SOME FACTORS AFFECTING THE DEVELOPMENT OF JONATHAN SPOT ON APPLES STORED IN CONTROLLED ATMOSPHERES

Thesis for the Degree of M. S.

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Wilhelmus Antonius Clerx

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By

WILHELMUS ANTONIUS CLERX

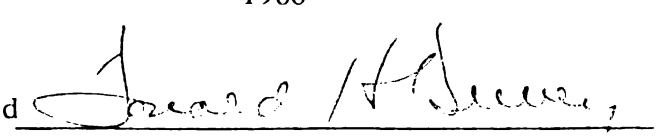
AN ABSTRACT

Submitted to the College of Agriculture, Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Horticulture

1960

Approved 

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WILHELMUS ANTONIUS CLERX ABSTRACT

Jonathan spot is a physiological disorder which may cause serious damage to several commercial varieties of apples during the cold storage period. Researchers have shown that spot may be controlled on Jonathan apples by utilization of controlled atmosphere (CA) storage in which high levels of carbon dioxide (usually 5 percent) and low levels of oxygen (3 percent) are employed in conjunction with a temperature of 32° F. Studies were made to determine whether the controlled atmospheres actually prevented the disorder or merely inhibited its development, and to gain further knowledge of the physiological factors affecting the development of the spot disease in the Jonathan apple.

Random samples of fruit grown in the Michigan State University orchard were stored in CA and regular storages during the 1958-59 and 1959-60 seasons. Apples were transferred from one storage to the other so as to provide CA and regular storage treatments of varying durations. It was found that controlled atmospheres inhibited the development of Jonathan spot only during the period when the fruit was in CA storage. Spot did not develop on apples in CA when the apples were stored immediately upon harvest under these conditions. Jonathan spot initiated during a regular storage period continued to develop when the fruit was subsequently held in CA. Fruit stored in CA developed spot when subsequently held in regular air at 32° F.

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The total amount of fruit which had developed spot at the conclusion of the total storage season was directly proportional to the duration of the regular storage period, and inversely proportional to the duration of the CA storage period.

Total acidity and pH changes of the flesh and skin of the stored apples were ascertained in relation to spot development throughout the second year of this study. The loss of acids, which is characteristic of most apples during storage, was greater during regular storage than during CA storage. Greater losses occurred in the skin than in the flesh of apples stored under both conditions. There was a highly significant correlation ($r = -.67$) of percentage total acid of the skin and the amount of fruit which developed Jonathan spot during storage; whereas the correlation value ($r = -.42$) for flesh acidity and spot was significant only at the 5% level. Acidity levels similar and lower than those associated with the appearance of spot in storage, when brought about by delayed harvest of the fruit or by holding the fruit at high temperatures, did not always cause the disorder to appear, however. It is doubtful there was a causal relation of acid changes and spot formation.

The location of the spots relative to open or closed lenticels in the fruit skin was examined as a possible clue to the effect of gas exchange through

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the lenticels upon the appearance of Jonathan spot. There was no evidence for such a relationship since spots developed adjacent to both types of lenticels. It was discovered, however, that fruit stored in CA had a larger proportion of open to closed lenticels than comparable apples in regular storage.

Mineral analysis of spotted and non-spotted skin tissues revealed there was a marked accumulation of K, P, Ca, Mg, Mn, B and Mo in the tissue afflicted with the disorder.

Two other disorders of Jonathan apples also were observed in the course of these studies. "Near spot", which appeared as a general bluish or brownish discoloration of the skin, occurred in CA and regular storage and was not related to the incidence of Jonathan spot. There was a small quantity of soft scald on apples in regular storage in one of the two seasons. The disorder was completely arrested when the afflicted apples were transferred to controlled atmospheres.

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INTRODUCTION

Jonathan is one of the most important varieties of apples grown in Michigan. Recent crop reports show that the production of Jonathan apples in this state amounts to three million bushels per year, of which approximately one-third is stored to enable marketing over a long period. Jonathan apples may be kept for four months in regular cold storage at 32° F, but at this temperature the apples are susceptible to soft scald, a serious storage disorder. At higher temperatures another physiological disorder, Jonathan spot, may cause serious losses. When Jonathan spot is found on apples in storage, it means that the further storage life of the fruit is limited, since the disorder is likely to increase rapidly and affect all red-colored fruit. Therefore, the apples must be inspected frequently and moved into market channels before Jonathan spot becomes extensive.

The storage life of Jonathan apples may be extended by modifying the gases of the storage room so that the oxygen content of the atmosphere is reduced to approximately 3 percent and the carbon dioxide is increased to 2 1/2 or 5 percent. Complete control of Jonathan spot and soft scald is obtained under these conditions.

While much experimental work has been concerned with Jonathan spot, there is as yet no satisfactory explanation as to its nature and cause except that it appears to be associated with over-maturity of the fruit and is

increased with further ripening during storage.

The previous studies concerning the control of Jonathan spot by utilization of increased levels of CO_2 and reduced levels of O_2 during cold storage have not revealed whether the controlled atmosphere (CA) actually prevented the disorder or merely inhibited its development. These studies were made, therefore, to determine whether CA prevents the initiation of Jonathan spot or merely retards its development, and to obtain further knowledge of the physiological factors responsible for the development of the spot disease in the Jonathan apple.

REVIEW OF LITERATURE

Jonathan spot is a physiological disorder that appears during storage of the apple and seriously affects the Jonathan variety. It also occurs on other apple varieties, especially Wealthy, Rome Beauty, and Esopus Spitzenburg.

The symptoms have been reported by numerous authors, including Hesler and Whetzel (1920), Brooks et al. (1920), Plagge and Maney (1924), Smock and Neubert (1950), Rose et al. (1951), Fisher and Porrit (1951), and Wright (1953). The composite picture drawn from the numerous descriptions characterize Jonathan spot as a localized darkening and blackening of the outer layer of anthocyanin bearing cells of the apple fruit. Jonathan spot primarily affects the epidermal and hypodermal cells, and sometimes the few pigmented cells beneath the skin. In the early stages of development the black spots are generally circular in shape and frequently center at the lenticels. During storage the spots increase in size and range from minute spots with a diameter of one-thirty-second to one-fourth of an inch or more. In later stages the spots appear in a greenish-brown color on the pale side of the fruit. The spots, with aging, become very slightly sunken, irregularly lobed, and the underlying tissue dries out. Fungi sometimes attack the spotted areas and cause rotting.

Although this disorder does not generally impair the cooking or eating quality of fruits, the unsightly appearance of the spots greatly reduce the

market value of the fruit (Plagge, 1924).

Suggestions as to the likely causes and factors associated with Jonathan spot were reported in 1920 by Brooks et al. They found that Jonathan spot developed more rapidly at relatively high storage temperatures than at low temperatures. This association with temperature was confirmed by Plagge and Maney (1936), Rose et al. (1951), and Fisher and Porritt (1951). There is also agreement that Jonathan spot may occasionally appear on fruit before it is picked, and some authors have assumed that spotting was associated with a varietal weakness in the epidermal tissue of the apple.

Pentzer reported in 1925 that the blue or black color of the spotted skin tissue resulted from a change in color of the anthocyanins in the cells and that the cells immediately below the spots were of lower acidity than the cells of adjacent areas. Association of the occurrence of Jonathan spot with the acid content of apples was also reported by Plagge and Gerhardt (1930), Plagge et al. (1936), and Wright (1953). Smock and Neubert (1950), however, stated that there is no proof that the color changes accompany pH of apple cells and question if acidity level and rate of loss of acids are the cause of Jonathan spot appearance.

Plagge and Maney (1924) concluded from ten years of study that size of apples, soil management, mean outside temperature, rainfall, sunshine, constant air movement, oil wraps, wax or tin foil wraps could be eliminated

as possible factors of influence on Jonathan spot development. Delay in placing the harvested fruit at 32° F, high storage temperatures, high humidity conditions, and low acidity of the fruit tissues were associated with the disease. These investigators suggested that the disorder is controlled by a composite factor based on several meteorological activities and storage conditions; and thereby determines the varying amounts of Jonathan spot from season to season.

A recent Australian report (CSIRO*, 1958) states that a positive correlation was found between incidence of Jonathan spot and average fruit size per tree. They also reported that hand thinning of fruit in the cell division stage resulted in increased cell division, reduced maturation rate, and reduced incidence of Jonathan spot.

Skin coatings applied to cold storage apples by means of alcoholic solutions of castor oil and shellac, oil emulsions or wax emulsions have partially controlled Jonathan spot, but since they adversely affect other quality characteristics, they are not reliable for commercial application (Hall, Sykes, and Trout, 1953).

The most practical method of Jonathan spot control has been by the use of controlled atmosphere (CA) storage. The combinations of carbon dioxide and oxygen of storage atmosphere at different temperatures listed

*C. S. I. R. O - Commonwealth Scientific and Industrial Research Organization.

below have proven satisfactory for its control (summarized from Ballinger, 1955).

Researchers	Year and Country of Studies	Atmospheres		Temperatures (°F)
		CO_2 (%)	O_2 (%)	
Smock and Van Doren	1941, U. S. A.	5	15)	
		5	10)	40, 45
		10	10)	
Van Hiele	1951, Netherlands	7	13	32, 36
Rasmussen	1951, Denmark	9	12	-
Vickery et al.	1951, Australia	5	16	-
Fisher	1954, Canada	4	16	-
Ballinger	1955, U. S. A.	0. 5	3)	
		2. 5	3)	32, 36, 40
		5	3)	
		7	13)	

CA storage offers certain benefits in addition to control of Jonathan spot. Apples may be held at least seven months without great loss of market quality and quite often maintain good quality for a longer period after removal from storage than apples from regular cold storage (Dewey et al., 1957).

Atomospheres containing 7 to 14 percent CO_2 with 1 to 12 percent O_2 surrounding apples enclosed in polyethylene or Saran crate liners have partially or completely controlled Jonathan spot (Plagge and Maney, 1941; Ballinger, 1955).

It has been reported that Jonathan apples from controlled atmosphere storage and sealed crate liners will remain free of spot during simulated retail periods after removal from storage (Smock, 1941; Ballinger, 1955; Workman, 1959).

Investigators (CSIRO, 1958) have attempted to control spot in Jonathan apples by treatment of the fruit with growth substances. They found that Jonathan spot was increased by preharvest applications of kinetin (significantly) but not by adenine, and was decreased when naphthaleneacetic acid was applied. Staden (1957) found that the development of Jonathan spot was reduced by application of diphenylamine.

A skin discoloration apparently caused by a change in color of the anthocyanin pigment from red to bluish or purplish-brown was first noticed and recorded as "near spot" by Bünemann (1957). The skin discoloration occurred in controlled atmosphere as well as in regular cold storage.

Soft scald seldom occurs on apples stored at temperatures above 38° F and is, therefore, normally classified as a low temperature injury (Wright, 1953). However, Dewey et al. (1957) reported that controlled atmosphere storage at 32° F was effective in reducing the occurrence of soft scald.

METHODS

Fruit

Fruits grown on the Horticulture Farm of Michigan State University were utilized for these experiments. They were harvested from trees planted in 1924 and grown under sod culture. In conjunction with a yearly mulching program, six of the trees supplying fruit for these studies received an application of ammonium nitrate on a basis of 300 pounds per acre, two trees received mixed fertilizer, 12-12-12, yearly at 300 pounds per acre. The spray program included applications of lime-sulphur, Glyodin, Cyprex, Sevin, and parathion. The trees had vigorous growth and healthy foliage.

The apples were harvested on September 30, 1958 and September 24, 1959. Randomized test samples were obtained by compositing the harvested apples following sorting for removal of fruit with serious defects, of excessively small and large size, and of poor red color.

Apples with less than 50 percent color were eliminated in 1958, but in 1959, due to unfavorable weather conditions, the picked apples were less mature and apples with 30 to 50 percent red color were selected.

The apples were held in temporary storage at 32° F for one day and then placed in the experimental chambers.

Experimental Set-up, Observations and Determinations

Experiment 1 involved the periodical transfer of fruit between regular and controlled atmosphere storages to investigate the influence of the duration of CA storage and the time of CA application on Jonathan spot initiation and development. The storage treatments used in 1958 are listed below. Two boxes of apples used for each treatment formed a replicate sample. Each box contained an average number of 150 apples. The treatments were accomplished by transferring the boxes to CA or regular storage on the dates given.

Treatment No.	Dates in CA Storage	Dates in Regular Storage
1	Oct. 15 - Dec. 15	Dec. 15 - May 1
2	Oct. 15 - Jan. 27	Jan. 27 - May 1
3	Oct. 15 - Feb. 26	Feb. 26 - May 1
4	Oct. 15 - March 25	March 25 - May 1
5	Oct. 15 - May 1	

Treatment No.	Dates in Regular Storage	Dates in CA Storage
6	Oct. 15 - Dec. 15	Dec. 15 - May 1
7	Oct. 15 - Jan. 27	Jan. 27 - May 1
8	Oct. 15 - Feb. 26	Feb. 26 - May 1
9	Oct. 15 - March 25	March 25 - May 1
10	Oct. 15 - May 1	

An additional ten boxes of apples of both series were held for the entire period (October 7 to March 4) in an atmosphere adjusted to an average of 7 percent CO_2 and 14 percent O_2 at 32° F. A comparable set of apples were held for the same period in 11 percent CO_2 and 10 percent O_2 at 32° F. At the end of the storage period, two boxes out of each of these two atmospheres were placed in rooms with constant temperatures of 32°, 46°, 60° and 75° F to determine the effect of these atmospheres on the subsequent appearance and development of Jonathan spot in normal air.

The fruit was sorted at each transfer date for Jonathan spot, "near spot", soft scald, decay, and other disorders. After opening of the CA storages, a final check was made of all the fruit involved in the experiment.

Experiment 1 was repeated in 1959. The same experimental design was followed, but the composition of the atmosphere and the date of fruit transfers differed from those in 1959. Also, three additional transfers were included.

The apples were stored for the following periods in an atmosphere adjusted to an average composition of 2.5 percent CO_2 and 3 percent O_2.

Lot	Dates in CA Storage	Dates in Regular Storage
1	Oct. 16 - Nov. 13	Nov. 13 - April 4
2	Oct. 16 - Dec. 11	Dec. 11 - April 4
3	Oct. 16 - Jan. 8	Jan. 8 - April 4
4	Oct. 16 - Feb. 5	Feb. 5 - April 4
5	Oct. 16 - March 4	March 4 - April 4
6	Oct. 16 - April 4	

Lot	Dates in Regular Storage	Dates in CA Storage
7	Oct. 16 - Nov. 13	Nov. 13 - April 4
8	Oct. 16 - Dec. 11	Dec. 11 - April 4
9	Oct. 16 - Jan. 8	Jan. 8 - April 4
10	Oct. 16 - Feb. 5	Feb. 5 - April 4
11	Oct. 16 - March 4	March 4 - April 4
12	Oct. 16 - April 4	

Lot	Regular Storage	CA Storage	Regular Storage
13	Oct. 16 - Dec. 11	Dec. 11 - Mar. 4	Mar. 4 - April 4
14	Oct. 16 - Jan. 8	Jan. 8 - Mar. 4	Mar. 4 - April 4

Lot	CA Storage	Regular Storage	CA Storage
15	Oct. 16 - Jan. 8	Jan. 8 - Mar. 4	Mar. 4 - April 4

At each transfer the fruit was sorted for the same disorders as in 1958; also, the pH and total titratable acidity of the skin and flesh for each lot of fruit was determined. Final determination of acids was made for each lot at the end of the storage period.

The CA atmosphere of 5 percent CO_2 and 3 percent O_2 was maintained by adding outside air to supply oxygen and by removing carbon dioxide with an abosrber apparatus. A closed-circuit air circulating pump was used to circulate the storage air through the sodium hydroxide solution in the absorber.

The atmospheres of 7 percent CO_2 and 14 percent O_2 and of 11 percent CO_2 and 10 percent O_2 were adjusted by ventilation with outside air.

Storage temperatures measured by thermocouples, and gas concentrations, determined with an Orsat gas analyzer, were read and adjusted as necessary to the required values each day.

The placement or removal of CA apples without marked disruption of the atmosphere was accomplished by entering the storage through a removable 2 ft. x 2 ft. window in the gas tight metal door. The opening was covered by a polyethylene sheet during the time required for the transfer. The person making the transfers used oxygen breathing equipment while in the CA atmosphere (see Figure 1).

Figure 1

The oxygen breathing equipment (top) and the square opening for entering the controlled atmospheres during the storage period (bottom).

Experiment 2 was designed for determination of a possible relationship between pH or total acidity of the fruit tissues and the incidence of Jonathan spot. Two boxes of apples were placed in rooms with the following constant temperatures: 32°, 45°, 60°, 75°, and 90° F. Three pickings were made 10 days, 20 days, and 30 days after the first harvest. The apples of the first two pickings were placed at 45° and 75° F. The third picking was placed in a constant temperature room of 75° F. The apples were examined every two weeks for Jonathan spot development. Upon the appearance of spots on 10 percent or more of the fruit, the pH and total acidity of the peel and flesh of a representative sample of fruit for the lot were determined.

Determination of pH and Total Titratable Acidity

The peelings and flesh used for acidity determinations were taken with an apple peeler which peeled the apple spirally and simultaneously removed a center cylinder of core 3/4 inches in diameter from the calyx to stem end of the fruit. The peel was weighed, placed in a Waring blendor with 200 cc distilled water, and blended for five minutes. The flesh, in quantities approximately equal for each apple, was taken from the center part, weighed and treated in the same way as the peels.

The blendate of the peel and of the flesh samples was strained through cheesecloth to remove cell wall debris. The pH of the filtered solution was determined and 50 cc aliquot was titrated to a pH of 8 with 0.1 N sodium hydroxide.

Experiment 3 was concerned with the relation of open lenticels to the disorder. In Experiment 3a, the method of Marcellin (1956) for identifying open lenticels was employed on thirty badly spotted apples to determine if the lenticels located in spotted areas were closed or opened, and for possible differences in numbers of opened lenticels in spotted areas and non-spotted areas of the fruit skin. When it was found that spotted skin areas contained opened lenticels, the experiment was made to study limited areas of fruit skin containing many spots and of the areas free of spot. The apples used had been in regular storage or in CA at 5 percent $C0_2$ and 3 percent 0_2 for approximately five and one-half months at the time of testing.

Marcellin Method for Determination of Open Lenticels

As it is difficult to establish with certainty the permeability of lenticels to gases either by microscopical examination or by vacuum treatment of the fruit, the method described by Marcellin (1956) for whole fruit was used. It is based on the reappearance on the exterior of the fruit of traces of oil previously absorbed by capillary movement into openings of the tissues. Each apple was dipped momentarily in a bath of mineral oil, Bayol-16-formula 2911, and the liquid allowed to penetrate the openings present in the surface of the apple. After the excess oil had dripped off, the apple was blotted carefully with filter paper without rubbing the surface. The drying was completed with anhydrous alumina powder (Al_2O_3) which was then rapidly removed by lightly

brushing with a piece of cotton. Finally, a new thin layer of powder was brought to the surface by the application of a mixture of aluminum-oxide and sudan IV oil stain.

The oil absorbed in the fissures of the tissues beneath the skin upon return to the surface contacted the stain to cause purple-red spots to appear at the outlet of each opening.

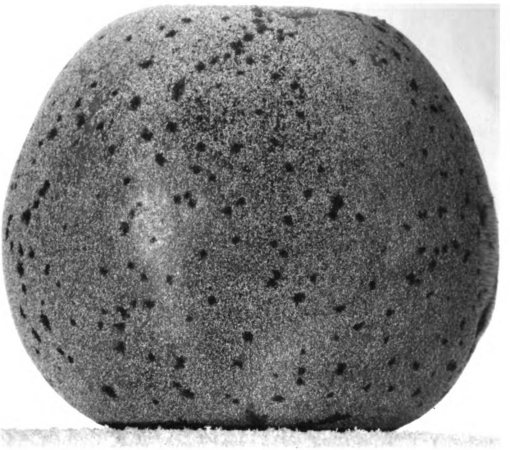
Two slight modifications in the method were made. The oil, Shell Mayoline 250T, used by Marcellin could not be obtained in the United States. A technical white oil, Bayol 16-formula 2911 from the Penola Oil Company, with apparently comparable properties, was employed. Instead of Sudan Black, the oil stain Sudan IV was used with satisfactory results.

Experiment 4 concerned the amount of mineral elements in spotted and non-spotted skin of Jonathan apples. The spotted areas of skin were very carefully removed in as thin a layer as possible from forty apples seriously affected with Jonathan spot which had been in regular storage at 32° F for five and one-half months. Approximately equal amounts of skin of the non-spotted surface between the spots were also removed from the same apples.

In addition, ten apples with spots on one side of the apple were peeled with an apple peeler. The peel, which consisted of the epidermal layer, the hypodermal layer and part of the cortex, was divided in two halves with and without Jonathan spot. The whole peel was also taken from ten apples without Jonathan spot.

Figure 2

The small dark spots show the location of open lenticels in this Jonathan apple. The spots were obtained by dipping the apple in oil and subsequently treating it with a mixture of aluminum oxide powder and oil stain.



Another 60 apples were divided into three groups for similar treatment. The peels were also removed from ten non-spotted apples which had been held in CA storage (5 percent CO_2 and 3 percent O_2) for five and one-half months and from ten spotted apples which had been kept for three and one-half months in the same storage followed by two months in normal cold storage at 32° F.

All peelings were dried at 105° F and ground in a Wiley mill through a 20-mesh screen. Weighed samples were analyzed for the following elements: N, K, P, Ca, Mg, Mo, Fe, Cu, B, Zn and Al. The nitrogen was determined by the Kjeldahl-Gunning Arnold method, potassium with the flame photometer, and all other elements spectrographically.

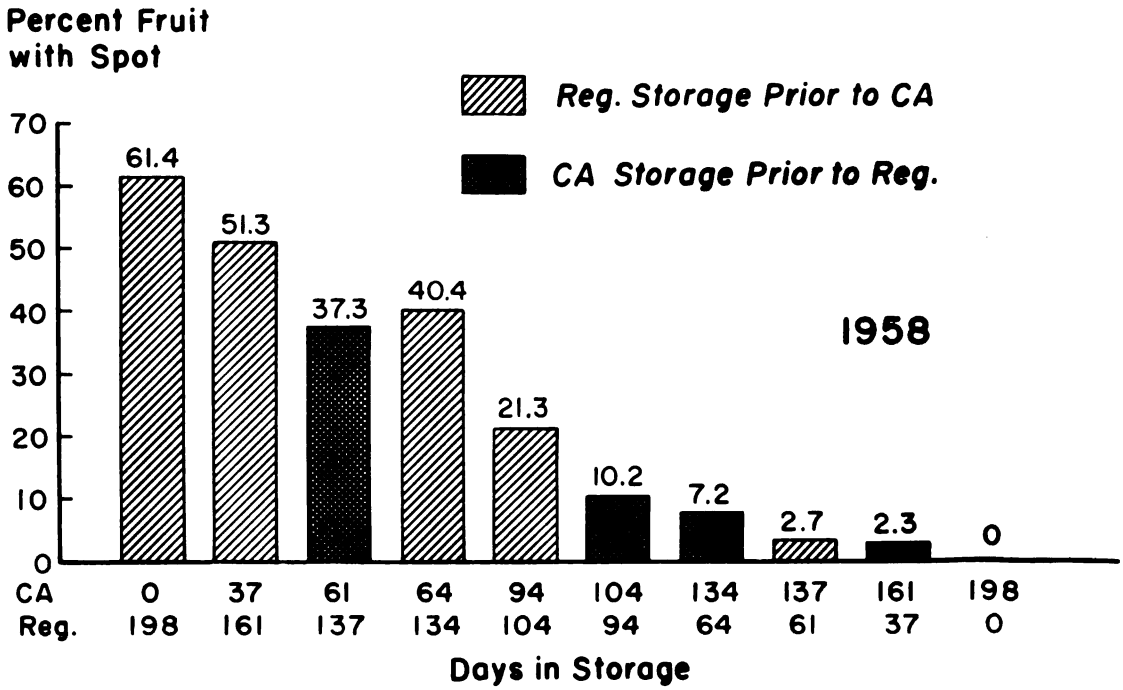
RESULTS

The apples which were stored in controlled atmospheres of 11 percent CO_2 - 10 percent O_2, 7 percent CO_2 - 14 percent O_2, 5 percent CO_2 - 3 percent O_2 and in regular storage at 32° F in 1958, and in a controlled atmosphere of 2.5 percent CO_2 - 3 percent O_2 and in regular storage at 32° F in 1959, did not develop Jonathan spot in CA storage when they were placed there immediately upon harvest.

The results of Experiment 1 concerning the periodical transfer of apples between regular and CA storages are summarized in Table I and graphically represented in Figure 3. Apples from controlled atmosphere of 5 percent CO_2 and 3 percent O_2 since harvest developed Jonathan spot when transferred to regular cold storage. Increasing the duration of the initial CA storage period decreased the amount of Jonathan spot at the end of the total storage season of seven months. The same general results occurred in 1959 for apples initially stored in an atmosphere of 2.5 percent CO_2 and 3 percent O_2. In 1958 one hundred four days of regular cold storage were required for Jonathan spot development when regular storage preceded CA. When the fruit was moved to CA, the spots continued to develop, but less extensively than during regular storage. Consequently, the quantity of spotted fruit at the end of the storage season was directly related to the length of time the apples had been in regular storage. The data of 1959 show

Figure 3

The development of spot on Jonathan apples during CA and regular cold storage.


Percent Fruit with Spot
Reg. Storage Prior to CA
CA Storage Prior to Reg.
1958
70
60
50
40
30
20
10
0
61.4
51.3
37.3
40.4
21.3
10.2
7.2
2.7
2.3
0
CA 0 37 61 64 94 104 134 137 161 198
Reg. 198 161 137 134 104 94 64 61 37 0
Days in Storage

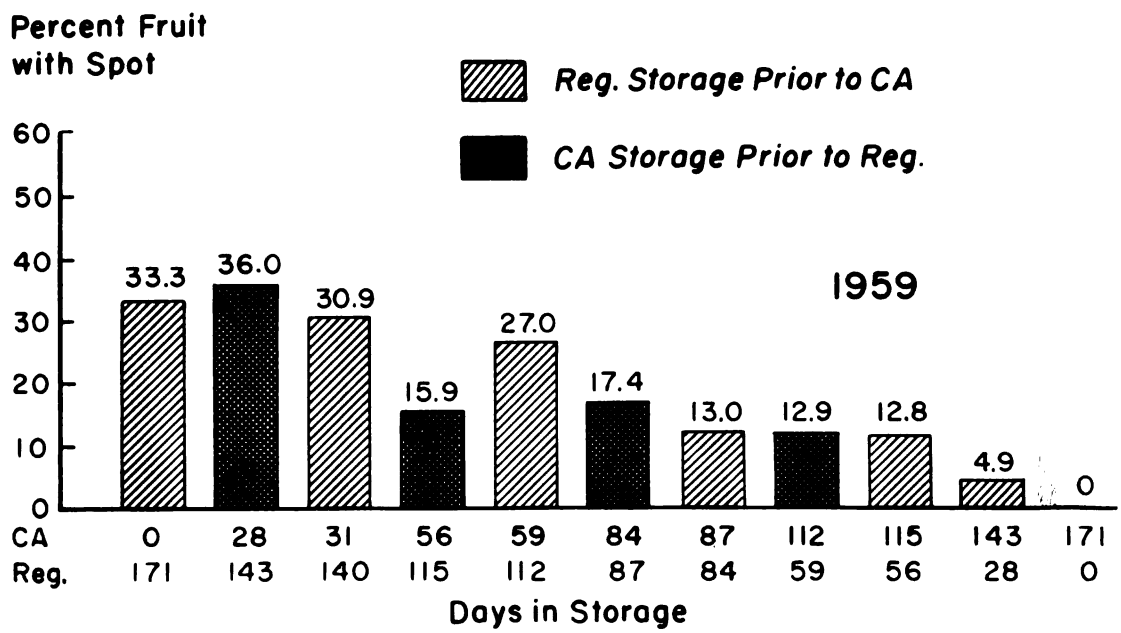

Percent Fruit with Spot
Reg. Storage Prior to CA
CA Storage Prior to Reg.
1959
60
50
40
30
20
10
0
33.3
36.0
30.9
15.9
27.0
17.4
13.0
12.9
12.8
4.9
0
CA 0 28 31 56 59 84 87 112 115 143 171
Reg. 171 143 140 115 112 87 84 59 56 28 0
Days in Storage

TABLE I

The Development of Spot on Jonathan Apples Stored for Different Periods in CA and Regular Cold Storage

1958

CA Storage Preceding Regular Cold Storage Days in		Fruit with Spot (Percent, Accumulative) Total Duration of Storage (Days)				
CA	Regular	61	104	134	161	198
61	137	—0	0	0	5.3	37.3
104	94	————	0	0	0	10.2
134	64	————	————	0	0	7.2
161	37	————	————	————	0	2.3
198	0	————	————	————	————	0

Regular Cold Storage Preceding CA Storage Days in		Fruit with Spot (Percent, Accumulative) Total Duration of Storage (Days				
CA	Regular	61	104	134	161	198
0	198	0	1.7	6.3	23.8	61.4
37	161				30.8	—51.3
64	134			15.8	————	—40.4
94	104		0	————	————	—21.3
137	61	0	————	————	————	2.7

1959

CA Storage Preceding Regular Cold Storage Days in		Fruit with Spot (Percent, Accumulative) Total Duration of Storage (Days)					
CA	Regular	28	56	84	112	140	171
28	143	—0	0	0	-	21.3	36.0
56	115	————	0	0	-	7.1	15.9
84	87	————	————	0	-	7.3	17.4
112	59	————	————	————	0	1.5	12.9
140	31	————	————	————	————	0	4.6
171	0	————	————	————	————	————	0

Regular Cold Storage Preceding CA Storage Days in		Fruit with Spot (Percent, Accumulative) Total Duration of Storage (Days)					
CA	Regular	28	56	84	112	140	171
0	171	0	0	1.7	-	17.5	33.3
31	140					13.3	—30.9
59	112				9.8	————	—27.0
87	84			.7	————	————	—13.0
115	56		0	————	————	————	—12.8
143	28	0	————	————	————	————	4.9

————Indicates duration of CA storage period.

a similar influence of CA storage on the final amount of Jonathan spot in that the amount of fruit with spot decreased as the duration of the CA period was increased. The amounts of fruit affected by Jonathan spot were smaller than in 1958; therefore, the effects of CA were less pronounced. A comparison of the development of spot on apples stored for approximately equal periods in CA when the CA period preceded or followed the regular storage period is given in Table II. It is indicated that a CA storage period of a given duration was equally effective in controlling spot regardless of whether it occurred in the initial or final portion of the season. There was considerable variation; in some cases CA storage at the beginning resulted in a smaller amount of spot, and in others CA storage towards the end of the storage period gave the smaller amount.

The results when CA or regular cold storage were employed only during the middle one-third of the total season are presented in Table III. Fruit initially placed in CA for 84 days, then in regular storage for 56 days followed by CA for 31 days developed spot during the final CA period. The apples placed initially in regular storage for 84 days developed spot during the subsequent 56 days in CA. When again placed in regular storage, spot development continued, but more rapidly than in the intermediate CA period. When the initial regular storage period was shortened to 56 days and the intermediate CA period extended to 84 days, Jonathan spot still developed in CA,

TABLE II

The Development of Spot on Jonathan Apples Stored for Approximately Equal Periods in CA and in Regular Cold Storage

1958

CA Storage Preceding Regular Cold Storage			Days in Cold Storage Preceding CA Storage		
Days in CA	Days in Regular	Jonathan Spot (percent)	Days in CA	Days in Regular	Jonathan Spot (percent)
134	64	7.2	137	61	2.7
104	94	10.2	94	104	21.3
61	137	37.3	64	134	40.4

1959

CA Storage Preceding Regular Cold Storage			Regular Cold Storage Preceding CA Storage		
Days in CA	Days in Regular	Jonathan Spot (percent)	Days in CA	Days in Regular	Jonathan Spot (perent)
140	31	4.6	143	28	4.9
112	59	12.9	115	56	12.8
84	87	17.4	87	84	13.0
56	115	15.9	59	112	27.0
28	143	36.0	31	140	30.9

TABLE III

The Development of Spot on Jonathan Apples Stored in CA between Periods of Regular Cold Storage or in Regular Air between CA Storage Periods

1959

CA Storage Preceding Regular Cold Storage			Fruit with Spot (percent)					
	Days in		Total Storage Duration (days)					
CA	Regular	CA	28	56	84	112	140	171
84	56	31	———	———	0	----------	0	——— 6.1

Regular Cold Storage Preceding CA Storage			Fruit with Spot (perent)					
	Days in		Total Storage Duration (days)					
Regular	CA	Regular	28	56	84	112	140	171
84	56	31	----------	----------	0	———	———	7.1 --16.7
56	84	31	-------	0	———	1.7	———	6.7 --23.8

———Indicates duration of CA storage period.
-------Indicates duration of regular cold storage period.

and the amount of spot increased very rapidly in the final 31 days in regular storage.

There was no evidence of "near spot", a blue or purple skin discoloration as described by Bünemann (1957), on the apples in 1959. In 1958 a high percentage of "near spot" developed in both CA and regular storage. The amount of fruit with "near spot" at the end of the storage season (Table IV) showed no relation to the duration the apples had been stored in CA regardless of whether the CA storage period occurred early or late in the season. An average of 14.8 percent of the apples developed "near spot" in the controlled atmosphere of 7 percent CO_2 - 14 percent O_2, and 18.2 percent showed this disorder upon removal from 11 percent CO_2 and 10 percent O_2.

Table V presents data on the incidence of Jonathan spot in relation to the presence of "near spot". The development of Jonathan spot was similar on fruit with and without "near spot" when the CA period took place at the beginning of the storage season. However, when the CA period followed regular cold storage, the rate of development was much higher on apples with "near spot".

The effect of holding the fruit after storage at temperatures above 32° F may be observed from the data given in Table VI. Apples stored in 7 percent CO_2 - 14 percent O_2 and 11 percent CO_2 - 10 percent O_2 developed Jonathan spot when subsequently held at 32° F in regular air for 23 days. At 46° F only apples of both atmospheres were free of spot when held at the higher temperatures

TABLE IV

The Development of "Near Spot" and Soft Scald on Apples Stored for Different Periods in CA and Regular Storage

1958

CA Storage Preceding Regular Cold Storage Days in		Fruit with "Near Spot" (percent, accumulative) Total Duration of Storage (days)					Fruit with Soft Scald (percent, accumulative) Total Duration of Storage (days)				
CA	Regular	61	104	134	161	198	61	104	134	161	198
61	137	— 0	9.9	18.9	44.4	50.3	— 0	0	0	0	0
104	94	———	9.5	14.6	34.9	51.7	———	0	0	0	0
134	64	———	———	29.8	42.3	51.7	———	———	0	0	0
161	37	———	———	———	50.8	55.3	———	———	———	0	0
198	0	———	———	———	———	24.1	———	———	———	———	0

Regular Cold Storage Preceding CA Storage Days in		Fruit with "Near Spot" (percent, accumulative) Total Duration of Storage (days)					Fruit with Soft Scald (percent, accumulative) Total Duration of Storage (days)				
CA	Regular	61	104	134	161	198	61	104	134	161	198
0	198	0	12.2	27.5	47.0	53.3	1.3	8.9	8.9	8.9	8.9
37	161				36.1	41.4				1.7	1.7
64	134			31.0	———	52.9			5.7	———	5.7
94	104		0	———	———	49.0		8.1	———	———	8.1
137	61	0	———	———	———	40.3	7.0	———	———	———	7.0

———Indicates length of CA storage period.

TABLE V

Development of Jonathan Spot on Apples with and without "Near Spot"

CA Storage Preceding Regular Cold Storage Days in		Spot on Fruit with "Near Spot" (Percent of apples) Total duration of storage (days)					Spot on Fruit without "Near Spot" (Percent of apples) Total duration of storage (days)				
CA	Regular	61	104	134	161	198	61	104	134	161	198
61	137	0	0	0	11.5	26.6	0	0	0	3.8	38.5
104	94		0	0	0	7.3		0	0	0	11.7
134	64			0	0	11.1			0	0	4.3
161	37				0	2.5				0	2.0
198	0					0					0

Regular Cold Storage Preceding CA Storage Days in		Spot on Fruit with "Near Spot" (Percent of apples) Total duration of storage (days)					Spot on Fruit without "Near Spot" (Percent of apples) Total duration of storage (days)				
CA	Regular	61	104	134	161	198	61	104	134	161	198
0	198	0	0	2.7	30.1	47.9	0	1.3	5.6	16.0	45.5
37	161				2.8	45.0				29.5	13.1
64	134			0		65.2			15.8		5.1
94	104		0			23.4		0			10.7
137	61	0				3.3	0				1.4

TABLE VI

Development of Jonathan Spot and "Near Spot" on Jonathan Apples Stored at Different Temperatures in Regular Cold Storage

	Subsequent Storage in Regular Storage (° F)	Jonathan Spotted Fruit (percent) Duration of Storage		"Near Spotted" Fruit (percent) Duration of Storage	
		0 Days	23 Days	0 Days	23 Days
Apples out of CA storage atmosphere: 7% CO_2 - 14% O_2	32	0	4.9	13.4	37.4
	46	0	2.6	19.0	38.6
	60	0	0	16.3	20.6
	74	0	0	12.5	50.2
Apples out of CA storage atmosphere: 11% CO_2 - 10% O_2	32	0	1.3	19.0	38.7
	46	0	0	20.9	38.6
	60	0	0	15.8	22.9
	74	0	0	18.6	43.4

of 60° and 75° F for 23 days. The temperature least favorable for "near spot" development was 60° F. No appreciable difference in amount of "near spot" development at the several post-storage holding temperatures existed between the two ventilated controlled atmospheres.

Soft scald did not develop on apples which had been held for 28 days or longer in CA storage immediately upon harvest. The development of soft scald which occurred on apples in regular cold storage was arrested upon transfer of the apples into CA storage (see Table IV, page 26).

Total Acid, pH and Jonathan Spot

The total acid in the skin of the apples for all samples was less than that of the flesh at harvest and remained at a lower level throughout the entire storage period (Tables VIIA and VIIB). This was also repeated in the pH readings, yet no correlation existed between percent toal acid and pH. The loss of acid in the skin of the apple was more rapid than the loss in the flesh, regardless of whether the apples were in CA or in regular cold storage. At the end of the storage period, the skin showed a greater total loss of acid than the flesh. Apples placed in CA directly after harvest had a higher total acidity of skin and flesh at the end of the storage than non-CA apples. It was found that the loss of total acid and the amount of Jonathan spot decreased when the length of time the apples were held in CA increased. These associations did not exist for apples which were placed in regular storage at the beginning of the cold storage period.

TABLE VIIA

Development of Jonathan Spot and pH of Jonathan Apples Stored for Different Periods in CA and Regular Cold Storage

CA Storage Preceding Regular Cold Storage		Fruit with Spot (percent accumulative)							pH Flesh							pH Skin						
Days in		Total Duration of Storage (days)							Total Duration of Storage (days)							Total Duration of Storage (days)						
CA	Regular	0	28	56	84	112	140	171	0	28	56	84	112	140	171	0	28	56	84	112	140	171
0	0	0							3.35							3.5						
28	143	——	0	0	0	-	21.3	36.0	——	3.4					3.6	——	3.55					3.95
56	115	——	——	0	0	-	7.1	15.9	——	——	3.45				3.6	——	——	3.65				3.85
84	87	——	——	——	0	-	7.3	17.4	——	——	——	3.5			3.6	——	——	——	3.7			3.8
112	59	——	——	——	——	0	1.5	12.9	——	——	——	——	-	3.5	3.55	——	——	——	——	-	3.8	3.9
140	31	——	——	——	——	——	0	4.6	——	——	——	——	——	3.45	3.65	——	——	——	——	——	3.65	3.9
171	0	——	——	——	——	——	——	0	——	——	——	——	——	——	3.55	——	——	——	——	——	——	3.6
CA	Regular CA																					
84	56 31	——	——	——	0	---	0	6.1	——	——	——	3.5	---	3.45	3.5	——	——	——	3.7	---	3.65	3.75
Regular Cold Storage Preceding CA Storage																						
Days in																						
CA	Regular																					
0	171	0	0	0	1.7	-	17.5	33.3	3.35					3.6	3.65	3.5					3.7	4.05
31	140						13.3	30.9						3.5	3.6						3.6	4.0
59	112				9.8	——	27.0						3.5	——	3.6					3.7	——	3.95
87	84				.7	——	——	13.0				3.55	——	——	3.65				3.8	——	——	3.9
115	56			0	——	——	——	12.8			3.45	——	——	——	3.5			3.65	——	——	——	4.0
143	28		0	——	——	——	——	4.9		3.4	——	——	——	——	3.55		3.6	——	——	——	——	3.95
Regular	CA Regular																					
84	56 31	----------			0	——	7.1	16.7	----------			3.5	——	3.55	3.6	----------			3.7	——	3.7	3.9
56	84 31	----------		0	——	1.7	6.7	23.8	------	3.4	——	——	——	3.6	3.6	--------	3.7	——	——	——	3.7	3.9

——————Indicates duration of CA storage period

----------Indicates duration of Regular cold storage period

TABLE VII B

Development of Jonathan Spot and Percent Total Acidity of Jonathan Apples Stored for Different Periods in CA and Regular Cold Storage

CA Storage Preceding Regular Cold Storage		Fruit with Spot (percent accumulative)							% Total Acid Flesh							% Total Acid Flesh						
Days in		Total Duration of Storage (days)							Total Duration of Storage (days)							Total Duration of Storage (days)						
CA	Regular	0	28	56	84	112	140	171	0	28	56	84	112	140	171	0	28	56	84	112	140	171
0	0	0							.78							.76						
28	143	—0		0	0	-	21.3	36.0	—.77						.58	—.74						.42
56	115	——		0	0	-	7.1	15.9	——		.70				.59	——		.67				.49
84	87	——		——	0	-	7.3	17.4	——		——	.65			.70	——		——	.61			.58
112	59	——		——	——	0	1.5	12.9	——		——	——	-	.65	.60	——		——	——	-	.61	.48
140	31	——		——	——	——	0	4.6	——		——	——	——	.70	.64	——		——	——	——	.59	.58
171	0	——		——	——	——	——	0	——		——	——	——	——	.70	——		——	——	——	——	.60
CA	Regular CA																					
84	56 31	——		——	0	-------	0—	6.1	——		——	.69	-----	.75—	.73	——		——	.56	-----	.67—	.62
Regular Cold Storage Preceding CA Storage																						
Days in																						
CA	Regular																					
0	171	0	0	0	1.7	-	17.5	33.3	.78					.66	.61	.76					.53	.45
31	140						13.3	—30.9						.63—	.63						.61—	.47
59	112				9.8	——	27.0						.74	——	.64					.69	——	.51
87	84				.7	——	—13.0					.56	——	——	.69				.49	——	——	.56
115	56			0	——	——	12.8				.72	——	——	——	.61			.56	——	——	——	.42
143	28		0	——	——	——	4.9			.77	——	——	——	——	.61		.71	——	——	——	——	.50
Regular	CA Regular																					
84	56 31	----------		0	——	7.1	--16.7		----------			.68—	.65	------	.69	----------			.61	——	.56	--.52
56	84 31	----------	0	——	1.7	—6.7	--23.8		---------		.63	——	.64	------	.64	---------		.62	——	——	.53	--.48

——— Indicates duration of CA storage period.

-------- Indicates duration of regular cold storage period.

The pH increase for apples in CA and regular storage as well as the decrease in acidity at the end of the storage were more marked in the skin than in the flesh. The ultimate loss of acid and increase of pH were greater in the skin than in the flesh whenever the regular cold storage period occurred between CA storage periods or the CA period occurred between regular cold periods. The apples which received a CA period between regular storage periods contained less total acid and had a higher pH at the end of the storage period than the apples which had a regular storage period between CA periods.

A linear correlation ($r = -.673^{**}$) was found for the percent total acid of the skin and the percent of fruit with Jonathan spot (see Figure 4). A relation existed between the total acid of the flesh and Jonathan spot development. This correlation coefficient ($r = -.42^{*}$), significant at the 5 percent level, is also shown in Figure 4.

The data of Experiment 2 showed no relationship of either total acid or pH of the skin and flesh to first appearance of Jonathan spot. The spot developed on apples stored at 32° F, 45° F, and on the delayed picked apples. The apples at the higher temperatures of 60° F, 75° F and 90° F did not develop any spot during their storage life. These apples shrivelled rapidly and were over mature in a short time. The total acidity of the apples at the higher temperatures decreased below those of apples stored at lower temperatures which did not develop spot. Except for fruit from the delayed pickings, the

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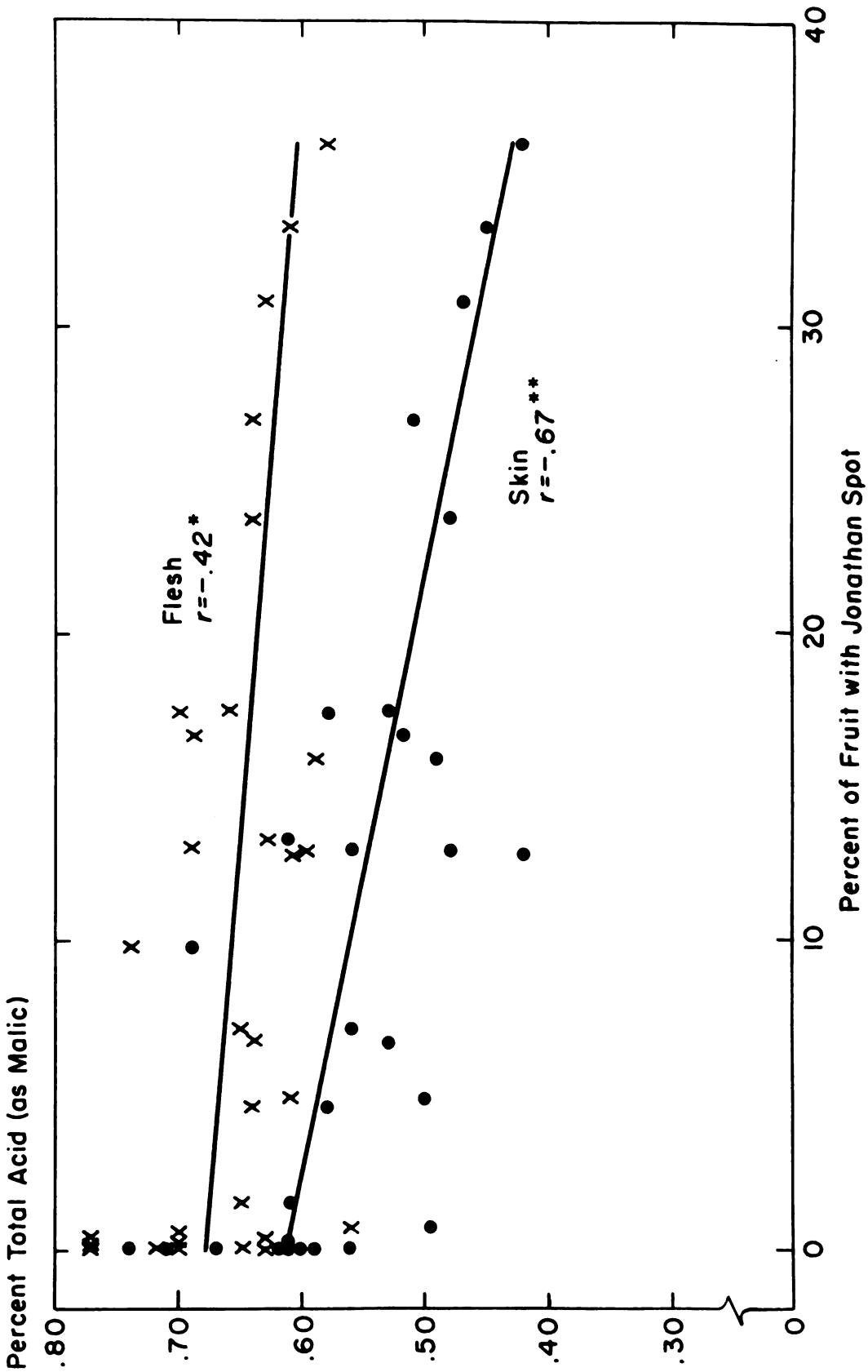
** Indicates significance at 1 percent level.

Figure 4

The linear correlation of Jonathan spot to the total acid of the skin and the flesh of Jonathan apples (skin: r = -.673**; y = .611 - .005x; s.e. of est: .062; flesh: r = -.42*; y = .679 - .020x; s.e. of est: .049).

x = percent of fruit with Jonathan spot

y = percent total acidity


Percent Total Acid (as Malic)
.80
.70
.60
.50
.40
.30
0
10
20
30
40
Percent of Fruit with Jonathan Spot
Flesh
r=-.42*
Skin
r=-.67**

apple skin had a lower total acidity and a higher pH than the flesh during the entire storage period; the reverse was true for the apples from the delayed pickings (see Table VIII).

In the studies of the relation of lenticels to the development of Jonathan spot, it was not possible to select skin areas on fruit from the several treatments which contained the same number of lenticels. Analysis of variance (Table IX) showed significant differences in the quantity of lenticels. Consequently, the data were tested by covariance analysis, the results of which are given in Table X.

There was no significant difference in the number of open lenticels for apples with Jonathan spot and apples free of Jonathan spot from regular cold storage. A highly significant difference was found for the amount of open lenticels between apples from CA and apples from regular storage. The former had twice as many open lenticels as the latter.

Table XI summarizes the results of the mineral element analyzer of the skin tissue of Jonathan apples. The results, based on dry weight, show a striking accumulation of the elements K, P, Ca, Mg, Mn, B and Mo in the spotted skin tissue over that in the in-between non-spotted tissue. The spotted tissue also contained a higher amount of N, Fe and Cu. The data for the skin of the apple halves with spots in relation to the corresponding non-spotted halves showed, except for the element P, an accumulation of the same

TABLE VIII

The pH and Total Acidity of Flesh and Skin Tissues of Apples at Time when at Least 10 Percent of the Fruit had Jonathan Spot

Treatment	Date	1959 % Total Acid Flesh	% Total Acid Skin	pH Flesh	pH Skin
Harvest	9-25-59	.78	.76	3.35	3.5
32° F	9-25-59	.78	.76	3.35	3.5
45° F	9-25-59	.78	.76	3.35	3.5
Delayed Pick 1	10-5-59	.71	.77	3.35	3.45
Delayed Pick 2	10-16-59	.70	.80	3.5	3.6
60° F	9-25-59	.78	.76	3.35	3.5
75° F	9-25-59	.78	.76	3.35	3.5
Delayed Pick 1	10-5-59	.71	.77	3.35	3.45
Delayed Pick 2	10-16-59	.70	.80	3.5	3.6
Delayed Pick 3	10-27-59	.63	.75	3.6	3.6
90° F	9-25-59	.78	.76	3.35	3.5

Treatment	Date	% Jonathan Spotted Fruit	% Total Acid Flesh	% Total Acid Skin	pH Flesh	pH Skin
32° F	3-19-60	11.0	.51	.41	3.65	3.95
45° F	3-19-60	26.4	.49	.38	3.65	3.85
Delayed Pick 1	12-15-59	12.2	.58	.55	3.65	3.85
Delayed Pick 2	12-15-59	41.8	.57	.42	3.8	4.0
60° F	12-15-59	0	.41	.39	3.8	4.0
75° F	12-15-59	0	.34	.32	4.1	4.2
Delayed Pick 1	12-30-59	31.0	.28	.30	4.3	4.5
Delayed Pick 2	12-28-59	41.5	.30	.26	4.3	4.4
Delayed Pick 3	12-30-59	26.5	.31	.29	4.2	4.45
90° F	10-22-59	0	.23	.18	4.2	4.5

TABLE IX

Analysis of Variance of the Total Number of Lenticels in the Counting Areas of the Skin of Jonathan Apples

Treatment	"Mean" Number of Lenticels in the Counting Area Chosen
Regular cold storage:	
With Jonathan spot - - - - - - - - - - - -	18.0
Without Jonathan spot - - - - - - - - - -	18.9
Controlled atmosphere storage - - - - - - - - (Without Jonathan spot)	25.2

L. S. D. 1% = 4.4
5% = 3.3

TABLE X

Analysis of Covariance of the Number of Open Lenticels in the Counting Areas of the Skin of Jonathan Apples

Treatment	"Mean" Number of Open Lenticels in the Counting Areas Chosen
Regular cold storage:	
With Jonathan spot - - - - - - - - - - - -	6.1
Without Jonathan spot - - - - - - - - - - -	6.0
Controlled atmosphere storage - - - - - - - - - (Without Jonathan spot)	14.1

L. S. D. 1% = 1.6
5% = 1.2

TABLE XI

Mineral Element Content of the Skin Tissues of Jonathan Apples

Tissue		Percent of Dry Weight					Ppm						
		N	K	P	Ca	Mg	Mn	Fe	Cu	B	Zn	Mo	Al
Jonathan spots	I	.53	1.12	.105	.175	.280	10	39	13.3	53.0	25	1.00	50
	II	.60	1.16	.124	.240	.440	20	132	11.0	63.8	12	2.15	86
Between Jonathan spots	I	.48	.68	.060	.080	.100	4	36	10.2	39.2	24	.30	57
	II	.56	.78	.076	.145	.155	14	125	6.4	44.7	8	1.70	78
Intact peel containing Jonathan spot (half apple)	I	.51	.86	.080	.130	.180	5	40	7.6	46.2	12	.75	57
	II	.50	1.08	.105	.160	.210	16	53	6.8	48.2	9	1.35	58
Intact peel free of Jonathan spot (half apple)	I	.42	.82	.073	.090	.110	2	34	7.2	38.0	12	.20	48
	II	.48	.70	.063	.085	.120	12	73	5.0	37.4	7	.65	92
Peel free of Jonathan spot (whole apple)	I	.33	.78	.060	.120	.100	4	47	6.6	33.0	12	.50	38
	II	.39	.78	.069	.175	.130	16	75	5.0	41.5	8	1.50	49
Peel of CA apples free of spot (whole apple)	I	-	-	-	-	-	-	-	-	-	-	-	-
	II	.48	.84	.069	.085	.130	12	35	8.0	36.8	9	.65	58
Entire peel of apples stored for 3 months in CA preceding regular cold storage, with Jonathan spot	I	-	-	-	-	-	-	-	-	-	-	-	-
	II	.38	.90	.076	.175	.180	16	124	8.4	50.3	13	1.35	40

elements as mentioned above for the spotted tissue. The spotted portions were also higher in N and Cu. The skin of spotted apples which had been held in CA storage for 84 days followed by regular cold storage was lower in N than comparable non-spotted skin, but showed an accumulation of the elements K, P, Ca, Mg, Mn, B and Mo, and a higher content of Fe, Al, P, Cu and Zn.

DISCUSSION

Jonathan apples, unless stored previously in regular air, did not develop spot while in controlled atmosphere. Fruit receiving CA storage in addition to varying periods of regular storage developed Jonathan spot, even in controlled atmosphere. However, the development of spot in controlled atmosphere was less rapid than in regular air storage. Apparently controlled atmosphere inhibits the changes necessary for spot, but will not prevent the formation of spots once these changes have been initiated. Furthermore, since CA apples upon placement in regular air eventually developed spot, there seemed to be no permanent residual effect of the atmospheres.

It is possible that cellular processes which lead to the development of Jonathan spot occurred in both controlled atmosphere and regular cold storage, but progressed at a much slower rate in controlled atmosphere. This is supported in part in that controlled atmosphere storage did not completely arrest development of Jonathan spot on fruit previously conditioned by regular cold storage. It is known that Jonathan spot is associated with senescence of the apples in that the apples must "age" somewhat before Jonathan spot will develop. The beneficial influence of controlled atmosphere storage consists of extending the storage life of the apples by retarding the life processes, such as respiration, breakdown of sugars and acids, change of pectins to soluble pectins, changes in fats, oils and waxes. In controlled

atmosphere storage the apple "ages" more slowly than in regular cold storage. The data presented here indicate that controlled atmosphere storage does not actually prevent the development of Jonathan spot, but rather retards the processes which lead to its development. In controlled atmosphere storage periods of normal duration, the apples apparently do not reach a stage of senescence which seems to be necessary for Jonathan spot development. Fruit stored for long periods in controlled atmosphere developed spots shortly after removal from controlled atmosphere.

Pentzer (1925) found that the tissues under the spotted skin contained less acid than the surrounding tissue and assumed that this unbalanced condition caused the forming of Jonathan spot. Several investigators (Plagge et al., 1924, 1930) have reported an association of total acidity with the amount of developed Jonathan spot. A high correlation of the skin acidity and a less significant correlation of the flesh acidity existed with the percentage of spotted fruit was found in this study. Smock and Neubert (1950) point out that this does not necessarily mean that Jonathan spot is caused by a decrease in acidity, but that the Jonathan spot may actually cause a decrease in acidity. The author agrees with the opinion of Smock and Neubert for several reasons. The total titratable acidity of the skin of the apples used in this research was always lower than that of flesh, regardless of whether the skin was spotted or not. The total acidity of the skin and the flesh of CA apples decreased

with time in storage. The skin and flesh of CA apples were higher in acids than that of the same tissue of apples held in regular cold storage for comparable periods. No Jonathan spot developed on CA stored apples even in those cases when the total acidity decreased to those levels at which Jonathan spot occurred on apples in regular cold storage. It may be postulated that, if acidity was the cause of Jonathan spot, the spotting should occur when the total acidity had decreased to a certain level at which the pigments turn color, regardless of the circumstances or means by which the acidity level was obtained. The apples which were stored in regular air at different temperatures developed Jonathan spot at different acidity levels.

It was very remarkable that in this experiment Jonathan spot did not develop on fruit held at the higher temperatures of 60° F, 75° F and 90° F. These apples ripened and shriveled rapidly. The total acidity of these apples decreased to the levels or to a lower level than those of the apples at lower temperatures which developed spot.

These results suggest that the correlation of total acidity of the apples with the amount of spotted fruit is due to the relationship that exists between age of fruit in storage and either total acidity or incidence of Jonathan spot, and not to a causal relationship of total acidity and Jonathan spot development.

The apple tissue located under the spots probably desiccates more rapidly than the surrounding tissue because of the deterioration of the cells affected by spot. A high transpiration rate of the spotted skin tissue could conceivably

result in an accumulation of the mobile elements in this tissue. The analysis of the different tissues showed that not only the mobile but also the immobile elements had accumulated in the spotted tissue. Apparently the spotted tissue had a higher transpiration rate and a higher respiration rate than the adjacent tissue at some stage of development of the disorder in order to accumulate these minerals against a concentration gradient.

Since no Jonathan spot develops on fruit placed in controlled atmosphere storage directly after harvest, it is possible that either the high carbon dioxide content or the low oxygen content of the internal atmosphere of the apple exerts an influence on the processes which cause the conditions resulting in spotting of the hypodermal tissue. Trout et al. (1942, 1953) found that during regular cold storage there is a marked decrease of the oxygen level in the internal atmosphere of the apples. After five months of storage, the oxygen level in Granny Smith apples had decreased to 6 to 8 percent. At the same time the carbon dioxide content increased from 1 percent to 4 or 5 percent. This indicates a considerable resistance to the passage of oxygen and carbon dioxide between the fruit and the surrounding atmosphere. Hall et al. (1955) observed that the atmosphere does not vary within an apple; therefore, the major part of the resistance to gas exchange is located in the cuticle and the small tier of packed cells underneath which compose the skin. The solubility of carbon dioxide in water and in oil is about twenty times greater than that of oxygen. They suggest that the marked increase of the resistance to oxygen, as a

function of age, is largely due to the considerable increase of the oil content of the cuticle. Because of the higher solubility of carbon dioxide in oil relative to oxygen, the modifications of the cuticle do not cause such a high increase in the resistance to passage of carbon dioxide. Hulme (1951) reported that the composition of the internal atmosphere of normal fruit is variable; also, Hall, Huelin et al. (1955) found a variability among apples of 16 percent for carbon dioxide and 20 percent for oxygen.

The lenticels, according to Marcellin (1955), play an important role in the respiration and transpiration processes since these control the entrance of oxygen into the fruit. Ulrich (1951), comparing the number of open lenticels of the William variety of pear with internal carbon dioxide content, found that the number of open lenticels varies in the same sense as the concentration of carbon dioxide gas in the intercellular spaces. Apples artificially coated with wax, oil, or analogous material and stored in regular air have internal atmospheres comparable to the atmospheres in controlled atmosphere storages (Trout, Hall and Sykes, 1953). The same results were found when only the calyx and the lenticels were covered. In controlled atmosphere storage the increased carbon dioxide concentration and reduced oxygen level retard the life processes of the apple. The constituents of the cuticle are the results of living processes, and it logically follows that their deposition occurs at a lower rate in controlled atmosphere storage.

Comparing the number of open lenticels for apples in regular cold storage and controlled atmosphere storage, the writer found that the apples in controlled atmosphere possessed a higher amount of open lenticels than apples in regular cold storage regardless of whether this fruit was spotted or free of spot. No difference in the amount of open lenticels existed between spotted apples and apples free of spot from regular cold storage.

In controlled atmosphere storage a conspicuous climacteric respiration maximum is not formed, in contrast with regular cold storage where a climacteric can be recognized by a maximum of carbon dioxide output (Behmer, 1958). It is possible that the increase in resistance to gas exchange with time is neglibible for controlled atmosphere apples and that the composition of the internal atmosphere approaches that of the external atmosphere.

Combining the above mentioned points and the results of this study, the author believes that in both controlled atmosphere storage and in coated fruit, the life processes are retarded rapidly. The apples continue to ripen normally, but at a low rate due to a favorably balanced internal atmosphere, consisting of a raised level of carbon dioxide and a reduced level of oxygen. Apples stored in regular cold storage may vary in the rate of change and composition of the internal atmosphere because of differences in the composition of the cuticle and the amount of plugged lenticels. This may cause an unequal retardation of the life processes in some apples which might lead to

toxic effects on certain metabolic processes resulting in the development of Jonathan spot. It is possible that low oxygen levels or high carbon dioxide levels, which might be produced in some apples, cause certain enzyme systems to function inadequately. It has been suggested by Hulme (1956) that high carbon dioxide levels in fruit stored under high concentration of carbon dioxide in the surrounding atmosphere results in accumulation of succinic acid which leads to carbon dioxide injury. Furthermore, Miller and Evans (1956) found carbon dioxide completely inhibited cytochrome oxidase of bean seedlings. Since sytochrome oxidase is an important enzyme in the respiration of apple tissue (Lieberman, 1958), carbon dioxide levels may exert a similar effect in this tissue. Perhaps the internal concentration of carbon dioxide relating to that of oxygen is the important consideration. The higher mineral content and the lower acidity of spotted tissue compared to non-spotted tissue of the apple may be indicative of a higher respiration rate during some phase of development of the disorder. The altered respiration resulting from unbalanced conditions in the fruit may lead to the development of Jonathan spot.

SUMMARY

Jonathan spot, a physiological disorder of apples, was studied in relation to some of the factors affecting its development and control. The experiments were made with Jonathan apples stored in regular air and controlled atmosphere (CA) during the 1958-59 and 1959060 cold storage seasons.

The storage of fruit in CA for variable periods of time, together with the transfer of fruit between CA and regular storage conditions, revealed that controlled atmosphere inhibited spot development only during the period in which the fruit was in the controlled atmospheres. Spot did not develop on apples in CA when the apples were stored immediately upon harvest under these conditions. Jonathan spot initiated during regular storage continued to develop when the fruit was subsequently held in CA. Fruit removed from CA storage developed spot subsequently when held in regular air at 32° F. The total amount of fruit with spot at the end of the season was directly proportional to the duration of the regular storage period, and inversely proportional to the duration of the CA storage period.

Soft scald, another storage disorder of Jonathan apples, was prevented by CA storage. Soft scald initiated in regular storage was arrested when the apples were transferred to CA.

The characteristic loss of acids in apples during storage was greater in regular air than in controlled atmospheres. Acid losses were greater in

the skin tissues than in the flesh tissues during storage. This was reflected by both total acidity and pH measurements. There was a highly significant negative correlation of percentage total acid of the skin and the amount of fruit that developed Jonathan spot during storage; whereas the correlation of flesh acidity and spot was significant only at the 5% level. Acidity levels similar and lower than those associated with spot appearance in storage, when brought about by delayed harvest or by holding the fruit at high temperatures did not always cause the disorder to appear, however.

Jonathan spots that developed in regular storage were not related to their proximity to open or closed lenticels in the fruit skin. CA storage apples had a larger proportion of open to closed lenticels than regular storage apples.

Fruit tissues afflicted with spot had higher percentages of K, P, Ca, Mg, Mn, B and Mo than adjacent and comparable tissues free of the disorder.

It was evident in these studies that Jonathan spot development is affected by a complex of factors, associated with aging and the approach of senescence in the fruit.

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