STUDIES IN STORAGE AND TRANSPORTATIONAL DISEASES OF FRUITS AND VEGETABLES DUE TO INCOMPLETE OXIDATION

Thesis for Degree of M. S.

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1926

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THESIS

Submitted in partial fulfillment of the
requirements for the degree of Master
of Science at the Michigan State
College of Agriculture and
Applied Science

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1926

General Considerations

The demand for fresh fruits and vegetables in the great central areas of consumption during the off season for production in these sections has kept apace with the marked increase in the growth of the vegetable and fruit industries of the semi-tropical regions. Fresh fruits and vegetables are now found on the city markets at all seasons of the year and, in addition, home-grown staple fruits and vegetables are available to the consumer over an extended market season. The production of fresh fruits and vegetables in such increased quantities in areas so distant from the central markets, and the extension of the market season of home-grown produce, has largely been brought about through the development and utilization of efficient refrigerator transport service and terminal storage facilities. Refrigerator transport and cold storage are indispensable aids to the fruit and vegetable industries and without these facilities the production and marketing of invaluable food supplies would be sharply limited. Partial failure in production, can, in a measure, be compensated for by more efficient handling and distribution, but deterioration of fruits and vegetables in storage and transportation results in much litigation for the transportation and storage companies, losses to the producer, and high prices to the consumer.

Modern intensive methods of production, marketing and storage of perishable fruits and vegetables have engendered new problems for solution. Perishable crops from the field to the consumer are subjected to destructive forces, the most important of which are plant diseases. The most important of the field diseases have been studied and control measures worked out. Most of the parasitic troubles that cause serious losses in transit and storage are well known. There are, however, some non-parasitic troubles that result from unfavorable storage or transportation conditions and some of these diseases are potentially equal in destructiveness to the most serious parasitic troubles. Notable progress has been made in the conquest of some of the most important diseases of this type. Further advancement of our knowledge and the working out of efficient control methods may safely be predicted as the result of future investigation.

This is the first of two papers dealing with the breakdown of certain fruits and vegetables under the conditions of storage and transportation. The investigation of these diseases was begun in 1919 in an attempt to find the cause of a leaf disease of cabbage prevalent in storage and in transcontinental shipments. Eventually it was found that other vegetables and also some fruits were affected by the same, or closely related troubles. Various citrus fruits were found to be severely affected. Progress made in the investigation of the vegetable diseases is reported in this paper.

Black Leaf Speck of Crucifers

Introduction

A disease of cabbage characterized by the occurrence of innumerable, small, sunken, black specks on all parts of the leaves has been observed in Michigan for several years. It was found frequently in late winter on cabbage from storage houses or pits out of doors. In some cases only the outer leaves were affected, while in others the spotting extended into, and often included, the leaves of the bud. The occasional occurrence of the specks on the youngest bud leaves practically eliminated the possibility of the disease being of parasitic origin. Microscopical examinations of sections and negative results from numerous attempts to isolate an organism from the lesions by the usual cultural methods early confirmed the belief that the disease was not caused by fungi or bacteria.

Observations made of shipments of cabbage arriving in Lansing from California in the spring of 1919 led to the discovery that a similar trouble was causing serious losses because of the close trimming of the heads necessary to make them salable. The disease in these shipments was not at first recognized as identical with that found on Michigan cabbage from storage because of the larger average size of the spots. The spotting of the leaves of the California cabbage was first thought to be due to the ring spot fungus (<u>Mycosphaerella</u> <u>brass-</u>

icicola). Collections of the material were brought to the laboratory and attempts made to isolate the causal organism, but all efforts to demonstrate the association of a fungus with the disease were negative. Subsequent experimental work and further comparisons of the configuration of the lesions established the identity of the disease on California and Michigan cabbage. Shipments from Florida and Texas were later observed commonly to be affected with the same trouble.

While inspecting car lot shipments of fruits and vegetables from all parts of the country in the railroad yards of Detroit in 1919 several cars of cauliflower from California showing severe spotting of the leaves were examined. Photographs were made and collections brought back to the laboratory where it was found that the great majority of the spots were sterile and could easily be distinguished from a few larger lesions caused by the ring spot fungus. Attempts by two separate workers to isolate an organism from the small specks were mutually unsuccessful. In appearance the spots on cauliflower and broccolli were practically identical with those found on cabbage and left no question as to their identity.

In the fall of 1921 a planting of chinese cabbage, (*Brassica pekinensis*), in the college horticultural gardens was found to be badly spotted with the typical black specks characteristic of the cabbage and cauliflower trouble. Practically all of the heads in the planting developed innumerable black specks on all parts of the leaves following an early severe frost and a period of sixteen to eighteen hours when the temperature was near, or below freezing. Microscopical examination of sections and tissue plantings on corn meal agar failed to reveal the presence of bacteria or fungi. No other observations have been made of the disease on this crop.

Brussells sprouts badly diseased with black leaf speck were collected on Cleveland markets in 1923. The specks occurred on all the leaves of the heads, and secondary rots were setting in.

Previous Observations

Only a few records are available of the occurrence of this disease and all previous observations have been made on cabbage. It has probably been confused with other leaf spot diseases for the spotting of the leaves is disfiguring enough to be conspicuous and so destructive that badly affected heads could not escape observation. It is possible that Osmun and Anderson (15) partially confused this disease with ring spot, (Mycosphaerella brassicicola) which they described as causing considerable loss in March and April shipments of cauliflower from California. The two diseases are not infrequently found on the same head and the lesions on the leaves might easily be mistaken for one and the same disease. Observations made in April 1922 of

California cauliflower on the Detroit market showed both diseases occurring on the same heads. Stewart (21) first reported the disease as generally prevalent on cabbage in the storage houses of New York and states that he has observed it since 1910. He also noted the disease in the field in the fall of 1910. Link and Gardner (12) record the presence of the disease on market cabbage from all regions. Link (11) found the variety Winningstadt affected in the field in California.

Name of the Disease

Stewart (21) first used the name black leaf speck in describing the disease on cabbage. Because of priority, and since this name also very well describes the usual appearance of the lesions observed on the leaves of other crucifers, it is proposed that it be used in designating the disease on all the crucifers affected with this trouble. Link and Gardner (12) used the name leaf speck to describe the disease as they found it on market cabbage. However, for the additional descriptive value it seems advisable to use the longer name. No other descriptions have been found in the literature.

Importance

Black leaf speck has been found in many shipments of cabbage and cauliflower which have been en route for a week or longer. The disease may or may not be visible upon first inspection of the car. This is due to the fact that the spots on the leaves do not always develop until the heads are removed from the car and held in the warehouse for several days. In other cases the disease is first noticed on local lots of cabbage and cauliflower that are sent out to retailers. For these reasons it is difficult to obtain first hand data on the losses caused by this trouble to these crucifers in transit.

On the other hand entire car loads of these vegetables may be seferely affected with black leaf speck fully developed at the time of the initial examination of the car. In the spring of 1919 it was almost impossible to find on the market either cabbage or cauliflower from the West entirely free from the disease. The first lot of California cabbage arriving in Lansing required the trimming away of more than half of the leaves to make the heads salable (Plate 1, Figs.1 and 2). Cauliflower was even more severely blemished. On March 29th, 1919, a car of broccoli from Oregon was inspected in the railroad yards of Detroit. The crates were loaded seven tiers high and no spaces were left between the tiers or at the doorway for ventilation. Black leaf speck seriously disfigured every leaf in all the crates it was possible to examine. Plate 2, Fig. 1 is from a photograph of one of these crates made at the time of inspection. On the same day crated cabbage was found generally affected with the disease in several of the wholesale houses. In 1920 the disease was common both on cabbage and cauliflower and has been observed each year since that time. In 1922 the disease was reported as especially severe on California cauliflower by railroad perishable freight inspectors and specimen heads were sent to the laboratory for confirmation of the diagnosis. The disease is not confined to western shipments but occurs as well on cruciferous crops from other sections.

In storage houses black leaf speck sometimes greatly reduces the value of the crop and makes a large portion of it unsalable. The disease appears at the time when the market value of the crop is usually at the peak and for this reason storage losses may be unusually severe. Regardless of the perfect condition of the crop at time of harvest the disease may entirely ruin the highest grade heads. Wastage in the storage houses of New York state is exemplified by the following report by Stewart (21),-"In April 1911, a cabbage grower of North Tonawanda sent to the station some specimens of diseased cabbage leaves accompanied by the following letter: ""I send you some cabbage leaves of the Danish Baldhead variety, and should like to have you tell me what it is that affects them. This is only an ordinary sample, some being much worse. There was quite a large proportion of the crop affected. It was unsalable. It does not show until January when the cabbage gets white. We store in the barn where the temperature is kept just above the freezing point. Wè first noticed a year ago on the crop of 1909."" The specimens sent were white leaves from the interior of a cabbage head." In the same article Stewart also reports the prevalence of the disease in Long Island storage houses in February 1915.

While no observations have been made of the disease in Michigan or Wisconsin storage houses it seems likely that it is just as serious here as in New York, since cabbage in the retail stores is commonly affected with the disease after January.

Local growers largely store their crop in pits out of doors. The disease is often especially severe on cabbage handled in this way. In Plate 3, Fig. 1 is shown a head of cabbage and a smaller head developed from an axillary bud badly blemished by black leaf speck. This cabbage was stored in a pit in the fall of 1919 and removed in early April 1920. Every head in this entire lot was badly diseased and worthless. The disease does not always affect all of the heads in this way although this condition is not uncommon. Heads so affected are not usable, having an unpalatable flavor when cooked. Secondary rots, mainly caused by Sclerotinia sp., occur generally on such heads. When only the outer leaves are affected the removal of these produces a salable head, although not always of good quality.

In storage cellars the disease affects winter cabbage in much the same way as in the pits out of doors.

It is, however, usually less serious. If the heads are piled up in large heaps, as is the usual way of storing where any considerable quantity is grown, the blemishing of the leaves reaches a maximum. One lot of cabbage taken from a large cellar storage in the winter of 1921 was observed to have fully fifty per-cent of the heads affected seriously enough to require extensive trimming to make them marketable.

As a field disease black leaf speck is probably of minor importance. Stewart (21) found it on cabbage in the field at Canandaigua, New York, in the fall of 1910 and reports that the inner, as well as the outer leaves, were affected. Link (11) reports the variety Winningstadt affected in the field in California. A planting of Chinese cabbage in the horticultural gardens of the Michigan State College was badly spotted with the disease in the fall of 1921. The disease developed after an unusually early and severe frost. Fully fifty percent of the heads in this planting were ruined by the effect of the spots on the leaves. Cauliflower grown on low land was affected with this trouble in the summer of 1924. Several observations have been made locally of the disease on cabbage in the fall. Early and severe frosts following favorable growing conditions appear to be correlated with its appearance at this time.

Description of the Disease

The chief characteristic of the black leaf speck disease on crucifers is the occurrence on all parts of the leaves of innumerable, small, depressed, lead-gray to black specks. In severe cases it is the usual thing for all of the leaves, including the small leaves of the bud, to show a general distribution of the black specks in the laminae and on the veins and midribs. In light cases of the disease only the outer layer of leaves are affected. The spots vary considerably in size, averaging less than 0.5 mm. in diameter. They occur singly or in clusters of myriad tiny dots scattered over the entire leaf surface. The most common picture presented by the disease on cabbage or cauliflower, in storage or transit, is the conspicuous disfigurement of the heads by the general occurrence of the black specks on the outer layers of leaves.

Observations of a considerable number of shipments of cabbage and cauliflower seem to indicate that the average size of the specks is larger when occurring on cabbage in transit, and that the lesions are smaller and more numberous on heads from commercial storage. For this reason the disease may be more conspicuous in its effect upon the heads than when observed in storage. Plate 3, Fig. 8 shows the appearance of a single leaf collected from a shipment of California cabbage in March 1919. This lot of cabbage was almost uniformly affected in this way, the outer leaves showing the large coalescing spots, while the inner leaves were covered with the smaller and less conspicuous lesions. As is shown in the photograph the large spots may result from the coalescence of several or many small lesions, or they may simply be due to the simultaneous death of a large number of neighboring cells.

The shape and contour of the spots also varies greatly. They may be circular, elliptical, oblong or distinctly angular. The majority, however, are spherical or oblong, with irregular margins as seen under low magnification with a hand lens. The spots may be considerably elongated, with the major axis either parallel or perpendicular to the veins of the leaf. All kinds of spots may occur on any portion of the leaf and the type of formation apparently bears no relation to their placement. Invariably the spots, large or small, are sunken below the level of the surrounding healthy tissue. They may be only slightly depressed, but the larger ones form crater-like areas in the tissues affected. The sunken character of the very small spots composing the colonies usually escapes notice unless magnified with a hand lens.

The leaves of cabbage taken in late winter from storage houses, cellars, or pits, are often entirely covered with the black specks. In cabbage pitted out of doors secondary growth often occurs, giving rise to small heads from the axilary buds. These are affected in the same way as the mother heads. This condition is shown in Plate 3, Fig. 1. Such effects of the disease render the heads worthless, the normal flavor is impaired or destroyed and the heads are, of course, unmarketable.

The disease severely disfigures the foliage of cauliflower in transit. California and Oregon shipments of cauliflower and broccoli arrive on the central and eastern markets often so severely affected with black leaf speck that their value and salability is seriously impaired. A photograph of diseased cauliflower is shown in Plate 2, Figs. 1 and 2, illustrating the condition in which shipments from the far West arrive at destination. Every crate in the car from which this sample was taken was affected in this way. Inspectors had been diagnosing this trouble as ring spot. The spots on cauliflower leaves are practically identical with those occurring on cabbage. The spots on the midribs appear to be more conspicuous and more sharply depressed than those of similar location and size on cabbage. A severe case of the disease on this crop often results in a yellowing and shedding of the leaves. When ring spot is present in any appreciable quantity, it is difficult to determine whether yellowing of the foliage is caused by the attack of the fungus or by black leaf speck and the conditions which cause it. This makes a complicated problem for the inspector who attempts to determine whether responsibility rests with the producer, the transportation company, or both. While the development of black leaf speck in shipments of cauliflower indicates unfavorable conditions within the car, the question arises as to whether or not these conditions are due to faulty methods of loading and stowing, or improper ventilation and refrigeration service. Either of these factors, or both, may determine the development of the disease while the crop is in transit. Delayed service in the movement of the cars will undoubtedly favor the occurrence of the disease. The flowers are not affected by the black leaf speck disease and so far as can be determined the quality is not greatly impaired.

Black leaf speck lesions may be visible from only one side of the leaf. The dorsal surface is most often the seat of the larger number of spots and if the specks are small they may be entirely invisible from the upper surface. Large spots can be seen from either side of the leaf. The very small specks are formed by the breaking down of comparatively few cells and the thickness of the leaf is a barrier to the free passage of light, so that until the leaves are removed from the head and examined separately the extent of the disease can not be determined. Stewart (21), also made this observation on specimens of cabbage from the storage houses of New York.

The specking is usually confined to the outer layers of leaves on heads affected in the field. In the only serious case observed in Michigan all of the leaves of heads of Chinese cabbage were diseased, the effect of which is shown in Plate 4, Fig. 1. As a field trouble the effect on the foliage is usually not severe enough to cause serious blemishing, besides the outer leaves are removed before marketing. The disease must, however, be distinguished from the effect produced by the attack of aphids.

Distinguished From Other Leaf Spots

Other diseases with which black leaf speck is likely to be confused are: ring spot (_Mycosphaerella brassicicola_); black leaf spot (_Alternaria brassicae_) and McCulloch's cauliflower spot (_Bacterium Maculicolum_).

It is distinguished from the first two by the sunken character of the lesions and the general irregularity of their outline. Lesions produced by the ring spot and black leaf spot fungi are usually larger, more regular and contain the fruiting bodies of the causal organisms. In neither of these fungous diseases are numerous lesions formed on the midribs and veins of the leaves as is the case with black leaf speck.

Infection by _Bacterium Maculicolum_ takes place through the stomata and this is one means of distinguishing this disease. A stoma will always be found dotting the center of the lesion while the bacteria may easily be demonstrated by microscopic examination.

Often the work of aphids on cabbage and cauliflower in the field causes small black spots somewhat similar to those produced by black leaf speck. However, instead of being sunken these spots are usually slightly elevated above the surrounding tissue. The absence of fungi and bacteria in the black leaf speck lesions makes microscopic examination a valuable means of identification.

Experimental Work

That black leaf speck was not caused by bacteria or fungi was early proven by repeated failures to isolate or demonstrate microscopically any organism on connection with the disease. Occasionally hyphae are found ramifying through the dead tissues and in severe cases of the disease, bacteria may cause secondary rotting, but ordinarily tissue cultures remain sterile. Further evidence that the disease was non-parasitic in its origin was shown by the failure of the lesions to increase in size or extent when held under conditions that favor the development of parasitic troubles.

The occurrence of black leaf speck in shipments confined for a week or longer in refrigerator cars where ventilation is restricted, in storage pits where air renewal is largely prevented, and in storage houses and cellars where cabbage is piled in deep piles of stored in large bins, suggested the probability that the disease might be similar in origin to black heart of potato, as described by Stewart and Mix (22). The disease had been noted by Dr. G. H. Coons, for several years previous to this investigation, upon cabbage stored locally in out-of-door pits. The writer had found it commonly in transcontinental shipments of cabbage from California. The disease was found abundantly on both cabbage and cauliflower on the local markets, often in destructive form. For a while black leaf speck was confused with ring spot, which also is found frequently on these vegetables from California and Oregon. However, critical examination and negative results from attempts to isolate Mycosphaerella brassicicola from the lesions led to the conclusion, that the disease as it occurred locally on cabbage in pits and cellars, was probably identical with the trouble prevalent on the California and Oregon cabbage and cauliflower.

Preliminary experiments were begun to determine, if possible, the general conditions which might produce the disease. Since it originated under conditions similar to those that produce blackheart of potato, the first experiments were designed to imitate these conditions. In all cases clean, sound heads of cabbage were selected for the experiments. In case of any question as to their previous history they were held at room temperature for at least three days. All wilted, discolored, or mutilated leaves were first removed so that the leaves were perfectly sound and free from blemishes. The effect of various storage conditions was determined by sealing seven lots of heads in large glass jars with ground glass tops and subjecting them to various conditions of temperature and aeration. In the early experiments, in order to shorten the period of storage, a portion of the oxygen was exhausted from the storage chamber by the use of pyrogallic acid and sodium hydrate solution. Not enough of the chemicals were used to exhaust all of the oxygen, tests showing about five to six percent of oxygen remaining in the jars. Later this procedure was discontinued. In Jars 1 and 2 no chemicals were used. These jars were filled with the heads and stored at the temperatures indicated in Table I. A portion of the oxygen was exhausted from Jars 3 and 4 by chemicals, an average of six per cent remaining after all absorption had ceased. Lots 5 and 6 were stored in carbon dioxide gas obtained from a Kipp generator by means of hydrochloric acid and lump marble. Lot 7 was aerated constantly by blowing a stream of air through the storage jar. All were stored at the temperatures given in the table. The 10° temperatures are approximate only, varying 1-2° daily.

In each of the lots in which the oxygen was partially exhausted and also in which it was not, complete breakdown occurred on all of the heads stored at 30° C. The leaves were soft, water-soaked and leathery and anaerobic bacteria were active in the tissues, giving rise to a strong krauty odor in the jars. In Lots 2 and 4 stored at 10° C. sunken lesions appeared upon the

Table 1.

Effect of various storage conditions in producing black leaf speck of cabbage

Lot No.	Storage condition	Temp.	Duration storage	Results
1	Air-tight glass jar	30°C.	7 days	Heads all soft. Severe breakdown
2	Ditto	10°C.	13 days	Heads normal in appearance when jar first opened. Developed red-heart condition in 1 hr. and black leaf speck in 3 days.
3	Oxygen partly exhausted from storage jar with pyro and NaOH	30°C.	6 days	Heads all soft, watery. Anaerobic rots present.
4	Ditto	10°C.	7 days	Heads normal in appearance when first exposed to air. Sunken lesions developed in 1 hour. Blackened in 3 days.
5	Air in storage jar replaced with CO_2	30°C.	5 days	Strong krauty odor in jar when opened. Heads developed slight red-heart in 12 hours.
6	Ditto	10°C.	7 days	All heads normal in appearance. No change in 5 days at room temperature.
7	Fresh air Blown through storage chamber constantly.	30°C.	6 days	Outer leaves moldy and softening. No specking developed in 5 days.

leaves soon after the heads were taken from the jars. After three days at room temperature these sunken spots darkened and finally were dark brown to black in color. These spots were neither so numerous nor so typical in shape or size as the lesions found naturally on cabbage and cauliflower. They were, however, characteristically sunken and colored like those found on cabbage in severe cases of black leaf speck. Colorless at first, these spots passed through various color changes from light pink to black. The black color of the lesions was apparent after three days. Lots 5 and 6 stored in carbon dioxide remained normal in appearance but developed a slight reddening of the heart leaves in those heads held at the higher temperature. In the heads of Lot 2, held for thirteen days at 10° C., this condition was very pronounced. This phase of the breakdown problem is discussed in Part 2 of this paper in connection with a similar trouble affecting head lettuce. In Lot 7, where fresh air was constantly blown through the storage jar, no specking nor any trace of red heart developed in three days after removal from the jar. The outer leaves of these heads were slightly moldy and beginning to soften from the effects of the fungus, (Mucor sp.).

In a general way these preliminary experiments indicated that one factor causing breakdown was operative under conditions of poor aeration. Black leaf speck developed on the heads stored in jars with the oxygen partially exhausted and also where the percentage of this gas in the storage air was not reduced previous to the beginning of the storage period. That the factor causing breakdown was not the accumulation of carbon dioxide in the jars was shown by the behavior of the heads stored at two different temperatures in pure atmospheres of this gas. These heads appeared perfectly normal upon exposure to the air and developed no specking in three days. The necessity for holding the heads for observation for several days after removal from storage was demonstrated by the delayed and gradual development of the lesions of black leaf speck.

It seemed desirable to determine approximately the rate of diminution of oxygen within the storage chambers. Two large glass jars were filled to about one-half capacity with small clean heads of cabbage, and a third jar to two-fifths capacity. The jars were sealed and connected with rubber tubing. A bellows apparatus was attached so that the air could be thoroughly stirred before sampling. Jar No.3 had almost double the capacity of Nos. 1 and 2. Analyses of the oxygen content of each jar were made at intervals by means of Ganong apparatus (7). The gas readings were corrected for error and are approximately as accurate as they can be made with this sort of apparatus. The jars were opened after five, eight, and ten days. The results are given in Table 2.

Table 2

Effect of length of storage period and composition of the air of storage jars upon the production of black leaf speck

Lot	Storage Condition	Duration of storage	Air Composition		Results
			Hours in storage	% O.	
I	Sealed glass jar 1/2 filled	5 days	24 hrs. 72 hrs.	2.5 2.0	Breakdown as sunken lesions 1 hr. after opening. Outer leaves developed typical black specks.
2	Ditto	8 days	24 hrs. 72 hrs. 96 hrs. 120 hrs.	2.0 1.5 1.0 0.5	Krauty odor in jar when opened. Large, sunken lesions formed on outer leaves 2 hrs. after opening. Blackened.
3	Sealed glass jar 2/5 filled	10 days	120 hrs. 168 hrs. 192 hrs.	2.5 1.0 1.0	Breakdown as very typical black specks in parenchyma and on veins. Developed gradually from sunken, colorless lesions.

Temperature 18-20 C.

Breakdown occurred after five days, during which time the oxygen content of the air in the jars was practically exhausted. Jar No. 3 was not opened until ten days after the beginning of the experiment. Due to the larger storage capacity and the relatively smaller amount of space occupied by the cabbage the oxygen supply was greater and consequently was not depleted as quickly as in Jars I and 2. Examination of the table shows that the sharp decline of oxygen in the jars occurred during the first twenty-four hours of storage.

In Jar No. 1 the reading after twenty-four hours of storage showed only 2.5 percent residual oxygen, while in Jar 2, for the same period, the amount was two percent. Following this marked decrease during the first twenty-four hours the decline thereafter was very slight, only 0.5 percent in each of the first two jars for the next forty-eight hours. The last reading for Jar No. 2 was made after one hundred and twenty hours of storage and showed 0.5 percent oxygen yet remaining. In Jar No. 3 with a larger capacity the initial reduction was almost the same for the first twenty-four hours but after eight days still showed 1.0 percent oxygen present.

The results of this experiment indicate that respiration is very active in cabbage stored at a temperature of 18° to 20° C. and that if the oxygen supply is not renewed at this temperature it quickly is reduced to a very low minimum. The condition of oxygen hunger was probably reached during the first twenty-four hours of storage in each of the three jars. It does not seem likely that exposure to an atmosphere containing less than two percent oxygen could long be endured by even parenchyma cells. The slow decrease in the oxygen after the first twenty-four hours is probably due to the suppressing effect upon respiration of the accumulated carbon dioxide. The chemical changes that occur during respiration may be greatly inhibited by the presence of a large amount of carbon dioxide in the air. Brooks and Cooley (5) have shown that apples stored in one to six percent carbon dioxide are thereafter immune from scald and attribute this to an inhibition of the life activities of the apple. The natural accumulation of this gas in the closed storage chambers probably results in a retardation of the rate of the gaseous exchange and thus prolongs the tolerance of the cells to the condition of oxygen hunger. However, after three days at this temperature the oxygen content had only been reduced ten percent below that reached after twenty-four hours of storage, and the disease developed on the heads following removal from the jar. Therefore, it seems probable that the base level of existence of these cells in a diminishing oxygen supply, even when the accumulation of carbon dioxide retards oxidation, may be reached within a relatively short time after storage if the oxygen pressure is not increased. The conditions of temperature and aeration of this experiment are often simulated in the bulk shipments of cabbage in refrigerator cars. Respiration increases the temperature and failure to ventilate promptly, or carelessness in following ventilation instructions, may result in the depletion of the oxygen content of the car below the minimum required for the continued existence of certain groups of cells. Under refrigeration the temperature inside the car may reach the level of 8-10 C. in the upper tiers of the load even when cabbage is shipped in crates. Very little air renewal is possible when the ventilators are closed, for regrigerator cars are practically air tight especially when new. Similar conditions must also occur in pits in which cabbage is stored, and aeration is probably largely prevented by the practice of piling in large heaps in the storage houses or cellars.

In the preceding experiment the most typical lesions of black leaf speck developed on the heads stored in jars, in which the volume of air was greater in proportion to the space occupied by the cabbage than in the other two jars. The length of storage was ten days in comparison to five and eight days respectively for jars 1 and 2. As a check on these first experiments, and also to increase the range of conditions of temperature, length of storage, aeration, etc., the next experiment was designed to test a wide range of these various factors. Three large aluminum jars with covers ground to fit tightly so that the containers were almost air-tight were filled about half-full of small heads of cabbage. These three jars were stored at temperatures of 8-10°, 18-20° and 30° C. Series No. 4 was placed in a similar container and connected with a pump to force fresh air through the jar constantly and held at 20° C. A large dessicator was used for the next lot, and in the bottom of this was first placed enough pyro and sodium hydroxide solutions to partially exhaust the oxygen present. A second dessicator contained heads stored over sodium hydroxide solution to absorb the carbon dioxide produced. Lot

No. 7 was stored in a large glass jar in which the air was displaced by carbon dioxide obtained from a Kipp generator. The last lot was placed in an open glass jar. Series 4 to 8 were all stored at the same temperature, 18-20° C. The results of these tests are given in Table 3.

Table 3.

Influence of various storage conditions upon the production of black leaf speck of cabbage.

Lot	Storage condition	Temp.	Duration storage	Results
1	In air-tight 25-liter jar	30°C.	5 days	Complete breakdown of all heads. All soft and attacked by anaerobic bacteria.
2	In 25-liter air-tight jar	10 to 12°C.	12 days	All heads developed a severe case of black leaf speck after 2 days at room temp. All outer leaves severely blemished.
3.	In 20-liter air-tight jar	20°C.	6 days	Typical black leaf speck lesions developed in 5 days at room temp. Numerous, minute, sunken colorless lesions visible when jars first opened.
4	In air-tight jar with fresh air blown through constantly	20°C.	6 days	Normal. Outer leaves slightly moldy. No specking developed in 5 days.
5	Stored in large glass jar over Pyro and NaOH	20°C.	4 days	Breakdown visible after 2 days as sunken spots in parenchyma. When opened after 4 days spots numerous, large, not typical.
6	Stored in large dessicator over NaOH	20°C.	6 days	Typical black leaf speck lesions completely developed after 5 days at room temperature. In parenchyma and on the veins.
7	In large air-tight jar filled with CO_2	20°C.	7 days	Normal. No change in 5 days. Strong pungent odor in jar when opened.
8	Stored in large, open glass jar	20°C.	10 days	Normal. No change in 5 days.

As in the previous experiments complete breakdown of the heads occurred at the temperature of 30° C. The leaves were soft, and watery and the storage jars filled with a strong krauty odor. At a temperature of 20° C. six day's storage was sufficient to produce typical lesions of black leaf speck. The numerous, small, sunken and colorless specks were visible when the heads were taken from the jar, but the characteristic dark brown to black color developed gradually during a period of five days. During this time the heads were held in the ice box at a temperature of 10° C. but frequently removed for observation. This somewhat delayed the appearance of the typical color of the lesions but was found desirable on account of the drying out of the leaves when exposed to the dry air of the laboratory. When removed from Jar No.2 after a storage of twelve days at a temperature of 10°-12° C., the heads of cabbage were apparently normal in every way. There was no unusual odor in the jar and the leaves of the heads were still quite green and bright. Moisture had collected slightly on the sides of the storage jar. These heads were left on a table in the laboratory and covered with moist absorbent toweling. Within twenty-four hours the outer leaves were showing numerous black specks, and at the end of two days after removal from storage they were badly blemished by innumerable, small, sunken black lesions that reproduced in every detail the normal appearance of the disease as found both in transit and in storage. Plate 5, Figs. 1 and 2 picture the general condition of all of the heads in this series. The spots were located on the veins and in the parenchyma and were visible from both sides of the leaf. The lesions affected several layers of leaves and the development of the specks continued progressively inward on the leaves for several days. Very typical lesions were also produced on the heads stored over sodium hydroxide for six days. These appeared slowly, requiring five days for complete development at ice box temperature (10° C.).

It was again demonstrated by the reaction of the heads stored in carbon dioxide that this product of metabolism is not the cause of black leaf speck. The heads stored in the gas were normal in appearance when taken from the storage jar and developed no signs of the disease when held for the usual observation period. Lots 4 and 8 were subjected to a continuous change of air and were sound except for the development of mold on the outer leaves. No lesions of black leaf speck appeared on any of the heads of these two lots.

Effect of Air Movement

In the preceding experiments it was shown that cabbage reacts somewhat similarly to apples when stored under conditions which are not favorable for ventilation and change of air. On the leaves of cabbage, breakdown occurs in the form of small black specks when the heads are stored where there is no renewal of the air. It was also shown that the accumulation of carbon dioxide which occurs under such conditions is not the cause of black leaf speck.

In invéstigating apple scald, Brooks, Cooley and Fisher (5) found that this disease could be prevented by stirring the air of the storage house and that this favorable action is not due to the composition of the air itself since renewal was not essential. They believe that scald is caused by the accumulation on the surface of the fruit of toxic volatile esters and that air movement dissipates these substances and prevents the development of scald. Since black leaf speck of cabbage occurs under storage and transportation conditions similar to those that produce scald of apples some of the experiments used by the above investigators were tested in these investigations. Provision was made for agitating the air of the chambers in which the cabbage was stored without effecting renewal. A special air-tight box was constructed which housed a motor-driven fan. The outlet from this was connected by means of glass and rubber tubing to a large glass storage chamber through which the air was forced around the heads of cabbage and then led back again through rubber tubing to the fan box. The set up of the apparatus used in this experiment is shown in Plate 6, Fig. 1. A similar lot of heads was placed in a large storage jar connected to a suction pump and fresh air drawn constantly through it. The temperature was approximately 20° C. throughout the duration of the experiment. The duration of the exposure of these two lots was six and ten days respectively. The results of this experiment are summarized in Table 4.

Table 4.

Effect of air movement upon the production of black leaf speck in stored cabbage

Lot No.	Storage Conditions	Duration	Results
1	Fresh air constantly drawn through storage chamber by suction pump	10 days	Heads all sound 5 days after removal from storage. Slight development of mold on surface leaves
2	Air in continuous circulation by means of motor driven fan, but not renewed	6 days	All heads with typical breakdown. Specks very small, located on veins and in parenchyma. Several layers of leaves affected.

Temperature 20°C.

The outer layers of leaves on all the heads of cabbage from Lot No. 2 subjected to storage for six days, where the air was in constant motion but not renewed, developed typical lesions of black leaf speck. The specking was quite general, affecting the vein tissues as well as the parenchyma between the veins. Practically all of these specks were very small and non-coalescent. In mild cases of breakdown, such as represented here, the specks that develop are usually very small, numerous and distinct, while in severe cases the lesions are more likely to be larger and coalescent. The heads bathed for ten days with a constant stream of fresh air did not develop any lesions of black leaf speck. The outer leaves that came in contact with the walls of the storage chamber were slightly moldy from the attack of a species of Mucor. The humidity in each of these chambers was high at all times and moisture was deposited as small drops on the walls of the jars. This favored the development of fungi which sometimes attacked the outer leaves.

There was no indication from the results of this experiment that air movement would prevent breakdown unless renewal was accomplished as well. However, further tests were made in order to fully establish this point. Variable conditions of temperature and air composition were provided. In Lot No.1 the heads were stored under conditions similar to those of the preceding experiment, the air of the storage chamber being constantly agitated but not renewed. Lots 2 and 3 were stored in large glass jars and the air displaced with nitrogen. These jars were stored at temperatures of 10°C. and 18°-20°C. Number 4 was connected to a suction pump and the air in the jar constantly renewed. Lot No.5, consisting of twenty-five or more small heads, was stored in a large low temperature incubator equipped with a motor-driven fan. The temperature was kept down to approximately 10°C. by means of ice in a chamber above the heads. When closed and all crevices sealed with modeling clay the chamber was practically air tight. The heads could be observed at all time through a glass door and temperature readings made from a projecting thermometer. The results of these tests are summarized in Table 5.

Table 5.

Effect of composition and movement of the storage air upon development of black leaf speck in cabbage.

Lot	Storage Condition	Temp.	Duration of storage	Results
1	Air constantly circulated but not renewed	22° to 25° C.	3 days	Breakdown on some heads, mostly as minute specks at margins of the leaves
2	Storage chamber filled with nitrogen	22° to 25° C.	2 days	Breakdown very severe. Outer leaves soft and heart leaves become red color on exposure to air.
3	Ditto	10°C.	7 days	Breakdown severe. Outer leaves beginning to soften. Heart leaves of all heads reddened upon exposure to air.
4	Fresh air drawn through storage chamber constantly	22° to 25° C.	3 days	All heads sound. No change in 3 days.
5	Air constantly circulated by motor-driven fan	10°C.	11 days	Mild breakdown on majority of heads. Typical black specks on outer leaves. Intumesences on cut stems.

In Lots 1 and 5 stored at temperatures of 20° and 10° C. respectively, black leaf speck developed in a typical way after removal of the heads from storage. At the higher temperature an exposure of only three days with the air in constant circulation inside the storage jar was sufficient to cause breakdown. In the preceding experiment on air movement the duration of the experiment had been six days. Here with the time shortened to three days the disease developed on the margins of the outer leaves, not so abundantly as in the previous test with longer exposure, but characteristically, with the small specks typical of the mild form of breakdown. At the temperature of 10° C. the heads were exposed for eleven days. No evidence of breakdown could be observed on the leaves before the heads were removed from the incubator. However, the black specks appeared on a majority of the heads shortly after removing them to the outer air and in addition small intumesences developed on the cut stems. The severity of the disease was much less than developed in an earlier experiment where the heads were stored for twelve days at approximately this same temperature in still air. However, the volume of air was greater in proportion to the volume of cabbage in this test than the former and it is also almost impossible to prevent some interchange of air in apparatus of this kind with the air inside the incubator in motion.

The cabbage in both nitrogen-filled jars was severely affected with breakdown. The outer leaves of the heads were soft and watery, becoming leathery on drying, and the heart leaves developed the redheart condition. In Lot No.4, exposed to a continuous stream of fresh air forced through the storage jar, the heads developed no breakdown in the usual observation period. The results of these tests seem to demonstrate conclusively that air movement in the storage house, without renewal, is not effective in preventing the development of black leaf speck, and that in this respect this disease differs from apple scald.

Effect of Accumulated Volatile Storage Gases Other Than Carbon Dioxide

Although in the experiments with air movement there was no indication that black leaf speck was similar to apple scald it was decided to test further the possibility by collecting the gases given off in storage and exposing sound heads to them. Small, sound heads of cabbage were sealed in a large glass jar, the air displaced with nitrogen and the jar held at a temperature of 20° C. for three days. A similar lot of heads was then placed in a second jar and the accumulated gases drawn over from Jar No. 1 by means of a suction pump. The gases were passed through a tower containing potassium hydroxide to remove the carbon dioxide. A third lot of heads was stored in a glass jar and fresh air drawn over them through a suction pump. The heads were removed from Jar No.2 after being exposed to the gases for twenty-four hours. The results are shown in Table 6.

Table 6.

Effect of Volatile compounds other than CO_2 given off by cabbage in storage upon the development of black leaf speck.

Lot	Storage conditions	Exposure	Results
1	Nitrogen gas for 3 days	0	Breakdown on all heads as sunken lesions on inner leaves. Outer leaves softening.
2	Sound heads in large glass jar.	Gases from Jar 1 drawn through KOH to Jar 2. Left 24 hours.	Heads removed after 24 hours and held at room temperature for 3 days. All sound.
3	Subjected to continuous stream of fresh air	3 days	All sound except for slight mold on one head at bottom of jar. No specking in 5 days.

Temperature 20° C.

The heads stored in nitrogen for three days were severely affected with breakdown. The outer leaves were softened and conspicuous; large, sunken spots were present, No specks or breakdown in any form appeared on the heads exposed to the gases from the original heads or on the heads bathed by a constant stream of fresh air during the period of storage.

The above experiment was repeated under similar conditions except that the heads of cabbage were merely stored in a tight jar and left for three days. A second jar was then filled with fresh, sound heads of the same kind of cabbage and partial vacuum created in the storage jar by attaching a vacuum pump and exhausting as much of the air as possible. This jar was then connected to Jar

No. 1 by means of very heavy rubber tubing and the stop-cocks closing each jar were then opened. In this way the gases from Jar No. 1 were drawn into Jar No. 2 until the pressure was equalized. The heads in Jar No.2 were then left for twenty-four hours at a temperature of 20° C. The results are shown in Table 7.

Table 7.

Effect of Gases given off by cabbage in storage upon the development of black leaf speck.

Lot	Storage period	Exposure period	Results
1	3 days	3 days	Breakdown as typical black specks developed on the leaves after exposing the heads to the air.
2	0	24 hours	Heads all sound after holding for 7 days exposed to air at room temperature.

Temperature 20° C.

The results of this experiment are similar to those of the preceding one and tend to show that toxic compounds of a volatile nature are not responsible for black leaf speck.

Effect of Low Temperature

In some of the preceding experiments black leaf speck was produced by storing heads of cabbage at comparatively low temperatures. The disease was reproduced most typically at temperatures below which the ordinary parasitic troubles fail actively to develop. The disease as it occurs naturally in storage houses and storage pits, as well as in refrigerator cars, develops at temperatures from about 0° to 10° C. This, however, does not represent the entire range of temperatures to which cabbage and the other crucifers are subjected in storage and transportation but are within the average range. Black leaf speck has been reported as causing serious losses in the storage houses of New York state (21), where the temperature was held near 0°C. Its occurrence has also been noted in the fall in the field by Stewart (21). Reference has been made (page 10) to observations by the writer on the occurrence of this disease following early, heavy frosts, both on cabbage and chinese cabbage. Link (11), reports the variety Winningstadt affected in the field in California but makes no reference to temperature. However, in the same article he refers to freezing injury to celery in the fields of the same vicinity. It is probable, therefore, that the occurrence of the disease in the field there was correlated with low temperature.

Other investigators have noted the fact that certain plants break down following exposure to low temperatures. Harvey (9), has shown that the leaves of tomato plants become spotted with brown spots if long exposed to a temperature of 5° C. It has been demonstrated by Molisch (14), that the leaves of tropical plants like Episcia, Physodeira, etc., become severely spotted with brown spots following exposures of twenty-four hours to temperatures of 1° to 5° C.

The effect of low temperature in producing black leaf speck was tested by storing a large number of heads of cabbage in a large refrigerating room where the temperature variation was within the limits 1.0 to 1.5° C. Part of these heads were in an open wire basket and an equal number in a loosely covered tin box. There was an abundant supply of fresh air in this room, admitted by the frequent opening of doors. Temperature records were kept with a thermograph and showed little variation from the above figures. A second lot of heads was placed in a fruit cold storage room held at a temperature of 5° to 7° C. Part of these heads were in an open wire basket and the rest were stored in a large aluminum cooker which, when closed, was practically air-tight. The effect of the cabbage in the open basket upon dairy products nearby made it necessary to remove Lot 2 from cold storage after three days. The results are given in Table 8.

Table 8.

Effect of low temperature upon breakdown of cabbage in cold storage.

Lot No.	Condition of storage	Temperature	Duration	Results
1	In covered tin box	-1 to +1°C.	17 days	Breakdown developed on all heads. Visible lesions on some heads when taken from storage.
2	In open basket	-1 to +1°C.	3 days	Slight breakdown on 2 heads.
3	In air-tight container	5-6°C.	17 days	Soft rot in nearly all heads. Inner leaves affected with characteristic black specks.
4	In open basket	5-6°C.	17 days	Sound

While it was not expected that breakdown would occur within three days, Lot 2, removed from storage after having been exposed to temperatures near 0°C. for this length of time, developed a few small black specks on the margins of the outer leaves after holding the heads at room temperature for three days. Lot No.1, enclosed in the loosely covered tin box, was allowed to remain in storage for seventeen days. At the end of this time breakdown was apparent on the outer leaves as small black specks. After holding for three days exposed to air at room temperature all of the heads were affected with the characteristic lesions of the black leaf speck disease. In addition to the small lesions the midribs of some of the outer leaves were sunken at various intervals in longitudinal areas of blackened tissue, two to four inches in extent. These lesions very much resembled those caused by <u>Bacterium campestre</u> and were at first thought to be due to infection by this organism. However sections and cultural methods failed to demonstrate the presence of any organism within the necrotic areas. It seems unlikely, moreover, that bacteria or fungi could develop to this extent at such low temperatures within seventeen days. These lesions were apparently a severe form of breakdown. Lots 3 and 4 were also removed from storage on the seventeenth day. All of the heads held in the open basket were normal in appearance and remained so during the usual observation period. The heads from Lot No. 4 held in the aluminum container were affected with soft rot on the outer leaves and there was a pronounced krauty odor present when the container was opened. Upon removing the outer layers of leaves black leaf speck developed on the newly uncovered leaves. This phase of the occurrence of this disease as an accompaniment of soft rot has been noted before and is discussed in Part II of this paper. Where cabbage is attacked by soft rot the layers of sound leaves beneath those affected with the rot are often found covered with the specks characteristic of breakdown. With the outer leaves destroyed by soft rot organisms the cells beneath are subjected to anaerobic conditions because of the consumption of the available oxygen by the bacteria at the surface area. These organisms are known to be voracious consumers of oxygen and where soft rot is present to the extent that all the surface area is attacked the partial or complete anaerobiosis that ensues results in some form of breakdown of the tissues deprived of oxygen.

The effect of low temperature upon the production of black leaf speck, even in the presence of an abundant supply of oxygen, is strikingly illustrated by this experiment. Slight spotting of the leaves occurred after the short exposure of only three days to an average temperature of 0° C. and severe breakdown followed a storage period under the same conditions for seventeen days. An exposure of three days at 0° C. is usually ineffective in producing black leaf speck. The evidence at hand seems to show that black leaf speck occurs at low temperatures, near 0° C., despite the presence of an abundant supply of oxygen, and that it also is produced at higher temperatures when for any reason a constant supply of oxygen is not available. The temperature range tested in these experiments cover those to which plants in storage and transportation are ordinarily subjected.

Whenever temperature or aeration conditions in storage or transportation are such as to restrict or make unavailable a constant supply of oxygen black leaf speck may be expected to result if these conditions are of sufficient duration.

II

Redheart of Cabbage and Head Lettuce

Introduction

During the course of the investigations reported in the first part of this paper it was found, that under certain conditions of poor ventilation and also low temperatures, the heart leaves of cabbage become affected with a trouble tentatively designated red-heart. Heads affected with soft rot were also subject to the same disease. The solid-headed types of lettuce are very commonly affected with this trouble and it is in this crop that the disease assumes considerable importance. It has been noted for several years in lettuce from the western states sold under the trade name of "Iceberg". It is most frequently seen in shipments on local markets and which have been held for sometime under refrigeration or under conditions which are unfavorable for thorough ventilation. It is also an accompaniment of soft rot and almost invariably heads that are badly attacked by soft rot show this type of breakdown in the heart leaves.

The disease had been observed prior to the initiation of the investigations on black leaf speck of crucifers, and the reaction of cabbage to ventilation and refrigeration experiments suggested the probable nature of the disease in lettuce. Experimental work was begun while the black leaf speck investigations were in progress and because of the distinct symptoms presented by this particular type of breakdown it has seemed advisable to present the results separately.

Economic Importance

No figures are available as to the losses caused by this disease. As a transportation disease on lettuce it seldom attains rank of major importance. It is only after the crates of lettuce have been sent out in lot shipments and are placed on the market by the retailer that the trouble is manifested. The disease has not been found in the original car lot shipments when examined at destination. However, entire crates have been seen in wholesale houses and the markets which were almost worthless because of the presence of redheart. The method of shipping the solid-headed varieties of lettuce is very conducive to the development of soft rot and it is following this disease that redheart is most serious. Usually the crates are repacked and filled with ice, resulting in a great deal of bruising, with the subsequent development of soft rot. Before such heads are offered for sale by the retailer the rotted outer leaves are trimmed away and the heads appear to be entirely sound. If, however, they are cut open the heart leaves often will be found to be reddened and the head worthless. The general practice of holding stocks of lettuce in refrigerators retards the development of soft rot but is apparently favorable to the occurrence of redheart.

In cars long delayed in transit or held for an unusually length of time before unloading,this trouble may cause considerable damage to head lettuce that will not be apparent for sometime after unloading. The disease is most conspicuous during the late winter and early spring months. Head lettuce bought after February,in a great many cases,is affected with redheart. The occurrence of the disease at this time of the year is probably correlated with higher temperatures and consequent development of soft rot.

The disease in cabbage occasionally causes serious damage. It was reported by the perishable freight inspection service of one of the large railroads as the cause of considerable loss in cabbage from the storage houses of New York state in 1920-21. Car-load lots of cabbage in transit after January 1921 were found to be commonly affected with redheart (Plate 6, Fig. 3). This cabbage had been held in the commercial storage houses in the usual way and apparently the disease was due to the conditions prevalent there during the period of storage. The disease has not been observed in commercial lots of cabbage in Michigan but it is probable that it is present in greater or lesser amounts each year. Since the conditions which are favorable for the production of black leaf speck have been shown also to produce redheart, it seems logical that where the former disease occurs in its more serious form that redheart will also be present. Where the storage houses are systematically aerated and the temperature is held a few degrees above 0° C., the disease is probably of little importance. Cabbage shipped in crates is unlikely to be affected unless excessive delay occurs or soft rot develops.

Name of the Disease

This disease has not been reported in the literature on cabbage or lettuce diseases. The name redheart is suggested since it describes the chief symptom of this type of breakdown. There is some variation in the color of the diseased portions of the heads of cabbage and lettuce, and while the name redheart more clearly describes the color of the cabbage leaves it is appropriate also for lettuce since the affected heart leaves are a chestnut brown color.

Description of the Disease

This type of breakdown is distinguished by the characteristic reddish color of the heart leaves both of cabbage and lettuce. In cabbage these leaves are a typical red color, while in head lettuce they are a deep chestnut brown. Under experimental conditions these colors have been observed to develop gradually, the affected leaves of lettuce passing successively through the color changes of walnut brown, neutral red, Indian purple to chestnut brown. In cabbage the leaves at first appear slightly infiltrated then a gradual change occurs from a light pink to a deep red color. In Plate 6, Fig. 3, is shown the half of each of two heads of cabbage, one sound, the other affected with redheart.

The outer leaves may or may not be affected with black leaf speck when redheart is present. Usually when the former disease is abundant on the outer leaves redheart is also found in various stages of development from only a small discolored area to the involvement of the entire central portion of the head.

Occasionally the outer leaves of head lettuce affected with redheart develop numerous pits on the midribs and veins and sometimes in the parenchyma between the veins. These pits are similar to the lesions of black leaf speck on cabbage but are a chestnut brown color like the heart leaves. These lesions are small and usually somewhat elongated with the longer axis parallel to the midrib. In Plate 6, Fig. 2 is shown this type of breakdown on a head of Iceberg lettuce. This phase of breakdown usually escapes notice because it most commonly occurs on the outer leaves and these leaves are removed before the lettuce is placed on the retail market. When it does occur the presence of these lesions is a good index of the condition of the heart leaves, for usually redheart is also present.

Experimental Work

Since the previous work with cabbage had indicated the importance of securing sound heads for experimental work all lettuce used was carefully selected and held at room temperature for observation for three days before storing. The variety commonly sold as Iceberg was used in all these experiments. In each case a number of heads were cut open to determine the soundness of the heart leaves and a large number of heads were used for checks. The first experiment consisted of storing sound heads in nitrogen and in air and subjecting them to aerations with the same gases. A third lot was inoculated with five strains of soft rot bacteria and held in loosely covered tin boxes lined with moist filter paper. About half a dozen heads were inoculated with each strain of the soft rot organism. Lots 1 and 2 were each stored in large glass jars and aerated twice daily for thirty minutes with nitrogen and air respectively. This was continued for four days while Lot 3, inoculated with the bacteria, was held until the soft rot was well developed, twelve days. Uninoculated checks were stored in a similar way. The results are given in Table 9.

Table 9

Effect of storage conditions upon the development of redheart in Iceberg lettuce.

Lot	Storage Conditions	Duration of storage	Results
1	In large glass jar connected to tank of nitrogen. Nitrogen changed twice daily.	4 days	When first cut open all heart leaves normal color. Change successively to walnut brown, neutral red, Indian purple, chestnut brown. Sunken pits on outer leaves, neutral red color.
2	Same as above but air used in place of nitrogen	4 days	Heads all normal in appearance. No redheart developed in 5 days. No pits on outer leaves.
3	6 heads in each of 5 tin boxes. Inoculated with 5 strains soft rot bacteria	12 days	All outer leaves rotted with slimy soft rot. Hearts of all heads typically affected with redheart.
4	Same as above but sterile water used for inoculation	12 days	Sound. No redheart in any heads.

Temperature 20°C.

Lots 1 and 2 were removed from the jars at the end of four days'storage and immediately cut open to determine the condition of the heart leaves. The heart leaves of all heads from Lot 1 when first examined appeared normal except for a slight infiltration, giving a dull appearance to the heart of the plant. In three minutes this had become a walnut brown color and passed successively through the following changes; in fifteen minutes neutral red; two hours Indian purple; three hours chestnut brown, after which no further change in color occurred. Ridgway color standards were used for comparison. In Plate 7, Fig. 1 is shown one of these heads three hours after cutting open. All the heads aerated with air were sound and developed no condition resembling redheart. All the heads inoculated with each strain of soft rot bacteria developed a typical case of slimy soft rot, the outer leaves becoming soft, slimy and largely disintegrated. Every head in these five separate lots was affected with a typical case of redheart, the disease being fully developed when the heads were first cut open. The uninoculated heads were sound at the end of the twelve days storage period and were free from redheart or any other symptoms of breakdown.

This experiment indicated that breakdown in the form of a reddening of the heart leaves and the development of sunken lesions of the same color on the outer leaves could be satisfactorily reproduced under controlled conditions very similar to those to which lettuce is exposed in transportation, storage and the open market. Poor ventilation, resulting in a progressive decrease in the available oxygen, is undoubtedly one of the causes of breakdown of this type and the universal presence of soft rot on head lettuce sold on the markets is probably one of the most common causes of redheart.

The production of redheart and black leaf speck in cabbage at low temperatures suggested the possibility that the same factor might be effective in producing the disease in lettuce. A crate of sound Iceberg lettuce was obtained for this experiment and divided into two lots. The heads of Lot No. 1 were placed in two large loosely covered tin boxes and stored in a cold storage room where the temperature ranged from -1.0° to 1.0°C. The heads of Lot No. 2 were placed in wire baskets in a refrigerator at a temperature of 8-10° C. Examinations were made of each of these lots at intervals of several days. At the end of three weeks one box was removed from cold storage and one from the refrigerator. The remaining heads of Lot No. 1 were left in storage for an additional three weeks, while those in the refrigerator were removed after a total storage period of four weeks. The behavior of these various lots of lettuce is shown in Table 10.

Table 10.

Effect of low temperatures upon the development of redheart in Iceberg lettuce.

Lot No.	Storage conditions	Temp.	Duration storage	Results
1	Loosely covered tin boxes in cold storage room	-1.0° to 1.0° C.	3 weeks	Sound. No redheart developed in 3 days after removal from storage.
2	Ditto	-1° to 1.0° C.	6 weeks	Redheart in 30% of the heads after 24 hrs. at room temperature. Sunken red lesions on the outer leaves.
3	Open wire baskets in ordinary refrigerator	8-10° C.	3 weeks	Redheart in 50% of the heads.
4	Ditto	8-10° C.	4 weeks	Soft rot and redheart in all heads.

Examination of the table will show that no redheart was produced in the lettuce stored for three weeks at a temperature of -1.0° to 1.0° C. but that a similar period of exposure to a temperature of 8°-10° C. resulted in the production of the disease in fifty percent of the heads. This is partially to be explained by the fact that no soft rot developed on the heads at the lower temperature while the heads developing redheart were also affected to some extent with soft rot. However, some of the heads that were affected with breakdown of the heart leaves did not develop sufficient rot to account for its occurrence. It will be noted, however, that one week later all of the heads at the higher temperature were attacked by slimy soft rot and that redheart was present in every one of these (Plate 7, Fig. 2). After six weeks of storage at the lower temperature thirty percent of the remaining heads in cold storage had developed breakdown which was evident within twenty-four hours after removal from storage. In addition the red sunken specks were abundant on some of the outer leaves. These lesions were similar to those of black leaf speck on crucifers except for coloration. The results of this experiment, while differing somewhat from those obtained with cabbage, are in agreement as to the production of the disease by exposure to low temperature even in the presence of an abundant supply of oxygen.

Typical redheart of the type that occurs in lettuce and cabbage in storage, and frequently develops after transcontinental shipment in refrigerator cars, has been reproduced by the conditions of the foregoing experiments namely: restricted aeration or limited oxygen supply; secondary effect of soft rot, which effectively inhibits free gas exchange and storage at low temperatures similar to those encountered in cold storage and certain areas within refrigerator cars. In some respects, especially reaction to temperatures near 0° C., lettuce appears more tolerant to unfavorable conditions than cabbage, but in general the response of these two vegetables to similar conditions of aeration and temperature is fairly uniform. The expression of this reaction apparently differs somewhat in the parts of the head most susceptible, in cabbage the outer leaves, in lettuce the heart leaves. That black leaf speck and redheart are similar expressions of a reaction to the same or closely related stimuli is shown by the fact that the reddening of the heart leaves of both cabbage and lettuce is a mutual response to the same physical conditions, and that the breakdown of certain areas of the outer leaves of cabbage in the form of black specks can also be produced in lettuce in the form of reddish specks of the same type, by prolonging the exposure to the unfavorable conditions which bring out these diseases. The existence of leaf speck and redheart in either a head of cabbage or lettuce is simply evidence of a compound reaction of these vegetables to similar unfavorable environmental conditions.

III

Surface Breakdown or "Button Rot" of Potato Tubers

Introduction

The peculiar surface pitting of potatoes that occurs in storage after January and also in transportation is a common phenomenon each year. The disease has been known for sometime by the trade as "Button Rot". By pathologists it has generally be considered as due to the attack of some species of Fusarium. Apparently no investigational work has been done to determine the cause of the disease, the occasional association of Fusaria with the necrotic tissues being the reason for the assignment of the associated fungus as the causal agent. The disease has either been increasing in importance or more attention has been directed to storage troubles the past few years, since inquiries concerning the nature of this trouble have been on the increase.

Importance

Attention has been directed to this disease because of the prevalence of the trouble in storage houses, and also in cellars or pit storage. The losses sustained by the growers who hold their potatoes for late winter or spring shipments has lifted this disease from the rank of a minor trouble to a plane where in some years it ranks as one of the most important storage diseases of this crop.

Actual losses are difficult to determine due to the fact that affected potatoes are not always unsalable but are reduced in value. Where blackheart is not a complicating factor the disease merely is an unsightly blemish, but in severe cases secondary rots, and occasionally blackheart, serve to render the affected tubers worthless. Kotila (10) has made some first hand observations on the prevalence and losses caused by this disease in the storage houses of Michigan. The disease was particularly severe in the late winter of 1923. Losses of twenty-five to thirty percent were not uncommon in some of the larger storage houses. While these figures can not be taken as indicating the average importance of the disease as a storage trouble they do represent the possibility of deterioration that may arise as the result of favorable conditions for the occurrence of this trouble.

As a transportation trouble the disease is probably correlated in most cases with the occurrence in storage. It may arise, however, as the result of unfavorable conditions within the car and in many cases is complicated by various secondary rots and internal breakdown as blackheart. The tubers may be exposed to the conditions necessary for the production of breakdown while in storage and show no symptoms until after removal. This makes a particularly difficult problem for inspectors who are confronted with the necessity of determining responsibility. In such cases storage house inspection is the only method of solving the difficulty and necessitates careful investigation.

As a field disease this type of breakdown has been observed only once. In the fall of 1924 Michigan potatoes in several counties were affected. Losses in some cases were serious. The disease was apparent at digging time, following an extended period of hot and very dry weather. The soil was thoroughly dehydrated and the tubers when dug were badly blemished and in most cases unsalable. The crops grown on the heavier soils were apparently most seriously diseased. The condition was first diagnosed as late blight rot, but no organisms could be demonstrated in the affected tubers, and in addition late blight was known to be absent from the fields in which the disease occurred. Certified fields were included among those showing breakdown of the tubers.

Symptoms

The disease is characterized by shallow surface pitting of the tubers, brought about by the death of certain cortical and adjacent cells and the subsequent dessication of the dead cells. The appearance of the disease is well illustrated in Plate 8, Fig. 1. All gradations in the severity of the disease in its effect upon the tubers may be present in the same lot of potatoes. The pits may be small and very numerous and only slightly depressed below the surrounding healthy tissues, but where large areas are involved the individual pits are usually fewer in number and extend deeper into the flesh. In the more severe forms of the disease this extension of the pitted area is often correlated with the secondary attacks of fungi, usually *Fusaria*. In the initial stages of the disease the pits are usually small, the surface is hard and the margins are fairly regular in outline with a grayish-blue color or metallic luster, and there may or may not be an accompanying brown discoloration of the flesh immediately beneath. In most cases of surface breakdown blackheart is infrequently associated with the pitting but this trouble may also be found in tubers showing severe surface injury. In many respects this surface pitting of the tuber resembles the injury caused by formaldehyde. It differs chiefly in the placement of these pits which bear no relation to the lenticels. The pits caused by formaldehyde injury are always lenticular in origin.

Previous Observations

Very few reports have been made of the occurrence of this disease in the literature of potato diseases. Stewart and Mix (22) noted the appearance on tubers, stored in tightly closed jars for ten to twelve days at temperatures near 70° F. of small, circular, dead depressed areas, 2-5 mm. in diameter and resembling the spots produced by fumigation with formaldehyde. They report the tubers severely injured by the occurrence of these spots. This trouble was probably identical with, or related to, the type of breakdown under consideration in this paper.

Link and Gardner (12) report this type of pitting on market potatoes and think it is the result of freezing injury. Coons and Kotila (6) report the general occurrence of the disease in Michigan storage houses and in potatoes stored in pits. The disease was particularly severe in the winter of 1922-23. Recently Bennett and Bartholomew (3) report the occurrence of surface injury as an accompaniment of blackheart produced under low temperature conditions. They do not describe the kind of injury produced and it may or may not have been of the pitting type.

Experimental Work

Following the successful production of black leaf speck in cruciferous plants by storing them under conditions of restricted aeration and also the production of redheart in cabbage and lettuce under similar conditions, the pitting of potato tubers in storage and transportation was suspected of being of like origin. In some of the experiments with cabbage, lettuce and fruits a few potatoes were enclosed in the storage jars and the effect of the various storage conditions noted. The first experiment, using potatoes exclusively, was set up in February 1921. Sound, clean tubers of the Rural variety were used and after being held at room temperature for several days they were stored as follows: lot 1 in a large glass jar sealed tightly with connections arranged for replacing air with nitrogen gas; lot 2, the same as lot 1 but arranged for aeration with air; lot 3 in an open basket.

In Table 11 are shown the results of this experiment. Lot 1, stored in nitrogen which was renewed by blowing fresh gas through the storage jar for thirty minutes daily, developed typical surface breakdown within two days after removal of the tubers from the jar. When taken from the jars the tubers were normal in appearance and apparently entirely sound.

Table 11.

Effect of aeration with air and with nitrogen upon development of surface pitting of potatoes

Lot	Storage	Duration	Results
1	In glass jar filled with nitrogen. Renewed daily.	3 days	Tubers normal in appearance when removed from jar, Surface pits developed within 48 hours at room temperature. No blackheart in any tubers.
2	In glass jar. Air blown through continuously	3 days	All sound. No change in six days.
3	Open basket	5 days	All sound.

Temperature 20-22° C.

No blackheart developed in any of the tubers. The tubers supplied with fresh air were sound and developed no trace of breakdown when held throughout at the usual observation period. Lot 3 in the open basket was unchanged. This experiment indicated that surface pitting could be produced under conditions similar to those causing black leaf speck of crucifers and redheart of cabbage and lettuce and that apparently the time exposure to these conditions required to produce surface pitting was less than that required to cause blackheart. Observations on the natural occurrence of surface pitting on tubers in the storage houses failed to show that blackheart was an accompanying symptom, in fact very few cases of blackheart have been observed where breakdown was apparent as depressed areas on the surface of the tubers. It seemed probable that these two kinds of symptoms might result from exposure of the tubers to similar conditions and that the varying response of the tubers could be explained on the basis of the duration of exposure to the storage conditions which would produce either surface breakdown or blackheart, or both.

The next experiment was designed to produce both surface breakdown and blackheart by prolonging the storage period over that of the preceding experiment. In this case the tubers were subjected to the same storage conditions as in the preceding experiment but allowed to remain in the storage jars for two additional days. An additional lot was stored in a large air-tight dessicator. The results are shown in Table 12.

Table 12.

Effect of length of storage upon the production of surface breakdown and blackheart in potato tubers

Lot	Storage	Duration	Results
1	Large glass jar. Nitrogen blown through 30 min. daily	5 days	Tubers moist on surface and slightly discolored. Upon cutting tubers cortex showed pink coloration, gradually changed to dark brown. Blackheart in nearly all tubers.
2	Same as 1 but air used for aeration	5 days	All normal. No change after holding 5 days.
3	Open basket	5 days	Normal after 10 days' observation.
4*	Stored in air-tight dessi-cator. 1 vol. of tubers to 6 volumes air	10 days	Normal in appearance when opened. All tubers developed surface breakdown as well as pitted areas and also blackheart.

Temperature 18-20°C.

* Air composition at end of ten days:

Oxygen - 3%
Carbon dioxide - 21%.

The tubers of Lot 1 stored in nitrogen for five days were moist on the surface when removed from the storage jar and brown discolored areas were visible on the surface. Some of the tubers were cut immediately and as soon as the flesh was exposed to the air the cambium began to discolor, becoming first slightly pink and gradually changing to a dark brown color. Pink areas appeared in various regions of the flesh and typical blackheart was produced in most of the tubers of the lot. Surface pitting occurred on part of the tubers, some of which were affected with blackheart and some free from it. The tubers exposed to a stream of fresh air were sound and showed no change after holding for ten days. The tubers in the open basket showed no change from the original condition. In Plate 8, Figs. 2 and 3 is shown the surface pitting developed on the tubers stored in the nitrogen, also the discoloration of the cortical cells and the production of blackheart in the flesh. The tubers from the dessicator showed severe surface pitting and every tuber was affected with blackheart. The gas analysis of jar 4 showed three percent oxygen and twenty-one percent carbon dioxide.

The conditions of storage have been modified in various ways and these experiments repeated with uniform results, namely the production of surface pitting, blackheart, or a combination these symptoms. In all cases the indications have been that surface pitting may be expected to develop after exposure to conditions of storage that are not severe enough to cause blackheart.

From two to five days storage in an atmosphere devoid of oxygen is sufficient to produce breakdown in the form of shallow surface pits on the tubers. Potato tubers are apparently more susceptible to this form of injury in the late winter than in the fall when they withstand much more severe storage conditions. Breakdown of the tubers in storage is almost exclusively a disease occurring in the late winter and has never been noted before January. This increase in sensitiveness to decreased oxygen supply may be correlated with increased physiological activity in the tubers toward the end of the normal rest period. It seems quite probable also that pre-storage conditions of the tubers may greatly influence their behavior under experimental conditions. Bennett and Bartholmew (3) have shown that tubers stored at low temperatures until they become sweet are much more liable to injury by high temperatures than non-sweet tubers and this is probably also true in their reaction to the conditions of storage that bring about surface breakdown.

In all the experiments attempted using freshly dug tubers of such varieties as Early Ohio or Bliss Triumph no surface breakdown or blackheart has been produced by exposing them to the conditions which have caused pitting or blackheart in other experiments with late varieties of potatoes. Recently dug tubers break down very quickly under storage conditions tried in the experimental work, either becoming watery or affected with soft rots. No surface breakdown or blackheart has ever been noted to occur naturally on the early varieties of potatoes but there seems no reason why blackheart could not occur in such tubers since this disease has been reported in the late varieties by allowing them to lie in the field after digging and exposed to the hot sun.

Gussow (8) reports an interesting case of blackheart produced in potatoes stored in a pit where the temperature varied from 32.5-46° F. and attributes the appearance of blackheart in the tubers to the effect of the low temperature. Moreover, he states that the pit was well-ventilated. Surface breakdown due to freezing injury has been reported by Link and Gardner (12). In a recent paper by Bennett and Bartholomew (3), they have shown that blackheart can be produced by exposing potatoes to temperatures at or near 0° C. At 0° C. tubers sealed in air-tight jars developed blackheart after thirty-seven days, thirty of which were required for the potatoes to exhaust the free oxygen from the jars. Seven days additional exposure to an oxygen-free atmosphere was necessary to produce blackheart. At 2.5° C. which approximates commercial storage house temperatures, forty-seven days, under similar conditions, elapsed before blackheart occurred, during the last eleven days of which the tubers were exposed to an atmosphere entirely depleted of free oxygen. In their summary they state, "The maximum period of exposure to an atmosphere devoid of oxygen before injury occurred was forty-two days at 5°C."

Concerning surface injury these same investigators report: "Surface injury never occurred, except in jars in which all the free oxygen had been exhausted, for sometime before its appearance. Under these conditions the accumulated oxygen apparently became well-distributed and was used up about equally in all parts of the tuber. Injury then occurred at any point in the tuber were oxygen deficiency occurred". In view of the widespread occurrence of surface breakdown in the form of shallow pitting and the absence of blackheart from affected tubers, it does not follow from the work of these investigators that low temperatures and an oxygen-free atmosphere are the sole conditions under which pitting may occur. In no case do they report the occurrence of surface breakdown in the absence of blackheart. Instead they regard the appearance of surface injury on the tubers as an index to the conditions under which blackheart was produced, namely, temperatures below 20° C. and storage air entirely depleted of oxygen. Such conditions are hardly realized in commercial storage houses. Commercial potato storage houses are usually held at about 3.3° C. (38°F.). This temperature is maintained by air ventilation. Blackheart has never been observed in storage houses where a high percentage of tubers were affected with surface pitting, so that this type of surface breakdown can not be used as an index of the internal condition of the tubers. Moreover, it is apparent that this surface pitting generally occurs as the result of conditions in the storage house that are not sufficiently severe to cause blackheart. However, it is possible that a more prolonged exposure to storage air conditions of a low but more or less constant oxygen content would bring about surface injury and cause no blackheart. Cortical cells contain a relatively larger amount of cytoplasm than older parenchyma cells and are, in consequence, in a more active physiological condition. It would seem that these cells of recent procambial origin and higher cytoplasmic content would be more sensitive to changes in oxygen concentration than the older parenchyma cells, which because of their less favorable location in respect to oxygen supply, are more accustomed to a lower concentration of this gas. This is suggested as the explanation of the occurrence of pitting both in the storage house and in the field. In the fall of 1924 surface breakdown caused rather severe damage in many fields of potatoes in various parts of the state and was reported as more prevalent in heavy soils. Certified fields sprayed for the control of leaf diseases were also badly affected. There was no precipitation over a period of several weeks. The soil was extremely dry and the sun shining on the exposed soil of the fields raised the temperature considerably above normal for the season. Under these conditions the tubers when dug showed typical breakdown as dead areas in the cortex and the subsequent drying and shrinkage of the killed tissues. No blackheart was found in any of the tubers. This sensitivity of the cortical cells to decreased oxygen supply seems a logical explanation of the physical cause of breakdown in the form of shallow surface pits. That this particular type of surface breakdown can not be used as an index of the occurrence of blackheart in the interior of the tuber, or of the conditions which have caused it is evident from a perusal of the type of conditions under which it occurs.

IV. Discussion

The results of the experiments reported in this paper point to the effect of abnormal physiological conditions upon respiration as the cause of breakdown in the form of leaf specking, redheart and surface pitting of the vegetables under discussion. That the physiological conditions which cause these diseases are initiated primarily by an inadequate, diminishing or unavailable oxygen supply seems to have been well-established in the results of the experimental work. The diseases considered are those that occur most commonly as the result of transport of vegetables in refrigerator cars or storage in commercial storage houses. A consideration of the conditions of temperature and aeration in the car and storage house together with a study of the reproduction of these diseases under controlled conditions of temperature and aeration warrants the conclusion that the primary physical cause of these diseases is sub-oxidation in cells most sensitive to changes in the oxygen concentration or availability. What the ultimate factor may be that causes the death of the cell is at present a problem for speculation.

It has been demonstrated that these breakdown diseases are a response to certain conditions prevailing under present methods of storing and transporting vegetables and that these conditions are inimical to the continued life of at least certain groups of cells or tissues. The range and fluctuations of temperature and aeration in the refrigerator car and storage house are certainly widely different from those to which plants are normally exposed. The confined spaces of the refrigerator car permit of but slight exchange of air, especially in the shipment of such vegetables as cabbage and potatoes which are not usually shipped under refrigeration and are, therefore, not subjected to the favorable influence of air exchange that occurs in the reicing of the cars at definite intervals. Practically the same conditions are prevalent in the storage house due to the methods of storing the vegetables in deep, unventilated bins or piling in heaps which prevent gas exchanges.

Bergman (4) has demonstrated the effectiveness of the refrigerator car in preventing free exchange of air. In his determinations of the air composition in iced dars loaded with strawberries he has shown that the CO_2 content may rise as much as 2.5 percent within eight hours after loading the car. No data are given as to the percentage of oxygen in the air. Considering the fact that the respiratory ration is at or near the minimum at temperatures approximating those of the refrigerator car, it is evident that the oxygen content of the air is very greatly reduced within a few hours after the cars are loaded. This condition must be more pronounced in loads of vegetables like potatoes and cabbage shipped in bulk and not ventilated. In Table 2 are given the results of gas analyses of cabbage stored in air-tight jars at a temperature of 18-20°C. The analyses show a very rapid consumption of the oxygen during the first twenty-four hours of storage. The much slower subsequent rate is probably due to the suppressing effect of carbon dioxide upon the respiratory processes and perhaps also to the relative unavailability of the small residual quantity of atmospheric oxygen.

The effect of low temperature in producing breakdown affords further indication of the role of sub-oxidation as a factor in the production of these storage diseases. The detrimental influence of low temperature upon certain plants has been observed by other workers. Molisch (14) noted the severe brown spotting of the leaves of certain tropical plants following a short exposure to temperatures of 1-4°C. He explained this type of breakdown as the result of the accumulation of toxic substances in the cells due to incomplete oxidation.

Harvey (9) has shown that tomato plants are injured or killed at a temperature of 5°C., and that this killing is not true freezing to death since there was no ice formation within the tissues. Black leaf speck of cabbage and redheart of cabbage and head lettuce have been produced at temperatures at or near 0°C. in the presence of an abundant supply of atmospheric oxygen. Black leaf speck and redheart occur in the commercial storage houses where the temperature is held just above freezing. Mention has been made of the occurrence of this type of leaf specking on chinese cabbage in the field following a sudden drop in temperature to near freezing. The same phenomenon has also been observed on cauliflower. The occurrence of black leaf speck on cabbage in the field in California as reported by Link (11) is probably correlated with low temperature.

Bennett and Bartholomew (3) have found that there is a progressive increase in the respiration rate as the temperature is lowered from 5° to 0°C. when potatoes are stored at these temperatures, and that at 0°C. the respiration rate is practically equal to that at 10°C. Blackheart was produced more readily at 2.5° and 0°C.than at 5°C. Gussow (8) reported blackheart in a pit of potatoes well ventilated but exposed to low temperatures. Bennett and Bartholomew, however, were not able to produce blackheart at low temperatures in the presence of oxygen. In their experiments no provision was made for the removal of the carbon dioxide produced and the suppressing effect of this gas upon the rate of respiration may account for the delay in the occurrence of blackheart until all the free oxygen was exhausted from the jars.

Potatoes in the commercial storage houses of Michigan, held at temperatures 3-4°C., are often seriously reduced in value late in the storage season by the surface pitting that disfigures the tubers. The relatively low temperature at which these houses are held is unquestionably not the only possible factor responsible for this trouble. Decreased oxygen supply in the bins is probably as important, or more so, than low temperature. The same may be true of the cabbage storage houses where black leaf speck occurs, although low temperature has been shown to cause this disease even in the presence of abundant atmospheric oxygen. Under transportation conditions decreased oxygen supply and low temperature are concerned and the influence of each will largely be determined by the degree of exclusion of fresh air, the temperature and the length of the haul.

At a temperature of 2-4° C. the respiratory ratio is at a minimum as shown in the results of Puriewitsch given by Palladin (17). At these temperatures oxygen absorption proceeds much faster than the elimination of carbon dioxide and, therefore, oxidation is not complete in the cells. The effect of low temperature must then be to slow up at some point the oxidation processes in the cell, although the quantity of atmospheric oxygen in the storage may continue to be depleted at a rapid rate. The process of oxidation of compounds in the cell may be so affected that toxic substances are formed which remain sufficiently long in the cell to cause injury to or death of the protoplast.

Air movement without renewal has not been effective in preventing these breakdown diseases. The rapidity with which black leaf speck was produced at low temperatures indicates that change of air may not be effective in preventing breakdown at temperatures near freezing. The negative results obtained by exposing sound heads of cabbage to the gaseous compounds produced by other heads stored in closed chambers seems further to indicate that intra-cellular compounds and not volatile compounds acting extra-cellularly are concerned in the production of the disease. In this respect these diseases differ from apple scald. Additional evidence of the dissimilarity of these sub-oxidation diseases and apple scald will be presented in the second series of papers dealing with fruit sub-oxidation troubles.

From an examination of the data obtained in the experimental work and observations of the occurrence of the various forms of these diseases, a satisfactory explanation can be framed for the variety of symptoms obtained under different storage conditions. It has been shown that surface pitting of potato tubers occurs in commercial storage houses in the absence of blackheart. The same thing is true of black leaf speck which is not necessarily an accompaniment of redheart. It was shown experimentally that under the most unfavorable conditions of storage redheart of cabbage might be produced. At a temperature of 37° C. redheart is produced very readily and undoubtedly is comparable to blackheart of potato. Redheart may also be produced at a temperature of 20° C. if the cabbage is stored long enough to deplete the oxygen. Specking of the outer leaves is a constant symptom also. In the potato experiments storage of sixty to seventy-two hours at a temperature of 20° C. in an atmosphere of nitrogen caused surface pitting of the tubers without the appearance of blackheart. Longer exposure resulted in both pitting of the surface and blackheart. Less severe conditions or shorter exposure cause surface injury before redheart or blackheart are produced.

As to the actual chemical changes in the cell which bring about death of the protoplasm only speculative ideas are available. Sub-oxidation in the cell brought about by low temperature, inadequate oxygen supply, or both, may result in the retention or accumulation in the cell of some toxic substance which affects enzyme activity and thus can not be removed by the normal processes of destructive oxidation. In this connection it is interesting to note that Bartholomew (2) was able to demonstrate an increase in the amino acids of potato tubers heated to produce blackheart. In 1910 Armstrong (1) and his co-workers demonstrated that under the influence of anaesthetic and certain substances called hormones reactions occur in the cells so as to bring the enzymes in contact with their substrata. One of the results of this mixing of enzymes and substrata was an oxidation which resulted in pigmentation. They also state that these phenomena are constantly occurring in the plant but that under normal conditions their products are passed off before becoming injurious. Under abnormal conditions these interaction products may accumulate and eventually cause the death of the cells. Sando (20) found that tomatoes, ripened under poor ventilation, were much higher in acidity and poorer quality in general than those ripened normally. Osterhout (16) in his work with anaesthetics showed that they caused a decrease in permeability which is reversible and that the subsequent increase is due to the accumulation of toxic substances. Pantanelli (18) has demonstrated that the cells of the endocarp of the Mandarin orange show a progressive increase in cell permeability when subjected to temperatures very near freezing.

All the diseases considered in this paper are found to occur under storage conditions where conditions are unfavorable for the normal oxidation processes in the cell. A general grouping of these troubles as sub-oxidation diseases seems desirable. As a tentative hypothesis, it is suggested that unfavorable temperature or oxygen supply, which are responsible for the sub-oxidation of compounds in the cell, bring about changes which result in the accumulation of toxic substances, perhaps acid in nature, and that these toxic substances exert a deleterious effect upon oxidative processes and eventually directly or indirectly cause the death of the protoplasm. The color changes which follow, resulting in black leaf speck, redheart, etc., are due to the mixing of the enzymes and their substrata.

Control

In attempting to prevent the development of storage diseases due to sub-oxidation it is necessary to have in mind the two important factors of causation, low temperature, and inadequate oxygen supply. Both are important and it can not safely be said that the regulation of one without attention to the other will control these diseases. A temperature of 0°C. with adequate ventilation may cause black leaf speck or redheart, while a temperature of 4-5°C. with the same ventilation may prevent their development.

Present methods of storage need to be modified so that stowing of vegetables in deep bins or solid heaps which prevent interchange of air is avoided. In the potato storage houses where surface pitting is being successfully combatted, air channels are being provided by the use of false floors and air chutes which carry fresh air up through the bins of tubers. This method of supplying oxygen to the tubers should be very effective in preventing losses from sub-oxidation troubles. The adoption of similar methods in the cabbage storage houses so that ventilation is secured in the heaps of heads will do much to prevent the development of black leaf speck and red-heart. In addition the temperature of the cabbage houses should be carefully regulated so that a mean of 3-5°C. be maintained in conjunction with good ventilation. This is especially important immediately after storage since it is probable that there may be some compensation for unfavorable conditions later in the season in thorough ventilation and well regulated temperature for a few weeks immediately following storage.

Potatoes appear most susceptible to breakdown troubles in late winter. This may be due to greater physiological activity of the tubers at that time or to the cumulative effects of unfavorable conditions during the long period of storage. Thorough ventilation should be supplied to prevent any possible predisposition to these trouble.

In the shipment of potatoes and cabbage in bulk some provision should be made for air circulation through the stowed vegetables themselves. False floors should be generally used and during cold weather periods when the temperature outside is too low to allow the ventilators being opened constantly during the journey they should be opened for a short time if possible at intervals. This is most important in the case of cabbage and cauliflower shipments from the Pacific coast and Florida.

The prevention of redheart in head lettuce is mostly a problem in the control of soft rot. Lettuce should not be held long in storage and the trimming away of the outer leaves attacked by soft rot organisms should be done frequently in case of delayed sale to prevent soft rot from developing and causing suffocation of the heart leaves of the head.

Summary

This paper presents the results of the investigation of certain non-parasitic diseases affecting crucifers, lettuce, and potatoes in storage and transportation.

Black leaf speck of crucifers is a disease commonly found on market and storage cabbage, cauliflower and other crucifers. It is important both as a storage and transportation trouble, often causing serious losses.

It may affect these crops in the field but is of minor importance as a field disease.

Redheart of cabbage and head lettuce occurs on these vegetables both in transportation and in storage. On cabbage redheart is often an accompaniment of black leaf speck when the latter is the result of very unfavorable storage conditions. Redheart in head lettuce mostly follows slimy soft rot but may be induced by prolonged storage at low temperature.

Surface injury to potato tubers in the form of numerous shallow pits is a disease similar in origin to black leaf speck and redheart. It is often a serious disease in commercial storage houses, may be troublesome in potatoes in transit and occasionally may cause much damage to the crop in the field.

All these diseases are symptoms of breakdown that occurs under the conditions of storage and transportation and are caused primarily by inadequate ventilation or by temperatures which prevent the utilization of the oxygen present.

These diseases may be produced under a wide range of temperature and air composition and both of these are important factors in causation.

Black leaf speck may be produced in the presence or absence of a supply of atmospheric oxygen if temperature conditions are favorable.

Air movement without renewal has not been effective as a preventive of breakdown and at low temperatures air composition appears to be less important than temperature as a cause of leaf speck and redheart of cabbage and lettuce.

Redheart of cabbage and head lettuce is apparently the expression of a severe reaction to prolonged or highly unfavorable storage conditions. Redheart may or may not be present in cabbage affected with leaf speck, its presence or absence being determined by the character or prolongation of exposure to the conditions that give rise to leaf speck.

Surface pitting of potato tubers may likewise be produced in the absence of blackheart by relatively shorter exposure to the conditions which produce blackheart. Surface injury of this type may be produced at either low or moderate temperatures.

The occurrence of surface injury of this type can not be used as an index of the conditions of storage which produce the injury, since it has been shown that this trouble may occur at either high or low temperatures, in the presence or absence of a supply of atmospheric oxygen.

These diseases appear to result from the incomplete oxidation of cell compounds, and reduction in oxygen pressure in storage and transportation and low temperature are factors which affect adversely the oxidation processes in the cell. It is suggested that toxic products accumulate in the cell because of sub-oxidation and that these toxic products are the cause of the death of the cell protoplasm.

As preventive measures it is suggested that storage methods be modified so that an adequate supply of atmospheric oxygen be always present and that the temperatures be adjusted so that this oxygen may be available to the cells.

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ACKNOWLEDGEMENTS

The writer is greatly indebted to Doctor G. H. Coons for helpful suggestions and criticisms given during the pursual of this investigation. For many suggestions and criticism of the manuscript the writer is indebted to Doctor E. A. Bessey.

P L A T E S

Plate I.

Black leaf speck on California cabbage

Fig.1. A head of cabbage with some of the outer leaves trimmed away to show the appearance of the black leaf speck lesions.

Fig.2. A head of California cabbage collected from a wholesale warehouse showing the appearance of the disease on outer leaves.

PLATE I.

Fig. 2.

Fig. 1.

Plate II.

Black leaf speck on a shipment of cauliflower from California

Fig.1. A crate of cauliflower taken directly from P. F. E. car No. 24 at Detroit in March 1921 and showing how black leaf speck affects trans-continental shipments of this vegetable.

Fig.2. A leaf and portion of a petiole from a head in the crate shown in Fig. 1, showing the characteristic lesions of black leaf speck.

PLATE II.

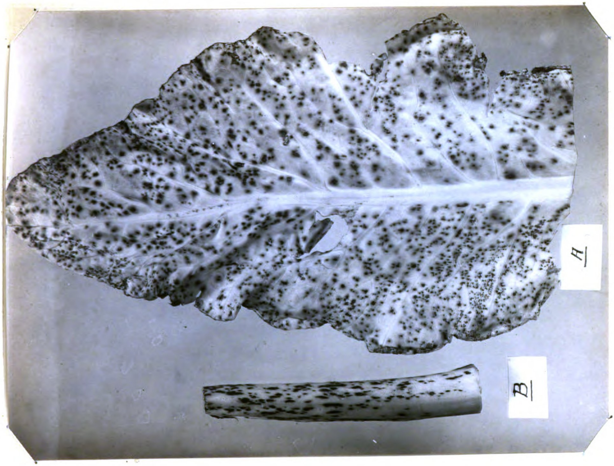

Fig. 2.

Fig. 1.

Plate III.

Black leaf speck on cabbage stored out of doors and from a transcontinental shipment.

Fig.1. Black leaf speck on a head of cabbage stored in pit out of doors. Note the small head developed from an axillary bud and also affected with the disease. The small size of the lesions is characteristic of cabbage stored in this way.

Fig.2. Black leaf speck on a leaf of cabbage from a California shipment showing the relatively large size of the lesions. These, in many cases, are due to the coalescence of smaller lesions.

PLATE III.

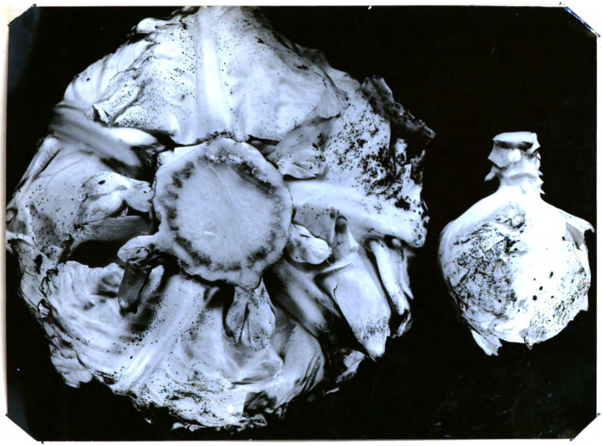

Fig. 1.

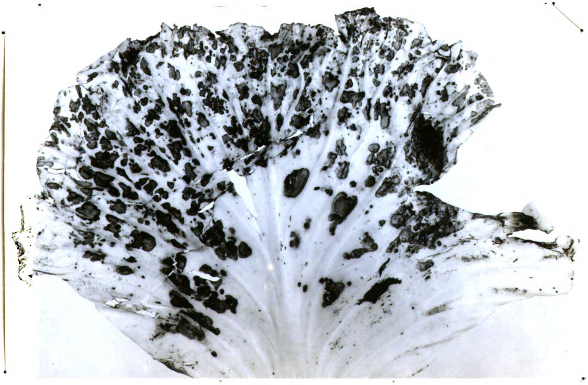

Fig. 2.

Plate IV.

Black leaf speck on Chinese cabbage. Developed on heads in field in September following a severe frost.

Fig. 1.

Plate V.

Black leaf speck produced in the laboratory

Fig. 1. Black leaf speck developed on a head stored in a tightly closed chamber at a temperature of 12° C. for thirteen days.

Fig.2. A similar head from the same lot with some of the outer leaves removed to show the effect of the disease upon the inner leaves.

PLATE V.

Fig. 2.

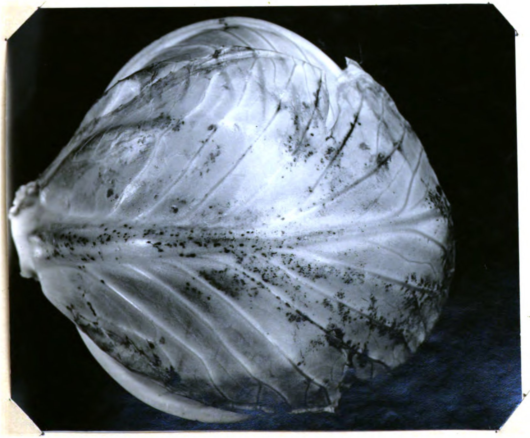

Fig. 1.

Plate VI.

Fig. 1. Apparatus used in some of the experiments to circulate air around the stored heads without affecting renewal.

Fig. 2. Leaf speck lesions developed on outer leaf of Iceberg lettuce following storage in Nitrogen for four days.

Fig. 3. Redheart of cabbage showing the reddened condition of the heart leaves and the appearance of a sound head from the same shipment.

PLATE VI.

Fig. 1.

Fig. 2.

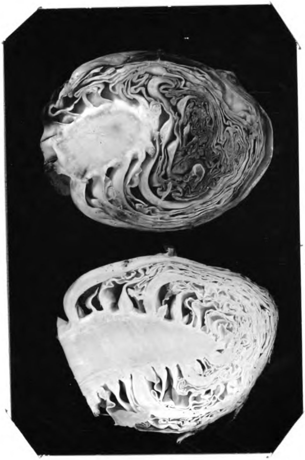

Fig 3.

Plate VII.

Redheart of head lettuce

Fig. 1. Redheart produced in Iceberg lettuce by storing the heads for four days in Nitrogen at a temperature of 20° C.

Fig. 2. Redheart produced in Iceberg lettuce by inoculating the heads with soft rot bacteria and storing at a temperature of 20° C. for twelve days.

Fig. 1.

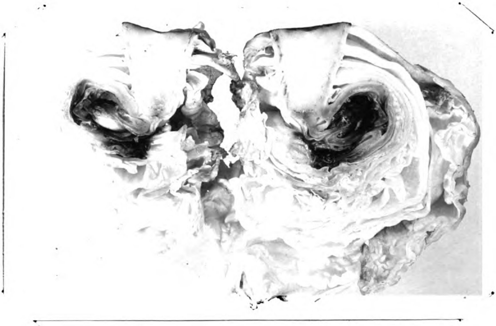

Fig. 2.

Plate VIII.

Surface breakdown of potato tubers

Fig. 1. A tuber from a shipment of Michigan potatoes to Chicago market in March 1922, showing the characteristic pitting of the surface by the breakdown lesions.

Fig. 2. Surface pitting produced on tuber by storage for five days in Nitrogen at a temperature of 20° C.

Fig. 3. Breakdown of the cortical tissues of the potato tuber and slight blackheart produced by storage in Nitrogen for five days.

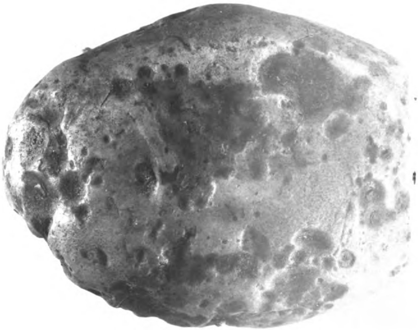

Fig. 1.

Fig. 3.

Fig. 2.