and controlled atmospheres. Unpublished Ph.D. Thesis, Michigan State University, East Lansing, Michigan.

_____, D. H. Dewey, and A. L. Kenworthy. 1959. The storage quality of Jonathan apples in relation to the nutrient levels of the leaves and fruits. Mich. Agr. Expt. Sta. Quart. Bull. 41:820-833.

Clements, H. F. 1935. Morphology and physiology of the pome lenticels of Pyrus Malus. Bot. Gaz. 97:101-117.

Clerx, W. A. 1960. Some factors affecting the development of Jonathan Spot on apples stored in controlled atmospheres. Unpublished M.S. Thesis, Michigan State University, East Lansing, Michigan.

Cook, M. T., and G. W. Martin. 1914. The Jonathan spot rot. Phytopathology 4:102-105.

Cromartie, R. J. T., and J. Harley-Mason. 1957. Melanin and its precursors. VIII. The Oxidation of methylated 5,6-Dihydroxyindoles. Biochem. J. 66:713-720.

Das, N. K., K. Patau, and F. Skoog. 1956. Initiation of mitosis and cell division by kinetin and indoleaceticacid in excised tobacco pith tissue. Physiol. Plantarum 9:640-651.

Davis, B. D. 1951. Aromatic Biosynthesis. I. The role of shikimic acid. J. Biol. Chem. 191:315-323.

Dewey, D. H., W. E. Ballinger and I. J. Pflug. 1957. Progress report on the controlled-atmosphere storage of Jonathan apples. Mich. Agr. Expt.Sta. Quart. Bull. 36:691-700.

_____, H. J. Raphael, and J. W. Goff. 1959. Polyethelene covers for apples stored in bushel crates on pallets. Mich. Agr. Expt. Sta. Quart. Bull. 42:197-209.

_____. 1962. Factors affecting the quality of Jonathan apples stored in controlled atmospheres. Proc. XVIth Intern. Hort. Congress. 438-445.

Ezell, B. D., and F. Gerhardt. 1942. Respiration and oxidase and catalase activity of apples in relation to maturity and storage. J. Agr. Research 65(10):433-440.

Flood, A. E., A. C. Hulme, and L. S. C. Wooltorton. 1960. The organic acid metabolism of Cox's Orange Pippin apples. J. Exptl. Bot. 11:316-334.

Fruton, J. S., and S. Simmonds. 1958. Microbial metabolism of phenulalanine and tyrosine, p. 832-835. In J. S. Fruton, and S. Simmonds, General biochemistry, John Wiley & Sons Inc., New York.

Haas, V. A., and E. R. Stadtman. 1949. Deterioration of dried fruits. Ind. Eng. Chem. 41:983-985.

Hall, E. G., S. M. Sykes, and S. A. Trout. 1953. Effects of skin coatings on the behaviour of apples in storage. Aust. J. Agr. Research 4:365-381.

Harbone, J. B. 1959. Chromatographic identifications of anthocyanin pigments. Chromatog. Rev. 1:209-224.

Hodge, J. H. 1953. Chemistry of browning reactions in model systems. Agr. Food Chem. 1:928-941.

Jacobson, L., and L. Ordin. 1954. Organic acid metabolism and ion absorbtion in roots. Plant Physiol. 29:70-75.

Joslyn, M. A., and J. D. Ponting. 1951. Enzyme-catalyzed oxidation browning of fruit products. Advances in Food Research 3:1-44.

Kidd, M. N., and A. Beaumont. 1925. An experimental study of the fungal invasion of apples in storage, with particular reference to invasion through the lenticels. Ann. of Appl. Biol. 12:14-33.

Krapf, B. 1961. Entwicklung und Bau der Lentizellen des apfels und ihre Bedeutung fur di Lagerung. (English summary) Landwirtschaflichen Jahrbuch der Schweiz. 2:389-435.

Luckwill, L. C. 1957. Studies of fruit development in relation to plant hormones. IV. Acidic auxins and growth inhibitors in leaves and fruits of the apple. J. Hort. Sci. 32:18-33.

Markakis, P., G. D. Livingston, and C. R. Fellers. 1957. Quantitative aspects of strawberry pigment degradation. Food Research 22:117-130.

_____, A. Jarczyk, and S. P. Krishna. 1963. Nonvolatile acids of blueberries. Agr. Food Chem. 11(1):8-11.

Martin, D., T. L. Lewis, and J. Cerny. 1961. Jonathan spot - three factors related to incidence: fruit size, breakdown, and seed number. Aust. J. Agr. Research 12:1039-1049.

Nagai, I. 1921. A genetico-physiological study on the formation of anthocyanin and brown pigments in plants. J. Coll. Agr. Univ. Tokyo Imp. Univ. 8:1-92.

Neal, G. E., and A. C. Hulme. 1958. The organic acid metabolism of Bramley's Seedling apple peel. J. Exptl. Bot. 9:142-157.

Neilands, J. B., and P. K. Stumpf. 1958. Determination of the pK , p. 13-14. In J. B. Neilands, and P. K. Stumpf, Outlines of enzyme chemistry, John Wiley & Sons Inc., New York.

Pentzer, W. T. 1925. Color pigment in relation to the development of Jonathan spot. Proc. Amer. Soc. Hort. Sci. 22:66-69.

Plagge, H. H., and T. J. Maney. 1924. Apple storage investigations. Fourth Progress Report I - Jonathan spot and soft scald. Iowa Agr. Expt. Sta. Bull. 222, 37p.

_____, and F. Gerhardt. 1930. Acidity changes associated with the keeping quality of apples under various storage conditions. Iowa Agr. Expt. Sta. Bull. 131, 32p.

_____, T. J. Maney, and B. S. Pickett. 1935. Functional diseases of the apple in storage. Iowa Agr. Expt. Sta. Bull. 329, 43p.

Rubin, B. A., E. V. Artsikhovskaya, V. E. Sokolova and T. M. Ivanova. 1952. (Chem. Abstr. 1953. Vol. 47, No. 695, d.) Doklady Akad. Nauk. S.S.S.R. 85:1123-1126.

Sass, J. E. 1958. Botanical microtechnique. The Iowa State Univ. Press, Ames, Iowa. 224p.

Sando, C. E. 1937. Coloring matters of Grimes Golden, Jonathan, and Stayman Winesap apples. J. Biol. Chem. 117:45-56.

Scott, W. M. 1911. A new fruit spot of apple. Phytopathology 1:32-34.

_____, and J. W. Roberts. 1913. The Jonathan fruit spot. U.S. Dept. Agr. Bur. Plant Ind. Cir. 112.

Siegelman, H. W. 1955. Detection and identification of polyphenol oxidase substrates in apple and pear skins. Arch. Biochem. Biophys. 56:97-102.

Siegelman, H. W., and S. B. Hendricks. 1958. Photocontrol of anthocyanin synthesis in apple skin. Plant Physiol. 33:185-190.

Smock, R. M., and A. M. Neubert. 1950. Storage troubles of the apple; 9. Jonathan spot, p. 233-234. In R. M. Smock and A. M. Neubert, Apples and apple products, Interscience Publishers Inc., New York.

Tomana, T. 1959. A study on the physiological diseases in Jonathan apples. 2. Nitrogen fertilization in relation to the nitrogen contents in flesh and the occurrence of the physiological diseases. (In Japanese, English summary.) Bull. Yamagata Univ. 3(1):279-289.

_____. 1960. Histological and physiological studies on the cause of Jonathan spot in apples. I. Varietal difference in skin structure of fruit in relation to the spot development. (In Japanese, translated into the English by Mrs. Midori Battistini). J. Hort. Assoc. Japan 29(4):273-280.

_____. 1961. Histological and physiological studies on the cause of the Jonathan spot in apples. II. Nitrogen fertilization and harvest time in relation to the spot development. (In Japanese, English summary.) Japan. Soc. Hort. Sci 30(2):117-124.

Trout, S. A., G. B. Tindale, and F. E. Huelin. 1940. Investigations on the storage of Jonathan apples grown in Victoria. Commonwealth, Australia, Council Sci. Ind. Research Organization. Bull. 135, 96p.

_____, E. G. Hall, and S. M. Sykes. 1953. Effects of skin coatings on the behaviour of apples in storage. Aust. J. Agr. Research 4:57-81.

Tukey, H. B., and J. O. Young. 1942. Gross morphology and histology of developing fruit of the apple. Bot. Gaz. 104:3-25.

Ulrich, R. 1941. Metabolism of nonvolatile organic acids in excised barley roots as related to cation-anion balance during salt accumulation. Am. J. Bot. 28:526-537.