AN INVESTIGATION OF CERTAIN STORAGE DISORDERS OF CELERY

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INTRODUCTION

The production of celery in Quebec is, at the present time, not well enough developed to supply the market demands in the province. This state of affairs is not due to soil or climatic conditions. There is sufficient area of muck soil, favourably located in this province, to produce enough celery for local demands and the climate is suitable for celery growing. The cause of this state of affairs is that Quebec celery growers have suffered serious losses in the storing and marketing of their crops. For this reason, celery production is comparatively undeveloped, and the great muck soil wealth of the province has not been turned to celery growing.

In Quebec, the same as in other celery producing areas, the storage life of celery varies from year to year depending to a certain extent on climatic and cultural conditions during growth. It is also well recognized that, for long storage life, the celery must be free from diseases and at the proper stage of maturity at the time of storing. However, the development and prevalance of certain storage disorders seem to be the most important factors in limiting the storage life of celery in this province.

This study was undertaken to investigate the storage disorders of celery in Quebec. An attempt has been made: (1) to record the prevalence of storage breakdown at different stages in the storage period, (2) to learn the causes of these disorders and (3) to suggest preventive and control measures which might be adopted to prevent losses from these disorders in storage.

In order to make a complete study of such a problem as storage rots, it was found necessary that other related factors be considered. Accordingly, an endeavour has been made to correlate the occurrence of rots and breakdowns with environmental conditions in the field, during harvest and in storage.

OUTLINE OF THE PROJECT

The work in this investigation is divided into two main lines of endeavour:

(1) Practical experimental work and observation in the field and in storage.

(2) Laboratory experiments, which were carried on to study the causes and progress of development of certain effects observed in the field and storage work.

Field and Storage Experiments

In the field work, celery was grown on both mineral and muck soils and the keeping qualities were later compared in storage. In the field work also, experimental plots receiving different fertilizer and spray treatments were set out, and the progress of disease development was followed throughout both the growing and storage periods.

In some of the storage experiments, various treatments were applied at the time of storing. Celery which received similar treatment in the field, was treated differently at the time of harvest, and the progress of rots and breakdowns was recorded throughout the storage period.

Storage experiments included the following studies: -

(1) The value of various fungicidal treatments.

(2) Delayed versus immediate storage.

(3) Effect of cutting back of the foliage.

(4) Effect of storing plants while wet.

(5) Effect of maintaining free moisture on plants in storage by sprinkling them with water from time to time.

(6) Effect of washing celery with water under pressure previous to storing.

(7) Effect of frost on keeping qualities.

(8) The effectiveness of various copper sprays and Bordeaux mixtures in inducing a storage disorder known as "collapsed lesions."#

In connection with the storage experiments much data on storage conditions was taken. Hygrothermograph records were taken throughout the whole storage period in various locations in the storage room. The following studies on storage conditions were carried out: -

(1) The effect of placing the crates on stands versus the usual method of piling the crates one on top of

"Collapsed lesions" are described on page 22.

the other with scantling between.

(2) The effect of spacing the crates so as to allow for circulation of air and evaporation of moisture.

(3) Temperature and humidity of the air of the storage room as compared to the temperature and humidity in the centre of the celery crates.

(4) Temperature and humidity variations at different heights and at various locations in the storage room.

Throughout the storage period, a number of bacterial and fungal pathogens were isolated from diseased celery, and their pathogenicity tested by reinoculation and reisolation.

Laboratory Experiments

A series of experiments was carried on in the laboratory to determine the cause and the predispositional factors affecting the development of collapsed lesions. The effect of treating the plants with various chemicals on collapsed lesion development was investigated. Sections were made of collapsed lesions and their anatomy studied. Another experiment was carried on to determine whether or not the epidermis over collapsed lesions is intact. Studies were conducted on the effect of wounding the celery with needle punctures, emery dust, and steel wool on the development of

collapsed lesions. The object of these investigations was to determine whether or not the chemicals which induced collapsed lesions in the laboratory penetrated by way of the stomata, or if they were dependent on bruises and injuries as channels of penetration.

Experiments were conducted in the laboratory in constant temperature and humidity chambers to determine the effect of these factors on collapsed lesion development.

An attempt was made to induce collapsed lesions on growing plants in the greenhouse by spraying with copper sulphate, copper nitrate, and a series of Bordeaux solutions with varying proportions of lime and copper sulphate. The progress of development of this disorder was observed in the different spray treatments, and records were taken on the rate of recovery of the plants in each treatment.

Both laboratory and greenhouse experiments were conducted to determine if there was a difference in the capacity of various Bordeaux mixtures, including a series made up with lime and a similar series made up with magnesium oxide and lime combined, in inducing collapsed lesions on storage celery and on growing plants in the greenhouse.

The Bordeaux solutions used in the field, greenhouse and laboratory experiments were then tested for soluble copper by the sodium diethyldithiocarbamate method, and the photoelectric

colorimeter was used in measuring the different color intensities. An attempt was then made to correlate the amount of copper ion in the Bordeaux solutions, with the effectiveness of these solutions in inducing collapsed lesions. An attempt was also made to correlate hydrogen ion concentration of the various Bordeaux mixtures, with their effectiveness in inducing collapsed lesions on stored celery.

Along this line of investigation, a series of experiments was conducted to determine whether or not collapsed lesions are induced from copper sulphate and Bordeaux residues on the celery by penetration of copper. In these experiments, the copper sulphate and Bordeaux residues were removed by washing with 1.0% H_2O_2 and 0.2% HNO_3 respectively, at various time intervals after the spray treatments were applied. A correlation could then be made between time of exposure to the spray and subsequent intensity of collapsed lesion development.

Spectrographic determinations for copper were made on collapsed lesion tissue and healthy tissue from similar locations on the same petiole. The copper content of the collapsed tissue was compared with that of the healthy tissue by measuring the intensity of the copper lines in a photometer. It was not possible in the time allotted to develop the spectrographic method to a degree where quantitative

determinations could be made, but the method gave very satisfactory qualitative results, and showed beyond doubt that the copper content of the abnormal tissues was higher.

An attempt was made to induce collapsed lesions by chilling the celery for various lengths of time and then storing at 32° F. No success was attained and the characteristic pits which have been reported on tomatoes, stone fruits and grape fruit as a result of chilling were not found (26) (38) (5). The effect of various carbon dioxide concentrations on the development of collapsed lesions was also investigated.

A final laboratory experiment was concerned chiefly with the following lines of investigation: -

(1) Symptoms of freezing injury on celery.

(2) Variations in the length of time required to freeze different parts of the celery plant at a room temperature of 26° F.

(3) The actual freezing temperature of celery and how it varies for different parts of the plant.

(4) The keeping qualities of celery after it has been subjected to freezing temperatures.

The temperature determinations in this experiment were made by the use of thermocouples. The cold point was kept at a temperature of 0° C. and the difference in

temperature was measured by the deflection of a galvanometer, which was set up outside the cold chamber. Thus, it was possible to record accurately the temperature in various parts of the celery plant, without having to open the cold chamber, which would have affected its temperature.

REVIEW OF LITERATURE

The Storage of Celery in General

Investigators seem to agree, that for satisfactory cold storage of celery, the product must be free from diseases and at the proper stage of maturity, at the time of harvesting. Any diseased, injured or discoloured plants should be discarded. It is essential that all crated plants be cleaned and adequately pre-cooled before going into storage; otherwise they are subject to early decay. Platenius (31) stated that celery may be pre-cooled in an iced tank, spring water or by being placed temporarily in a cold room. Zinck (46) describes a method whereby celery is cleaned first by a warm water spray, after which it is passed through a water tank held at a temperature of 35° F. The crates are then drained and are ready for storage. Williams (44) advocates post-cooling as well as pre-cooling in the storage of vegetables. He states that vegetables should not be taken from a low to a high temperature, without being kept for several hours at an intermediate temperature (55 - 60° F.). His claim is that rapid heating and condensation on the surfaces of the vegetables are harmful and shorten the life of the produce after it is placed on the market.

It is also generally understood, that the storage life of celery varies from year to year, depending to a certain extent on climatic and cultural conditions during growth. Corbett and Thompson (11) have shown that nitrogen-fertilized celery is much more subject to attack by microorganisms than celery fertilized with phosphorus and potash. They claim that with celery heavily fertilized with nitrogen salts, the more resistant components of the middle lamella such as calcium pectate and pectose are rapidly changed to the less resistant pectic acid and pectin. Corbett and Thompson thought the changing of the calcium pectate of the cell wall to pectin and pectic acid decreased the mechanical resistance of celery as storage progressed. Curtis (14), on the other hand, states that there is no decrease in mechanical resistance of the celery tissues during storage. He claims that the vascular tissues show increased mechanical resistance and the collenchyma strands show no change. He suggests that the vascular bundles may get greater strength by lignification. He disproves the work of Corbett and Thompson on the basis of the unreliability of their method, and points out that the decreased mechanical resistance observed by them, may be due to softening from storage diseases. White-Stevens (41), in agreement with Curtis, failed to show any well defined correlation between pectic hydrolysis and storage maturity,

apart from the cytolytic effects of fungal and bacterial pathogens.

Curtis (14) found that high moisture supply produces stronger collenchyma tissue and therefore increases stringiness. Low moisture supply produces weaker collenchyma tissue and thereby decreases this defect, although the vascular bundles are stronger when the moisture supply is low. His experiment showed that plants,which were trimmed and wrapped,lost the least amount of water during storage.

There is a difference of opinion among writers as to the effect of soil on the quality of celery produced. Watts (39) says that celery grown on muck soil is inferior in quality to celery grown on upland soil. Thompson (37) believes that muck soil will produce celery of superior quality to that grown on mineral or upland soils. He adds that, to be of the best quality, celery must have a continuous growth and this is more likely to occur on muck soil than on mineral soil,owing to the better physical condition and the greater water-holding capacity of the muck soil.

There is also a variation in the length of storage life of different varieties of celery. Platenius et al. (30) have found that the late green varieties of celery usually have the longest storage period. White-Stevens states, it is possible,that such factors as variety, cultural treatment, time of planting and harvesting may affect the loss of sugars

markedly. Since sugar content is a major property of food value in stored celery,and since loss of sugars shortens the storage period, it can be easily understood why these factors affect the storage life of the product. Norton (27) states that quality in celery is primarily a question of the relationship of the parenchymatous tissue to the fibrous tissue. This is determined in part by varietal differences, and within varietal limits by certain external conditions such as soil moisture, plant food, rate of growth, light, warmth, pest control and blanching. Mills (25) claims that quality in celery is based on its flavour, crispness and lack of stringiness. He thinks that flavour is associated in some way with the chlorophyll in the petioles,because the green varieties are usually better flavoured than the self-blanching types. Flavour would then be associated with rate of blanching and type of celery. He agrees with most other investigators that lack of crispness, which is directly caused by low water content of the tissues, may be indirectly caused by too high temperatures during the growing season, too little nitrogen in the fertilizer, checking of growth due to disease, or by too rapid growth which matures the crop too early in the season.

Platenius et al. (30) point out that if the celery is harvested in good condition, the next step is to haul it

to the cold storage as soon as possible, in order to prevent wilting or heating. In the storage room, provision should be made for air circulation around the crates. The usual practice is to place 2" x 2" scantling on the floor under the crates and also between the tiers. Space should also be reserved between the rows, to ensure proper ventilation and circulation of the air. Celery should not be piled more than four or five crates high in ordinary storage rooms, and precaution should be taken to leave a space of at least two feet between the top of the highest crate and the ceiling of the room. Thompson (36) observed that celery stored in large crates, always began to decay in the centres of the crates; and also, that there was considerable injury to the celery due to breaking of the crates. In every instance, decay was much less in small partition crates than in the standard crates. Furthermore, celery in the upper tiers in storage showed a much larger proportion of decay at the end of the storage period than did celery in the lower tiers.

Physiological Breakdown of Celery in Storage

Translocation and breakdown of sugars due to respiration, are probably the most important factors influencing the gradual lowering in quality and deterioration of celery in storage. Rose *et al.* (32) emphasized the fact that fresh

fruits and vegetables undergo changes during harvesting and storage. The most important changes are produced by respiration, a process resulting in the liberation of carbon dioxide, water and heat by the product at the expense of carbohydrate, which is broken down into simpler compounds. The amount of breakdown and energy released increases with the temperature,and it might also be mentioned here,that injured produce respires much more rapidly tnan produce which is free from injury. Rose and his associates point out that celery releases nearly four times as much energy in the form of heat at a temperature of 40° F. as at a temperature of 32° F. Hence it is important that celery be held at 32° F. in storage.

Corbett and Thompson (11) first showed that associated with respiration of celery in storage is a translocation of carbohydrates from the outer to the inner petioles. White-Stevens (43) criticises their work on the basis that they omitted to determine the carbohydrate changes of the crowns in relation to those of the petioles ,and he states that they presented no concrete evidence that sugars moving from the outer petioles actually entered the hearts. White-Stevens first showed that sucrose content increased markedly in the early part of the storage period, ultimately declining with a simultaneous increase in hexose. The increase in hexose reached a maximum and it also

finally declined. Basing food value on sugar content, he states that the optimum storage period for celery at 32° F. is between 60 and 100 days. He indicates a possibility that factors such as variety, cultural treatment and harvesting may markedly influence the reduction, translocation and respiration of sugars.

White-Stevens (43) found that hexose sugars decline in the outer petioles and crowns, but increase in the inner petioles. Disaccharides decrease quite rapidly in both inner and outer petioles, while in the crowns they increase markedly at first and subsequently decline rapidly. Polysaccharides decline slowly in both inner and outer petioles, but fluctuate in the crown, ultimately declining there also. Total carbohydrates calculated as hexose, decline in the outer petioles quite steadily. In the inner petioles, they fluctuate at first but, in general, show a decline as is the case in the outer petioles. This translocation of carbohydrates together with respiration, he thought to be responsible for decreasing the concentration of solutes in the cell sap; or it was, as he himself termed it, the "lowering of the osmotic value". Lowering of the osmotic value was more rapid in the outer petioles than in the inner petioles, and also, more rapid in the central than in the outer cells of an individual petiole. White-Stevens found that this lowering of the concentrations of solutes in the cells, is concurrent with the development of pithiness.

Platenius et al. (30) point out that chemical changes are likely to be most responsible for lowering of the eating quality of vegetables such as celery, which owe their taste largely to high sugar content.

Bourque (6) found that hexose sugars increased gradually until the end of the storage period, while sucrose showed a slight decline.

Young (45) records a definite movement of material from the outer leaf stalks to the heart stalks of Pascal celery. He claims that the outer petioles are of definite value in promoting the growth of the inner petioles during storage.

Besides the natural physiological breakdown of celery, due to respiration, certain other definite disorders in storage must also be classed as physiological breakdowns, because they are not caused by pathogenic organisms. These disorders have been termed collapsed lesions, and basal rot in this investigation. The symptoms of these disorders are described later in this paper, and they are shown to be due to the toxicity of Bordeaux residues, which become chemically activated by the high humidity of the storage room. In the present review of literature, it is sufficient to state that to date, very little has been published on these disorders and their cause has not been determined. White-Stevens (43)

described the symptoms of collapsed lesions and suggested that frost might be the causal factor. Both White-Stevens (40) and Ayers (2) were unable to isolate pathogenic organisms from these disorders.

Storage Rots Caused by Pathogenic Organisms

Very little has been done relative to the actual pathogenic rots of celery in storage. White-Stevens (40) found four fungal and eight bacterial types of organisms causing decay in stored celery. Fungi found by White-Stevens to be pathogenic included Botrytis spp. (three strains of the cinerea type), Sclerotinia sclerotiorum (two strains), Alternaria sp. and Cladosporium sp. All Botrytis forms isolated were found to make slight growth on potato-dextrose agar in 10 days at -6° C. and growth was slow at -0.6° C. Similar results were obtained upon inoculation into celery tissue. Sclerotinia sclerotiorum did not develop at 0° C. and growth at 3° C. was slow. White-Stevens concluded that Sclerotinia sclerotiorum will not develop in storage at 0° C. Neither Alternaria sp. nor Cladobotryum sp. were found to grow at 0° C. The late blight organism Septoria apii, although not inducing storage decay, was studied also by White-Stevens. No growth was detected at 1.75° C. in 24 days.

Bacterial organisms isolated and found pathogenic by White-Stevens were of the Erwinia caratovora type and fell into the genera Phytomonas and Erwinia S.A.B. Five of the eight types were found to make slight growth at -0.6° C. in beef-peptone broth at the end of 129 hours.

Ayers (2) isolated the same organisms and found that all except Sclerotinia sclerotiorum and Septoria apii showed active growth in culture at -0.5° C. He also found that the growth rate was more than twice as rapid in cultures placed inside crates as in those near the outside. He explained this by his finding that the temperature in the outer rows of a crate is approximately 0° C., while the temperature at the centre is usually more than 1° C. above this.

The review of literature presented above includes all the work which is concerned definitely with the storage of celery. In some instances however, it was found necessary, in connection with certain methods and procedures used in these investigations, to review additional literature, which had no direct bearing on the storage of celery. This literature will be cited later in the appropriate places in this paper.

FIELD AND STORAGE EXPERIMENTS 1937-1938

The objects of these experiments was to determine whether or not the development of storage disorders is affected by: -

(1) The kind of soil on which the celery was grown.

(2) The field fertilizer treatment.

(3) Subjection to field frosts before harvesting.

(4) Fungicidal treatments at the time of storing.

(5) Holding the celery at room temperature for three days before storing.

(6) Trimming or cutting back of the foliage.

(7) Thoroughly washing with water previous to storing.

(8) Maintaining free moisture on the plants throughout the storage period.

Materials and Methods

The celery used in these experiments was grown within a 25 mile radius of Montreal.

One hundred and forty-eight crates in all were used; one hundred and forty-four of these were grown on the muck soils of the Ste. Clothilde area and four crates, grown on mineral soil, were obtained from M. Achille Lafrance at Marieville. The Ste. Clothilde muck soil

celery was purchased from the following growers and received the following field treatments: -

Grower	No. of crates	Treatment in field
Girard Ampleman	103	Sprayed in field, regular fertilizer treatment.
Girard Ampleman	32	Sprayed in field; from a nutritional experiment using a series of fertilizer treatments with different amounts and proportions of nitrogen, phosphorus and potash.
Monsieurs Potvin and Cardinal, St. Remi	9	Unsprayed in the field (regular fertilizer treatment).

All of the celery, except 12 crates which were subjected to field frosts, was harvested on September 29 and stored on September 30. Four crates of frosted celery were harvested on the day following each of the first three severe fall frosts. The frosts occurred on the following dates: October 5, October 9 and October 11.

The celery was stored in the Harbour Cold Storage in Montreal in a room held at an average temperature of 32° F. and an average relative humidity of 96%. The crates were placed on stands and well spaced so that ample room was afforded for air movement and for making observations. Further data on storage conditions are recorded

under the heading "Conditions in the Harbour Cold Storage Warehouse" on page 49.

This celery was treated in various ways at the time of storage as indicated in Tables I to VIII. Four crates were used to each treatment, and these were examined at four different time intervals throughout the storage period.

Development of the Storage Disorders and the Method used in Recording Their Severity.

Collapsed lesions were the first disorders observed in storage in the fall of 1937. The celery was stored on September 29. On October 26, it was observed that collapsed lesions had already begun to develop on the celery treated with 1% $CuSO_4$ and 4-4-40 Bordeaux at the time of storage. On November 4, collapsed lesions were found on celery that had been sprayed with Bordeaux in the field, but had received no treatment at the time of storage. The check plants at this time did not show any collapsed lesion development. This indicates that the $CuSO_4$ and Bordeaux intensified and increased the rate of development of collapsed lesions. "Collapsed lesions" is a term given to depressed areas, which occur on either side of the petioles, and are confined chiefly to the region below the first node. Those on the convex side are elongated with the vertical axis of

the petiole, and the depressed area is restricted to parenchymatous tissue lying between the collenchymatous ridges. On the concave side of the petiole, the lesions are more circular in outline with the epidermis loosened, but unruptured on the surface. In all cases collapsed lesions develop a nut-brown colour and an unsightly appearance, and their development may be severe enough to render the plants unmarketable.

On November 4, some foliage rot and basal rot were also observed on the celery treated with $CuSO_4$ and Bordeaux. Foliage rot and basal rot seemed to develop simultaneously. At that time, foliage rot had appeared as small necrotic spots on the foliage, and basal rot showed merely as a browning of the vascular bundles, which could be seen only by breaking the petiole at the base. The foliage rot which developed in the early part of the storage period is due entirely to Bordeaux injury. At this time it seems the micro-organisms are incapable of initiating infection. They do however, gain entrance through the lesions caused by Bordeaux injury and so cause secondary infection. As time goes on the celery tissue becomes less turgid and its resistance to attack is probably weakened by respiration. The result is that at the end of six weeks storage the micro-organisms become capable of causing the primary infections

and from that time on, foliage rot progresses very rapidly. On November 9, it was observed that collapsed lesions, basal rot and particularly foliage rot were making rapid advance in the crates treated with Bordeaux and $CuSO_4$. Foliage rot had caused the leaves to become wilted and soft and bent down over the sides of the crates. Basal rot at this time, appeared as sunken, greenish depressions around the base of the petiole. An attempt was made at this time, to isolate pathogenic organisms from all three disorders with no success. Foliage rot did not develop in the check crates until six weeks after storing and no collapsed lesion development was recorded in these crates at that time. Thus it appears: (1) that collapsed lesions and basal rot are not caused by pathogenic organisms, (2) that pathogenic organisms are not responsible for the primary infections of foliage rot in the first six weeks of storage, and (3) that development of all three storage disorders, namely, foliage rot, collapsed lesions and basal rot is greatly increased by the application of 1% $CuSO_4$ and 4-4-40 Bordeaux sprays at the time of storage.

Four complete examinations, using one crate of each treatment at each examination, were made throughout the storage period at the following dates: December 4, December 18, January 4 and January 19. Brief examinations

without involving the discard of any crates, were also made at intervals of one week throughout the storage period. At each of the four complete examinations, a crate was opened and each plant was scored for the following storage disorders: late blight, stem cracking, foliage rot, collapsed lesions, and basal rot. The prevalence and intensity of these disorders was recorded by examining each plant in all crates opened. The figures 0, 1, 2 and 3 were used in this connection as class values and have the following significance:

0 - 100% marketable

1 - 75% marketable

2 - 50% marketable

3 - Unmarketable

The average degree of severity of each disorder was calculated as follows:- The class values (figures of intensity) were multiplied by the number of plants so infected (figures of prevalence) and all of such products from a single crate were added together and the sum was divided by the number of plants in the crate. An example may be outlined as follows: -

There are 50 plants in a crate.

20 are 100% marketable, having a class value of 0

20 are 50% marketable, having a class value of 2

10 are unmarketable, having a class value of 3

$$20 \times 0 = 0$$

$$20 \times 2 = 40$$

$$10 \times 3 = \underline{30}$$

$$70$$

$$70 \div 50 = 1.4$$

This figure (1.4) is an average figure of severity for a single examination. The figures recorded in the tables were arrived at by averaging the results of the four examinations and they designate the average severity of each disorder, in each treatment, over the whole storage period.

Results

The results of these experiments are recorded in Tables I to VIII.

Table I - Effect of applying various fungicidal treatments at time of storage upon development of storage disorders - Plants sprayed in field.

Fungicidal treatments	Disorders expressed as degrees of severity				
	Late blight	Stem cracking	Foliage rot	Collapsed lesions	Basal rot
1% sodium benzoate (dip)	0.00	0.03	2.99	0.33	0.40
5% sodium bicarbonate (dip)	0.00	0.04	2.98	0.50	1.54
1% borax + 1-300 formalin (dip)	0.00	0.06	1.42	0.56	0.83
1% borax + 1% chloride of lime (dip)	0.00	0.10	1.57	0.50	0.24
2% borax (dip)	0.00	0.00	1.69	1.02	1.45
1-300 formalin (dip)	0.00	0.08	1.56	0.29	0.80
1% $CuSO_4$ (dip)	0.00	0.00	2.74	2.99	1.98
4-4-40 Bordeaux (dip)	0.00	0.00	1.51	2.32	0.34
Nickel hydrate (dust)	0.00	0.00	1.36	0.13	0.93
Sulphur (dust)	0.00	0.05	1.09	0.03	0.81
Check - no treatment	0.00	0.00	1.21	0.11	0.98

Table II - Effect of time of storing after harvest upon development of storage disorders - Plants sprayed in field.

Time of storing after harvest	Disorders expressed as degrees of severity				
	Late blight	Stem cracking	Foliage rot	Collapsed lesions	Basal rot
Plants held 3 days at room temperature before storing	0.00	0.20	2.09	0.17	0.36
Stored immediately	0.00	0.05	1.59	0.18	0.94

Table III - Effect of cutting back of the foliage before storing on subsequent development of storage disorders - Plants sprayed in field.

Treatment	Disorders expressed as degrees of severity				
	Late blight	Stem cracking	Foliage rot	Collapsed lesions	Basal rot
Short trim ($\frac{1}{4}$ of leaf blades removed)	0.00	0.29	1.55	0.20	1.10
Long trim ($\frac{1}{2}$ of leaf blades removed)	0.00	0.12	0.71	0.30	0.65
Check - no treatment	0.00	0.23	1.50	0.18	0.91

Table IV - Effect of washing plants, of wetting plants at time of storage, and of maintaining free moisture on plants in storage on development of storage disorders - Plants sprayed in field.

Treatment	Disorders expressed as degrees of severity				
	Late blight	Stem cracking	Foliage rot	Collapsed lesions	Basal Rot
Watering only at time of storage	0.00	0.59	1.75	0.15	0.60
Watering throughout storage	0.00	1.23	2.33	0.20	0.34
Washed	0.00	0.57	2.16	0.82	0.93
Check - no treatment	0.00	0.03	2.18	0.18	1.20

Table V - Effect of subjection to field frosts previous to storing on development of storage disorders - Plants sprayed in field

Treatment	Disorders expressed as degrees of severity				
	Late blight	Stem cracking	Foliage rot	Collapsed lesions	Basal rot
Received one frost	0.10	0.00	1.93	0.21	0.72
Received two frosts	0.95	0.03	2.00	0.27	0.58
Received three frosts	1.20	0.09	1.82	0.23	0.42
Check - no treatment	0.02	0.00	1.18	0.09	0.55

Table VI - Effect of soil on which celery was grown in field on development of storage disorders - Plants sprayed in field.

Treatment	Disorders expressed as degrees of severity				
	Late blight	Stem cracking	Foliage rot	Collapsed lesions	Basal rot
Mineral soil	0.00	0.14	0.64	0.25	0.69
Muck soil	0.00	0.09	1.71	0.16	0.97

Table VII - Effect of fertilizer treatments in the field on development of storage disorders - Plants sprayed in field.

Treatment	Disorders expressed as degrees of severity				
	Late blight	Stem cracking	Foliage rot	Collapsed lesions	Basal rot
Fertilizer Formula N - P - K					
0-0-0	0.00	0.00	1.24	0.05	0.45
4-0-0	0.00	0.03	1.01	0.07	0.65
0-0-32	0.00	0.00	1.19	0.03	0.55
0-16-0	0.00	0.00	1.67	0.08	0.46
4-0-32	0.00	0.00	1.97	0.03	1.05
0-16-32	0.00	0.00	1.97	0.14	0.87
4-16-0	0.00	0.07	1.80	0.08	0.78
4-16-32	0.00	0.07	1.98	0.56	1.26

The data recorded in Tables I to VII concern celery which received Bordeaux sprays at regular intervals in the field for the control of late blight. The treatments shown in Table VIII were applied at the time of storage to celery which received no spray treatments in the field.

Table VIII - Effect of treatments applied at time of storing to celery which was not sprayed in the field.

Fungicidal treatments	Disorders expressed as degrees of severity				
	Late blight	Stem cracking	Foliage rot	Collapsed lesions	Basal rot
1% $CuSO_4$ (spray)	0.00	0.00	1.82	1.97	0.21
4-4-40 Bordeaux (spray)	0.00	0.00	2.21	2.16	0.57
1% $CuSO_4$ (dip)	0.00	0.00	3.00	3.00	2.32
4-4-40 Bordeaux (dip)	0.00	0.00	2.29	2.90	0.69
Check - no treatment	0.00	0.08	1.68	0.05	0.05

Observations and Analysis of Recorded Data

Collapsed lesions, basal rot and foliage rot are the three storage disorders with which we are mostly concerned. Collapsed lesions and basal rot are not caused by pathogenic organisms. Pathogenic organisms are not responsible for the primary infections of foliage rot in the first six weeks of storage. Development of all these disorders is greatly increased by the application of 1% $CuSO_4$ and 4-4-40 Bordeaux sprays at the time of storing.

Foliage rot first appears as small necrotic spots on the leaf blades. It progresses slowly in the first six weeks of storage but later may involve the entire petiole. The small necrotic spots are due to the toxicity of Bordeaux residues and they afford entrance for micro-organisms which cause secondary rot. The action of micro-organisms on stored celery, however, is not entirely secondary because after six weeks of storage the organisms cause primary infection. After this time foliage rot develops rapidly. The organisms most responsible for foliage rot are three species of bacteria of the Bacillus caratovorus type, three species of Botrytis, an Alternaria sp. and Sclerotinia sclerotiorum.

The data in Table I show that sulphur dust is the only fungicidal treatment which proved significantly better

than the check, taking all storage disorders into consideration. The nickel hydrate dust treated plants were about the same as the checks, but all other treatments appeared to increase the severity of one or more of the storage disorders. Foliage rot was most severe with the sodium benzoate, sodium bicarbonate and $CuSO_4$ treatments, and least severe on the check and sulphur dust treatments. The severity of collapsed lesions was increased greatly by $CuSO_4$, Bordeaux and borax treatments, but was slightly reduced by sulphur and nickel hydrate dusts. Basal rot was increased by copper sulphate, sodium bicarbonate and borax, but the borax + chloride of lime treatment decreased the amount of basal rot quite appreciably.

The data in Tables I and VIII show simultaneous development of foliage rot, collapsed lesions and basal rot, that is, in treatments where one of the disorders was more severe, the others were likewise more severe. Both $CuSO_4$ and Bordeaux treatments very significantly increased the severity of all three disorders, and dipping the celery in these solutions was more effective than spraying with the same solutions in increasing the severity of foliage rot, collapsed lesions and basal rot.

Delaying storage for three days after harvest, increased the amount of foliage rot and shortened the length

of time for profitable storage (Table II). Delay in storing also very significantly decreased the amount of basal rot.

Cutting back of the foliage did not alter the storage life of the plants (Table III).

Storing the plants while wet decreased the severity of basal rot, but had no effect on the occurrence of collapsed lesions or foliage rot. Maintaining free moisture on the plants in storage, by sprinkling them with water from time to time, decreased the severity of basal rot, had no effect on collapsed lesion development and slightly increased foliage rot. Washing the celery with water under pressure previous to storing, increased slightly foliage rot and basal rot, and very markedly collapsed lesions (Table IV).

Celery receiving one, two and three frosts, suffered more severely from foliage rot and collapsed lesions than did celery stored before frosts occurred (Table V).

There was no difference in the keeping quality of celery grown on mineral and muck soils (Table VI).

Foliage rot, collapsed lesions and basal rot were more prevalent on celery which received a complete fertilizer mixture, than on celery which received a fertilizer having one or two of the three essential components lacking. From the results of this experiment, it was not possible to determine the relative or absolute importance of each of the three main elements supplied in the fertilizer; namely,

nitrogen, phosphorus and potash; in affecting the prevalence of storage disorders (Table VII).

There seemed to be no correlation between the occurrence of stem cracking and the development of collapsed lesions. One might expect that the stem cracks would provide an excellent means of penetration for any chemicals, which might induce or increase the prevalence of collapsed lesions. However, this did not seem to be the case; and the records taken show that stem cracking may be quite severe, while collapsed lesions are not of any great significance.

Stem cracking and late blight did not make any advance after the celery was stored. Development of both these disorders was confined to the field. Stem cracking is a nutritional disorder caused by lack of boron and does not develop after active growth ceases.

Pithiness was not a factor in the keeping of celery during the 1937-38 season. Several plants might have been recorded as pithy, but this disorder caused no significant reduction in marketability.

FIELD AND STORAGE EXPERIMENTS 1938-1939

The object of these experiments was to determine the effect of various Bordeaux sprays on: -

(1) Disease development in the field.

(2) Keeping quality in storage.

Observations made on celery in storage during the winter term of 1937-38, suggested very strongly that three very important storage disorders; namely, collapsed lesions, basal rot and a foliage rot (not fungal or bacterial) might be caused by the dissolution of copper from Bordeaux residues. At this time collapsed lesions had never been recorded in the field, but no definite observations along this line had been made during the first year of this investigation. Thus, it was in order to carry on an experiment to determine whether or not these storage disorders occurred in the field as well as in storage; and also, if changing the proportions of copper sulphate and lime in the Bordeaux mixtures would affect their development in storage. This experiment at the same time made it possible to try out another series of Bordeaux mixtures with like concentrations of copper sulphate, but containing magnesium oxide as a partial substitute for hydrated calcium lime.

Materials and Procedure

An acre of celery grown on muck soil at Macdonald College was divided into plots, and these plots were sprayed at approximately weekly intervals throughout the summer with the following Bordeaux mixtures: 2-6-50, 4-6-50, 8-6-50, 4-3-50 and 4-12-50. These mixtures read in the following order: $CuSO_4$ - hydrated lime - water. Another series of plots were sprayed with Bordeaux mixtures of the same formulae, but having three parts by weight of calcium hydroxide to one part of magnesium oxide. (Ca 2-6-50, Ca 4-6-50, etc. indicate Bordeaux mixtures made up with calcium hydrate alone and similarly Mg 2-6-50, Mg 4-6-50,etc. indicate Bordeaux mixtures made up by using one part of MgO to three parts by weight of calcium hydrate). The acre of celery consisted of 27 rows, of which 7 were unsprayed checks, and the remainder were allotted to the 10 spray treatments at the rate of two rows to each treatment. In the fall of 1938, four crates from each treatment and 25 crates from the 7 check rows were harvested. The 40 crates from the spray treatments were labelled and placed in storage for observation without further treatment. The 25 crates from the check rows received the following treatments at the time of storage: -

Treatment	No. of crates
Ca 2-6-50 - spray	1
Ca 4-6-50 - spray	1
Ca 8-6-50 - spray	1
Ca 4-3-50 - spray	1
Ca 4-12-50 - spray	1
Mg 2-6-50 - spray	1
Mg 4-6-50 - spray	1
Mg 8-6-50 - spray	1
Mg 4-3-50 - spray	1
Mg 4-12-50 - spray	1
Long trim ($\frac{1}{2}$ of leaf blades removed)	3
Short trim ($\frac{1}{4}$ of leaf blades removed)	3
Watering only at time of storage	3
Watering throughout storage period	3
Check - no treatment at time of storage	3

The progress of development of storage disorders was recorded at various intervals throughout the storage period.

Method of Recording Severity of Storage Disorders

Four complete examinations, using one crate of each treatment at each examination, were made on the celery harvested from the field experiment. These examinations were made at the following dates: December 6, December 22, January 4 and January 20. With the celery treated at the time of storing, there were less than four crates in each treatment, and consequently, the number of examinations was limited accordingly. At each examination, a crate was

opened and each plant was scored for the development of the following storage conditions: late blight, stem cracking, foliage rot, collapsed lesions and basal rot. However, in the 1938-39 storage period, only foliage rot and collapsed lesions were of importance, and hence, they are the only disorders for which figures are recorded.

The prevalence of these disorders is recorded in Tables IX to XIII as percentage of the plants infected. The intensity of development of each disorder is recorded according to the class values 0, 1, 2, and 3, the significance of which is given as percentage marketability on page 25.

Results

The results of these experiments are recorded under headings A, B and C, and in Tables IX to XIII.

A. Development of Storage Disorders on Celery Harvested from the Field Experiment

Table IX - Effect of composition of Bordeaux mixtures used in the field on development of storage disorders.

Bordeaux formula	Date of examination	Intensity and prevalence of storage disorders			
		Foliage rot		Collapsed lesions	
		Degree	%	Degree	%
Ca 2-6-50	Dec. 6	.03	3.0	0.02	2.0
	Dec. 22	1.40	100.0	1.00	84.3
	Jan. 4	1.55	100.0	-	-
	Jan. 20	2.50	100.0	-	-
Ca 4-6-50	Dec. 6	1.00	50.0	0.26	19.2
	Dec. 22	1.20	100.0	-	-
	Jan. 4	2.00	100.0	-	-
	Jan. 20	2.60	100.0	-	-
Ca 8-6-50	Dec. 6	0.00	0.0	0.20	15.6
	Dec. 22	0.82	62.0	1.70	100.0
	Jan. 4	1.70	100.0	-	-
	Jan. 20	2.30	100.0	-	-
Ca 4-3-50	Dec. 6	0.00	0.0	0.20	15.0
	Dec. 22	1.00	95.5	1.55	83.5
	Jan. 4	1.70	100.0	-	-
	Jan. 20	2.50	100.0	-	-
Ca 4-12-50	Dec. 6	0.00	0.0	0.11	21.1
	Dec. 22	1.10	99.0	1.00	86.4
	Jan. 4	1.65	100.0	-	-
	Jan. 20	2.60	100.0	-	-
Mg 2-6-50	Dec. 6	0.07	7.0	0.02	2.0
	Dec. 22	1.00	100.0	0.51	37.7
	Jan. 4	1.45	100.0	-	-
	Jan. 20	2.30	100.0	-	-
Mg 4-6-50	Dec. 6	0.00	0.0	0.15	15.0
	Dec. 22	1.10	100.0	0.90	89.3
	Jan. 4	1.80	100.0	-	-
	Jan. 20	2.40	100.0	-	-

Table IX (continued)

Bordeaux formula	Date of examination	Intensity and prevalence of storage disorders			
		Foliage rot		Collapsed lesions	
		Degree	%	Degree	%
Mg 8-6-50	Dec. 6	0.04	4.0	0.18	14.0
	Dec. 22	1.00	95.5	1.20	86.6
	Jan. 4	2.23	100.0	-	-
Mg 4-3-50	Dec. 6	0.04	2.0	0.14	14.0
	Dec. 22	1.10	99.0	1.20	87.6
	Jan. 4	1.83	100.0	-	-
	Jan. 20	2.40	100.0	-	-
Mg 4-12-50	Dec. 6	0.04	4.0	0.30	26.0
	Dec. 22	1.10	100.0	1.10	83.7
	Jan. 4	1.70	100.0	-	-
	Jan. 20	2.10	100.0	-	-
Check - no treatment	Dec. 6	0.06	6.0	0.00	0.0
	Dec. 22	1.40	100.0	0.16	16.0
	Jan. 4	2.10	100.0	-	-

B. Development of Storage Disorders on Celery Which Was Treated at the Time of Storing.

Table X - Effect of application at the time of storage, of Bordeaux mixtures containing calcium and magnesium lime on the subsequent development of foliage rot and collapsed lesions during storage.

Bordeaux formula	Date of examination	Intensity and prevalence of storage disorders			
		Foliage rot		Collapsed lesions	
		Degree	%	Degree	%
Ca 2-6-50	Dec. 6	1.0	100.0	1.14	66.0
Ca 4-6-50	Dec. 6	1.0	100.0	2.10	91.0
Ca 8-6-50	Dec. 6	1.3	100.0	1.50	100.0
Ca 4-3-50	Dec. 6	0.9	85.0	1.60	100.0
Ca 4-12-50	Dec. 6	1.0	100.0	1.30	100.0
Mg 2-6-50	Dec. 6	1.4	100.0	1.60	100.0
Mg 4-6-50	Dec. 6	1.3	100.0	1.80	100.0
Mg 8-6-50	Dec. 6	1.1	100.0	1.90	100.0
Mg 4-3-50	Dec. 6	0.9	90.0	1.60	100.0
Mg 4-12-50	Dec. 6	1.2	100.0	1.60	100.0

Table XI - Effect of cutting back of the foliage on development of foliage rot and collapsed lesions during storage.

Treatment	Date of examination	Intensity and prevalence of storage disorders			
		Foliage rot		Collapsed lesions	
		Degree	%	Degree	%
Short trim ($\frac{1}{4}$ of leaf blades removed)	Dec. 6	0.15	15.0	0.02	2.0
	Dec. 22	1.07	100.0	0.09	9.0
	Jan. 4	1.70	100.0	-	-
Long trim ($\frac{1}{2}$ of leaf blades removed)	Dec. 6	0.20	20.0	0.24	19.7
	Dec. 22	1.60	92.0	0.10	10.0
	Jan. 4	1.40	100.0	0.03	3.0

Table XII - Effect of storing plants while wet, and of maintaining free moisture on the plants in storage on development of storage disorders.

Treatment	Date of examination	Intensity and prevalence of storage disorders			
		Foliage rot		Collapsed lesions	
		Degree	%	Degree	%
Storing plants while wet	Jan. 4	1.60	100.0	-	-
	Jan. 20	2.75	100.0	-	-
Sprinkling of plants in storage at intervals of one week	Jan. 4	1.40	100.0	-	-
	Jan. 20	2.60	100.0	-	-

C. Development of Storage Disorders on Celery Which Received No Treatment in the Field or at the Time of Storing.

Table XIII - Effect of applying no treatment either in the field or at the time of storing on development of storage disorders.

Treatment	Date of examination	Intensity and prevalence of storage disorders			
		Foliage rot		Collapsed lesions	
		Degree	%	Degree	%
Check - no treatment	Dec. 6	0.06	6.0	0.0	0.0
	Dec. 22	1.40	100.0	0.16	16.0
	Jan. 4	2.10	100.0	-	-

Observations and Conclusions

Collapsed lesions and foliage rot were the only disorders of any importance in the 1938-39 storage period. Basal rot was found on only a few plants, and these were distributed evenly throughout all treatments (one or two plants in each crate at the last two examinations). Thus, there was no indication that any treatment increased or decreased the severity of basal rot. Late blight and stem-cracking were not recorded in a single case.

Foliage rot, as referred to heretofore, is a decay which begins in the leaf blades and gradually works its way down into the petioles. In the 1937-38 storage period, a large percentage of the foliage rot was caused by three fungal organisms, namely, Botrytis spp., Sclerotinia sclerotiorum and Typhula spp. In the 1938-39 storage period, these three fungi were not observed in a single case and all of the foliage rot was due either to Bordeaux injury or to bacteria.

Collapsed lesions, as in the 1937-38 session, were the first storage disorders observed. Particularly was this so, with the celery which received Bordeaux spray treatments at the time of storing. On December 6, the plants in these crates (harvested on October 6) showed a great deal of damage. Collapsed lesions, as usually observed, are more prevalent on the concave than on the convex side of the petiole; but another peculiarity was that in this case, the collapsed lesion development was confined almost entirely to the convex or outer sides of the petioles. The disorder appeared to be a very extensive development of collapsed lesions and it has been recorded as such. Sometimes, practically the whole outer surface of the petiole was involved, and it was further observed that development of this disorder was most severe on the outermost petioles and diminished inward. There was

little or no development on the innermost petioles or on the inner surfaces of all petioles. On December 6 also, it was observed that foliage rot was more severe in this lot of celery than in other lots. As in the previous storage season, it was observed that collapsed lesions began to develop shortly after the celery was stored and this development continued until a maximum was reached about December 22. After this time, no further development was recorded.

From the recorded results of the combined field and storage experiments, the following observations were made: -

(1) There was slight development of collapsed lesions on celery which received no spray treatment either in the field or at the time of storage (Table XIII). This shows that collapsed lesions may develop on celery which received no chemical treatment of any kind and at the present time no explanation can be given for this development. The development of collapsed lesions is very much less severe on the unsprayed celery than in any of the Bordeaux spray treatments (Tables IX and X). This indicates that all of the Bordeaux mixtures used, whether applied during the growing season or at the time of storing, greatly increase the severity of collapsed lesion development.

(2) Collapsed lesion development was much more severe on celery which was sprayed at the time of storing, than

on celery which was sprayed only in the growing period (Comparing Tables IX and X). Thus, it appears that the more Bordeaux residue on the celery at the time of storing, the greater will be the collapsed lesion development in storage. It might, therefore, be advantageous for celery growers to refrain from spraying late in the growing season; and a further recommendation might be that, wherever possible, the celery should be washed before it is placed in storage.

(3) In all Bordeaux mixtures, those containing the largest amounts of $CuSO_4$ in proportion to the lime content, caused the most severe development of collapsed lesions in storage (Tables IX and X).

(4) In nearly all cases, the Bordeaux mixtures containing calcium hydrate, caused greater development of collapsed lesions than those containing a chemically equivalent amount of calcium hydrate plus magnesium oxide ($\frac{3}{4}$ $Ca(OH)_2$ and $\frac{1}{4}$ MgO)(Tables IX and X).

From these last two observations, it appears that the severity of collapsed lesions in storage could be lessened by reducing the amount of $CuSO_4$ used in the Bordeaux mixtures in the field spraying, and also by using burnt dolomitic limestone as a partial substitute for hydrated calcium lime.

(5) Without resorting to statistical analysis, the development of foliage rot does not appear to be

significantly affected by the various spray treatments (Tables IX and X).

(6) Cutting back, or trimming, of the foliage did not alter the storage life of the celery, and therefore was not considered a worth while practice (Table XI).

(7) Neither storing the plants while wet, nor maintaining free moisture on them during storage, significantly affected the development of foliage rot and collapsed lesions (Table XII).

CONDITIONS IN THE HARBOUR COLD STORAGE WAREHOUSE

In connection with the storage experiments, some data on storage conditions were recorded.

Hygrothermograph records were taken in the room in which the celery was stored over a period of six weeks. The temperature ran at approximately 32° F. with surprising uniformity, in the period in which the records were taken. It fluctuated between 33° F. and 31° F. and the graphs show that it was more often above than below 32° F. The relative humidity fluctuated between 97 and 94% and the average relative humidity over the period, was slightly more than 96%.

Slight variation in temperature was observed at different heights in the storage room. A thermometer, placed on top of the upper row of crates (5 ft. 6 in. from the floor), read 0.5° F. higher than a thermometer placed 2.5 ft. from the floor. No variation in temperature could be recorded between the room temperature and the temperature in the centre of the crates. This may be due to the fact that the celery under observation was placed on stands and well spaced, so that ample room was afforded for air movement. Also, it may be due to the fact that the crates used in these experiments were not the standard size employed by Thompson (36). The measurements of the crates used in this investigation were 21" deep x 25" long x 10" wide, while the standard crates are 23" deep x 24" long x 16" wide.

ORGANISMS ISOLATED FROM CELERY IN STORAGE

Foliage rots, due to three species of Botrytis, an Alternaria sp., Sclerotinia sclerotiorum and bacterial forms of the Bacillus caratovorus type, were as important as the physiological disorders in causing breakdown of the celery. Bacterial rots were probably the most prevalent, but these were only slightly more prevalent than rots caused by Botrytis spp. Sclerotinia sclerotiorum, although its sclerotia were found, was not very destructive in cold storage. Laboratory experiments showed it to be a very destructive rot at slightly higher temperatures. A review of the reports of the last few years would seem to indicate that bacterial rots are becoming more important from year to year.

Three bacteria, all causing soft rot of the foliage were isolated. Three species of Botrytis, isolated quite frequently, seemed to produce a very destructive rot and were quite often found in the fruiting condition. Sclerotinia sclerotiorum was isolated from its sclerotia several times, but on the whole its sclerotia were observed much less frequently than those of either Botrytis spp. or Typhyla sp. Sclerotia of Typhula sp. were very common and were found on decayed plants as early as November 15, but it was not possible to establish pathogenicity of the organism at 32°F.

in the laboratory. Alternaria sp. was isolated from decaying foliage during the storage period, and Septoria apii was isolated from blighted celery at the time of storage. Four species of Penicillium and one Rhizopus species were isolated from decaying petioles and foliage.

The pathogenicity of these organisms was tested by inoculating them into healthy celery, and then reisolating and comparing the final culture with the original isolation. Three bacteria of the Bacillus caratovorus type, all causing soft rot of the foliage, were found to be pathogenic on inoculation. Botrytis spp., Alternaria sp. and Sclerotinia sclerotiorum were the only fungi that caused definite decay of celery in the laboratory at 32° F. Sclerotinia sclerotiorum, although not very prevalent in cold storage, was shown by laboratory experiments, to be a very destructive rot at slightly higher temperatures. Typhula sp., although very prevalent in cold storage was found to be non-pathogenic in the laboratory at 32° F., which was the only temperature tried. The four Penicillium species and the single Rhizopus species were not pathogenic. It was concluded that all the fungi isolated, except Botrytis spp., Alternaria sp. and Sclerotinia sclerotiorum are secondary organisms in celery breakdown; and that they become effective only after physiological breakdown has set in.

Experiments on the effect of temperature on the

growth of the fungi isolated, showed that Botrytis spp., Alternaria sp., Sclerotinia sclerotiorum and Typhula will grow in culture at 0° C. Septoria apii, on the other hand, will not grow at this temperature.

A bacterial organism causing a soft, brown, crown rot in the field, was isolated during the summer of 1937. Reinoculated into growing plants in the greenhouse, it produced typical symptoms of the disease. This organism was not isolated in storage, probably because an attempt was made to discard all diseased plants at the time of crating.

An attempt was made to isolate organisms from basal rot and collapsed lesions, but no success was attained. The conclusion agrees with that of former investigators (2) (40), namely, that these disorders are not caused by pathogenic organisms.

LABORATORY EXPERIMENTS AND OBSERVATIONS

Laboratory experiments were carried on throughout both years of this investigation, to determine the cause and to study the progress of development, of certain reactions observed in the field and in storage.

Experiment I.

Object: To study the anatomy of collapsed lesions.

Sections were made of collapsed lesions and their anatomy studied. A collapsed lesion, whether it is on the concave (inner) or convex (outer) side of the petiole is essentially a collapsing and browning of the parenchyma tissue. Always, the epidermis is thickened and the parenchyma cells under the epidermis are crushed. In the early stages of development only a few rows of cells are involved, but later the depressions may extend so far into the petiole as to render it unmarketable.

On the concave side of the petiole collapsed lesions are merely depressions of the epidermis because of collapsing of the underlying parenchyma tissue (Plates I and II). They are quite circular in outline and the depression itself is regular and spherical in shape. Also, the epidermis remains smooth and intact so that the surface of the depressed area presents a glossy appearance.

On the convex side of the petiole also, collapsed lesions are depressions of the epidermis because of collapsing of the underlying parenchyma tissue, but the manner of collapsing and the final appearance of the lesions are affected by the presence of the collenchymatous strands which characterize this side of the petiole. Collapsed lesions on the convex side of the petiole are not so regular in outline, nor is the tissue so uniformly depressed. The collenchymatous ridges being composed of stronger material protrude after the surrounding parenchyma tissue has collapsed (Plates III and IV) and give the surface of the collapsed lesion a wash-board appearance. The parenchyma tissue frequently collapses to such an extent that the collenchyma strands break through the epidermis and appear as loose strands, entirely outside of the petiole. Finally, the collenchyma strands may break and coil up giving the collapsed lesion a ragged appearance.

An experiment was carried on to determine whether or not the epidermis over a collapsed lesion is intact. Petioles showing collapsed lesions, were submerged in a 0.1% solution of methylene blue. At the end of 24 hours, it was found that the dye had penetrated and collapsed lesions, in such a manner as to detect the presence of very small collapsed lesions, that could not be seen previously. More stain

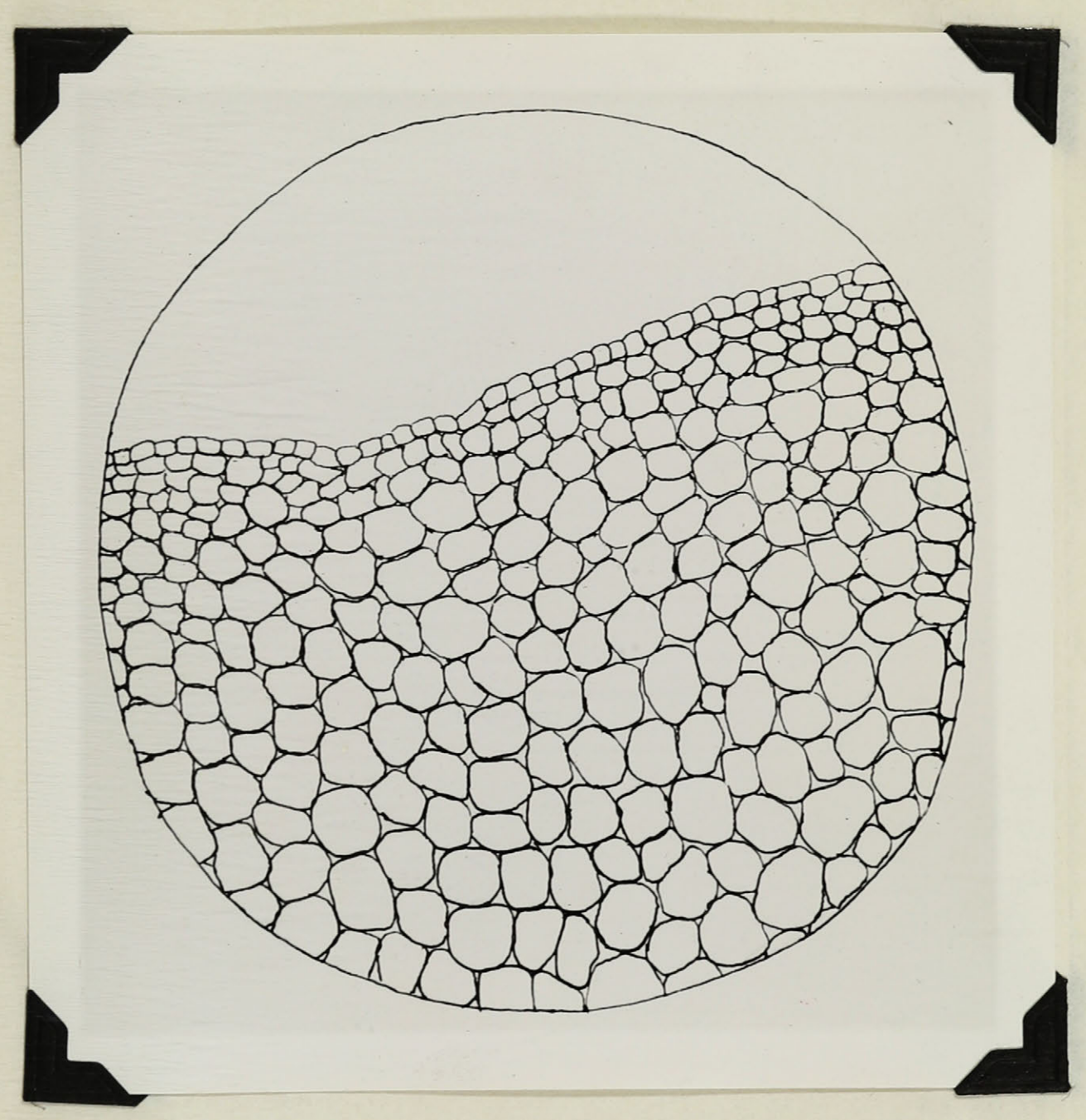

Plate I. - Semi-diagramatic transverse section of the concave (inner) side of a celery petiole showing healthy epidermis and parenchyma tissue.

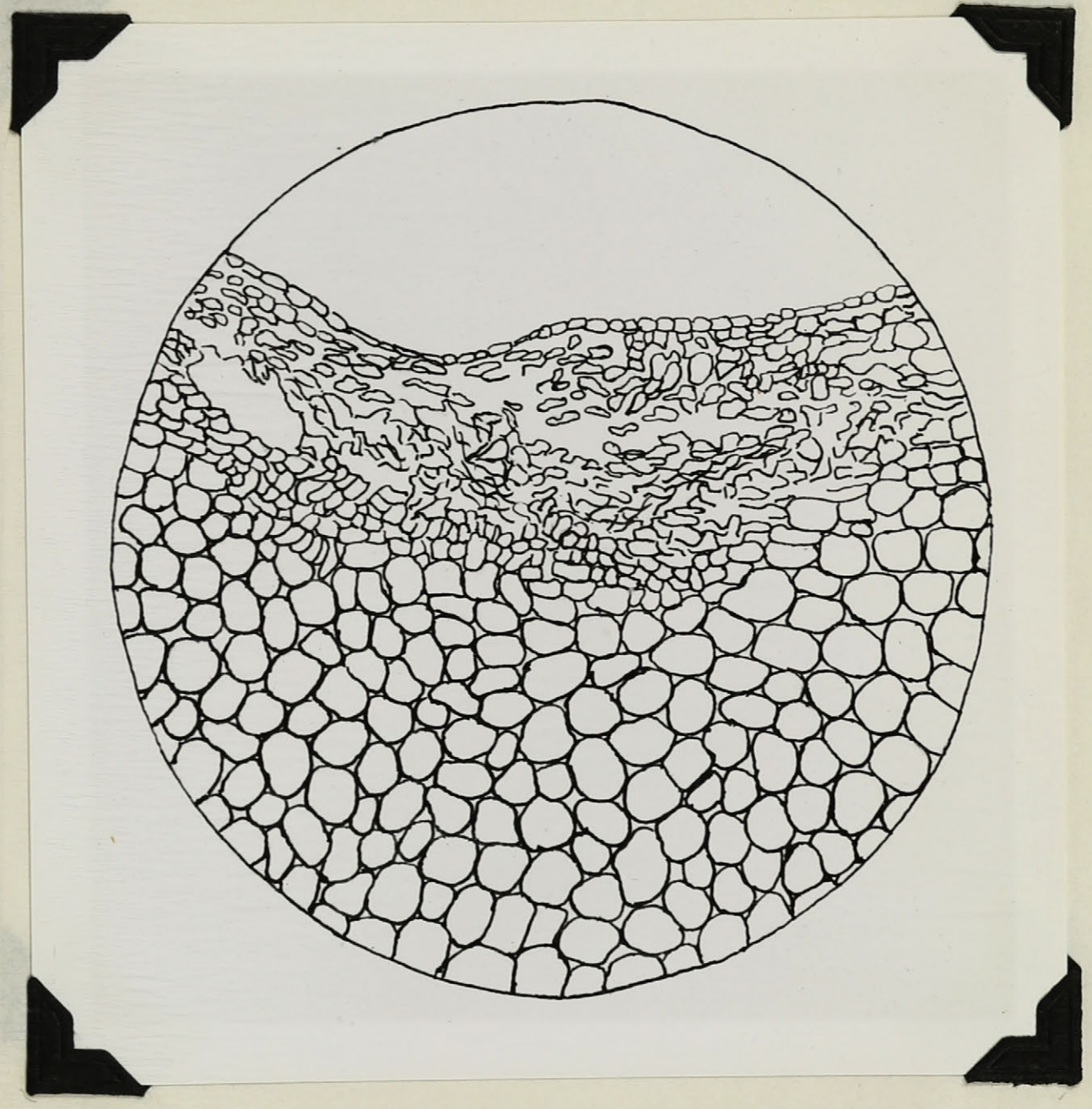

Plate II. - Semi-diagramatic transverse section of collapsed lesion on the concave (inner) side of a celery petiole. Observe that the epidermis is not broken although some of the cells are distorted. The underlying parenchyma cells are severely collapsed and many of the cell walls are disrupted.

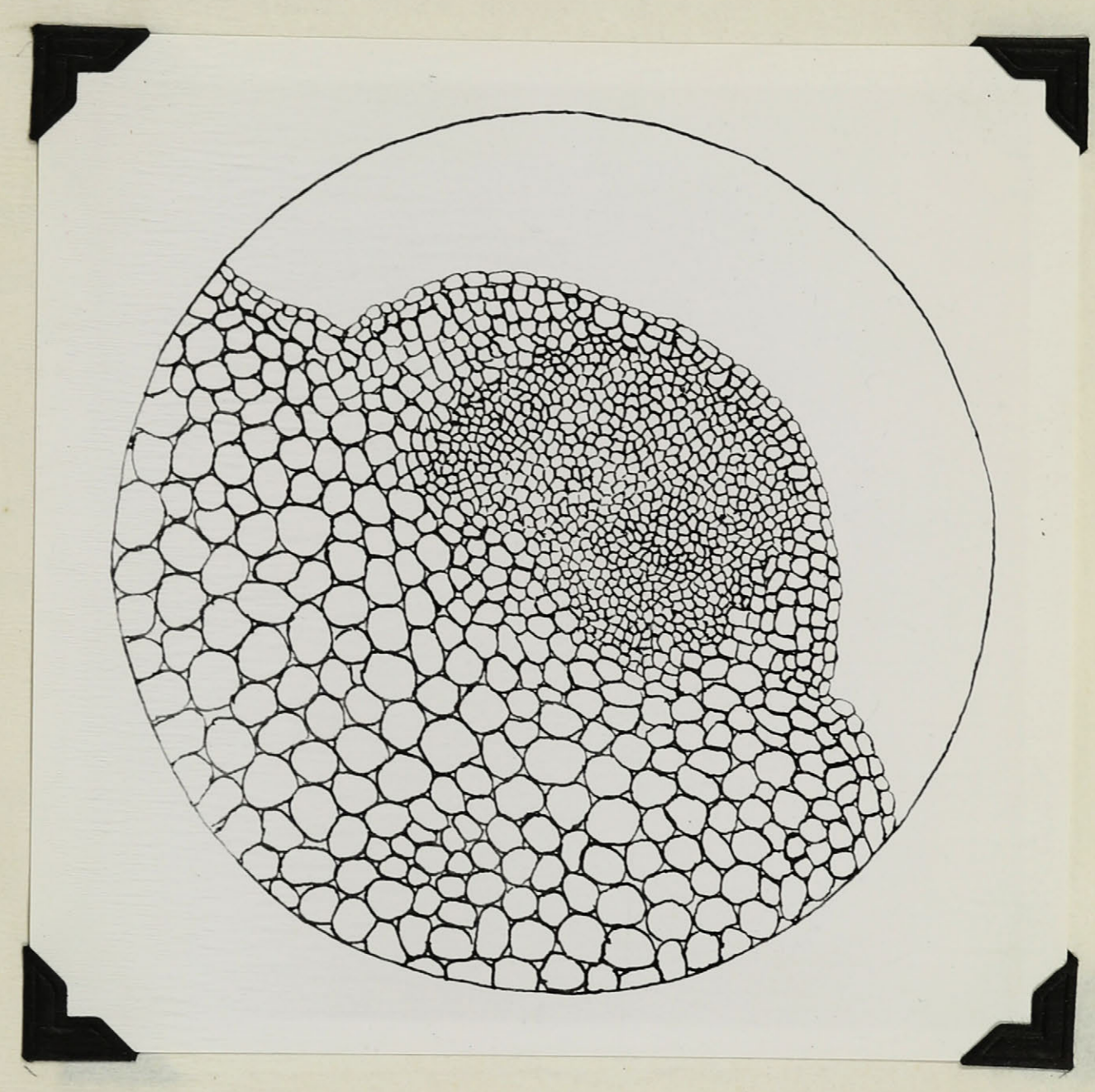

Plate III. - Semi-diagramatic transverse section of a normal collenchymatous ridge on the convex (outer) side of a celery petiole. Observe the small compact cells of the collenchyma or strengthening tissue in contrast to the large, rounded cells of the underlying parenchyma tissue.

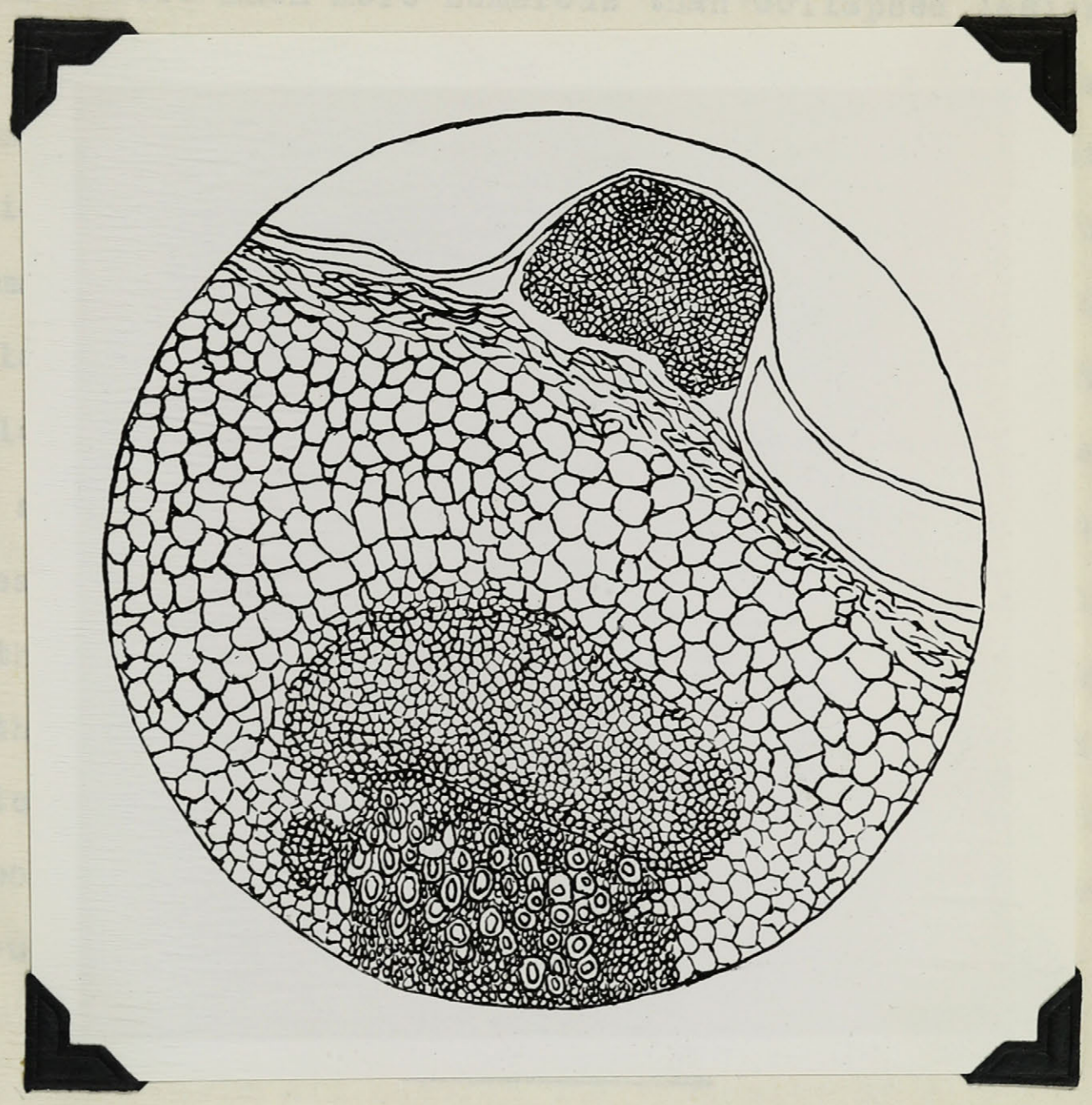

Plate IV. - Semi-diagramatic transverse section of a collapsed lesion on the convex (outer) side of a celery petiole. Observe the loosened epidermis and the protrusion of the collenchymatous strand because of the collapsing of the surrounding parenchyma tissue. The vascular tissue is not affected.

penetrated the larger lesions.

A strip of the stained celery epidermis was examined under the microscope, and it was observed that the stomata were much more numerous than collapsed lesions. In the centre of each collapsed lesion was a heavily stained stoma, and this was surrounded by the more lightly stained lesion tissue. In nearly all cases the guard cells of the stomata were stained, but only in collapsed lesions did the cells surrounding the guard cells become stained. These cells could be detected, not only by their acceptance of the stain, but also by a more or less shrunken and collapsed appearance. These observations indicate that the collapsing of the tissue favoured the penetration of the stain by way of the stomata. A quite feasible explanation is that some toxic substance may have previously penetrated the stomata of collapsed lesions, killed the cells, and so made conditions favourable for penetration of the stain.

Experiment II.

Object: The artificial induction of collapsed lesions by spraying with various chemicals.

Materials and Methods.

In this experiment, the celery was sprayed with various chemicals, and then stored at the same temperature

and humidity (32°F. and 98% r.h.) as would be found in the Harbour Cold Storage Warehouse in Montreal. Metal flower boxes, tightly covered with rubber sheeting, were used as constant humidity chambers and constant humidities were maintained by saturated salt solutions (9) (17) (35) (34).

The intensity of development of collapsed lesions, was scored according to the percentage area of the petioles covered with lesions. Estimations of the percentage area covered with lesions were made by using the chart shown in Plate V.

Results

Collapsed lesions developed in 12 hours on celery which was sprayed with a 1% solution of $CuSO_4$ and then stored at 32° F. and 98% r.h. The Bordeaux spray, with which celery is commonly sprayed in the field (4-6-50), produced collapsed lesions at the end of 20 days under similar conditions.

Table XIV records the intensity of development of collapsed lesions on celery petioles treated with the various chemicals as shown.

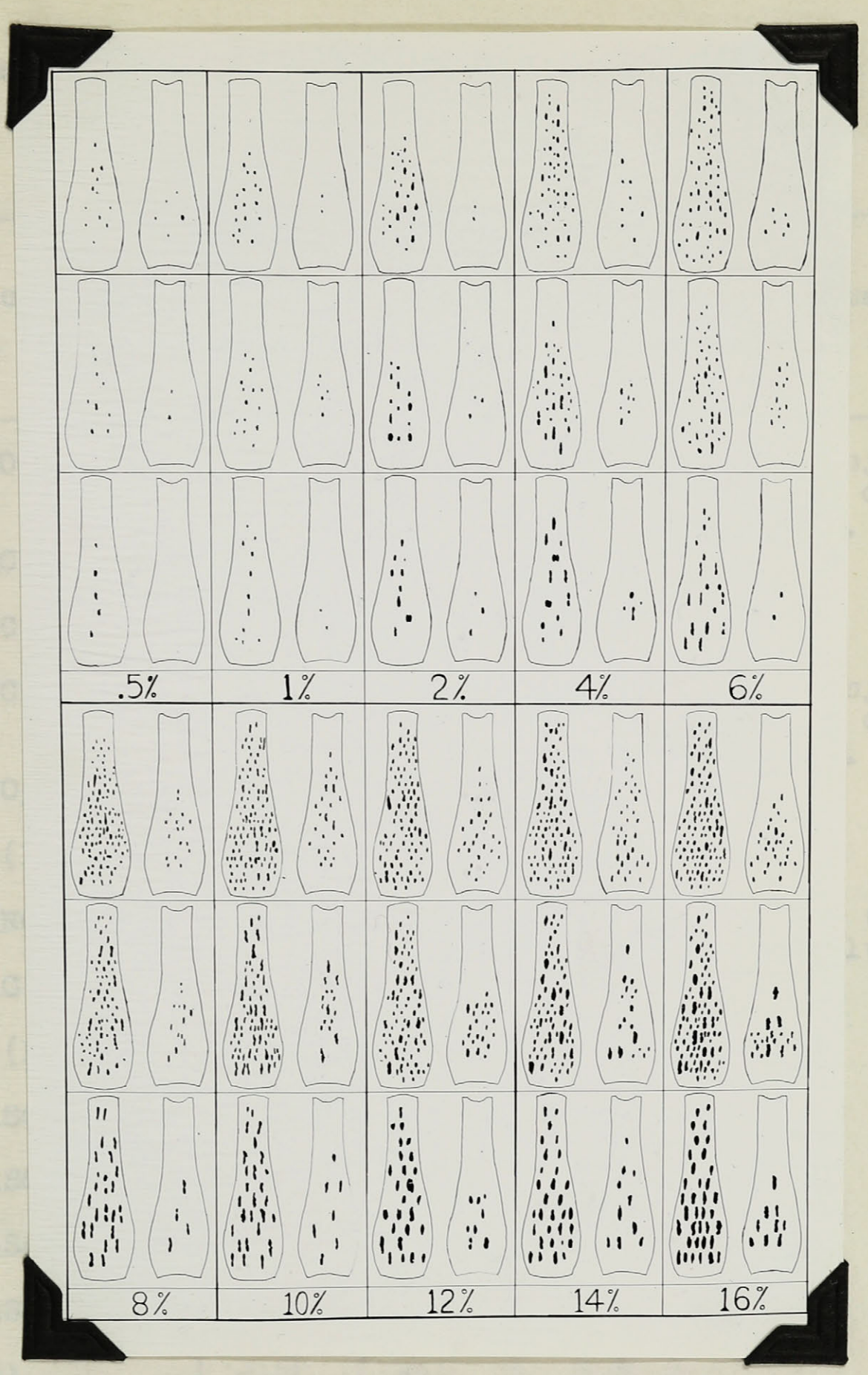

Plate V. - Chart used in estimating intensity of collapsed lesion development. The range 0.5% to 16% denotes percentage of the total area covered with lesions. Three cases of percentage coverage are represented with lesions of varying size.

Table XIV - Effect of various chemicals upon collapsed lesion development, at 32°F. and 98% r.h.

Chemical	Percentage of total petiole area covered with lesions after various time intervals			Remarks
	24 hrs.	48 hrs.	72 hrs.	
1% $Hg(NO_3)_2$	6.2	8.6	13.4	Severe injury; not all typical collapsed lesions.
1.0% $CuCl_2$	3.6	8.0	11.6	
1.0% $ZnCl_2$	3.0	7.0	10.0	
1.0% $HgCl_2$	4.4	7.2	10.0	Severe injury; not all typical collapsed lesions.
0.5% HNO_3	2.0	6.0	9.6	
1.0% $Cu(NO_3)_2$	1.7	5.0	8.4	
1.0% $AgNO_3$	2.0	5.0	8.2	Petioles soft and wilted, with silvery appearance.
1.0% $AlCl_3$	1.9	4.4	8.0	
1.0% $Zn(NO_3)_2$	1.0	4.0	7.6	
1.0% $CuSO_4$	1.9	4.0	7.6	
0.5% $CuSO_4$	0.8	1.4	6.0	
0.1% $CuSO_4$	0.0	0.5	0.7	
1.0% $ZnSO_4$	1.8	2.4	7.0	
0.5% HCl	1.5	4.0	7.2	
0.5% H_2SO_4	0.9	1.8	3.0	
1.0% Ag acetate	1.0	1.5	2.6	Petioles slightly soft and wilted.
1.0% Cu acetate	1.0	1.5	2.0	
1.0% Cu tartrate	0.0	0.5	0.7	

Table XIV (continued)

Chemical	Percentage of total petiole area covered with lesions after various time intervals			Remarks
	24 hrs.	48 hrs.	72 hrs.	
1.0% KNO_3	0.0	0.4	0.6	Petioles soft and wilted.
1.0% KCl	0.3	0.5	0.5	" " " "
1.0% $NaNO_3$	0.0	0.5	0.5	" " " "
1.0% NaCl	0.0	0.0	0.0	" " " "
1.0% $Ca(NO_3)_2$	0.0	0.0	0.0	
1.0% $CaCl_2$	0.0	0.0	0.0	
1.0% Ca acetate	0.0	0.0	0.0	
1.0% Ca bimalate	0.0	0.0	0.0	
1.0% $BaCl_2$	0.0	0.0	0.0	
0.5% NaOH	0.0	0.0	0.0	(Petioles badly wilted with indentations along collenchyma strands, but these were not typical collapsed lesions.
0.5% KOH	0.0	0.0	0.0	

Observations

From the results in Table XIV, the following observations can be made: -

(1) Salts of Cu, Zn, Hg and Ag are more effective in causing collapsed lesions than are salts of the more active

elements, K and Na. The latter elements according to Mendelejeff's table, are more active chemically and have lower atomic weights than the former: -

Elements in order of activity	Atomic weights
K	39.2
Na	23.1
Zn	65.4
Cu	63.6
Hg	200.0
Ag	197.2

Thus, a general statement may be that the elements with highest atomic weights are most effective in producing collapsed lesions. Furthermore, elements near the centre of the periodic table are more effective than those at either end. This agrees with the work of McCallan and Wilcoxon (23) on the toxicity of various elements to fungous spores.

(2) There seems to be some correlation between toxicity of salts and the valencies of their basic components. Salts of univalent elements are not as effective in causing collapsed lesions as are salts of bivalent and trivalent elements. However, salts of the bivalent elements Hg, Cu

and Zn cause greater intensity of development of collapsed lesions than salts of the trivalent element, Al.

(3) Chloride salts of Cu, Zn and Hg are more effective than nitrate salts, and these again are more effective than sulphate salts of the same elements. Weakest of all, are salts of the organic acids; acetic and tartaric.

(4) The Cu, Zn and Hg salts of hydrochloric and sulphuric acids are more effective in causing collapsed lesions than the acids themselves.

(5) HNO_3 is more effective than either HCl or H_2SO_4. Also, HNO_3 is more effective than nitrate salts.

(6) NaOH and KOH cause severe injury and softening of the collenchyma strands, but do not produce typical collapsed lesions.

(7) Severe injury to the celery was caused also by 1% solutions of $HgNO_3$, $HgCl_2$ and $AgNO_3$, but the injury did not appear in the form of typical collapsed lesions. The injury in all three cases involved the entire surface area of the petiole, and was not restricted to localized areas as is the case with collapsed lesions. However, very small, but typical collapsed lesions, developed near the bases of the petioles in all three cases. The petioles sprayed with $AgNO_3$ solution had a silvery appearance. The solution when applied was quite clear, so this suggests that the silver was set free in some manner.

Discussion

It seems that both positive and negative ions exert a toxic effect. H_2SO_4 produced definite collapsed lesions, so it can be concluded that sulphate ions are toxic. However, $CuSO_4$ is more effective than H_2SO_4 in producing collapsed lesions; therefore copper ions must also be toxic. From this it seems feasible that hydrolysis of $CuSO_4$, $Cu(OH)_2$, cupric trioxy sulphate, basic sulphates or other complex salts from Bordeaux residues on stored celery, might put into solution copper and sulphate ions which would cause collapsed lesion development.

Experiment III.

Object: To determine the effectiveness of different Bordeaux mixtures in causing collapsed lesions.

Materials and Methods

Celery petioles were sprayed with Bordeaux mixtures having varying proportions of $CuSO_4$ and lime as follows:- 4-0-40, 4-1-40, 4-2-40, 4-3-40, 4-4-40, 5-4-40, 6-4-40, 7-4-40, 8-4-40, 9-4-40 and 10-4-40. Two series of Bordeaux mixtures of the above formulae were used; the first containing calcium hydrate, and the second series containing equivalent amounts of a mixture made up of three parts of calcium hydrate to one

part by weight of magnesium oxide. After receiving the different spray treatments, the celery was stored at 32° F. and 95% r.h.

The degree of collapsed lesions was scored according to the percentage of the total petiole area covered with lesions (Plate V, page 61).

Observations

The results are recorded in Table XV.

Table XV - Degree of development of collapsed lesions with various Bordeaux mixtures over a 21-day period, at 32° F. and 98% r.h.

Bordeaux formula	Percentage of total petiole area covered with lesions
# Ca 4-0-40	16.0
Ca 4-1-40	13.0
Ca 4-2-40	6.0
Ca 4-3-40	1.5
Ca 4-4-40	0.5
Ca 5-4-40	1.5
Ca 6-4-40	2.4
Ca 7-4-40	2.8
Ca 8-4-40	4.4
Ca 9-4-40	5.6
Ca 10-4-40	8.0
Mg 4-0-40	16.0
Mg 4-1-40	7.2
Mg 4-2-40	4.0
Mg 4-3-40	1.3
Mg 4-4-40	1.0
Mg 5-4-40	1.2
Mg 6-4-40	1.8
Mg 7-4-40	2.8
Mg 8-4-40	2.6
Mg 9-4-40	4.2
Mg 10-4-40	5.8

See Plate VI, page 68

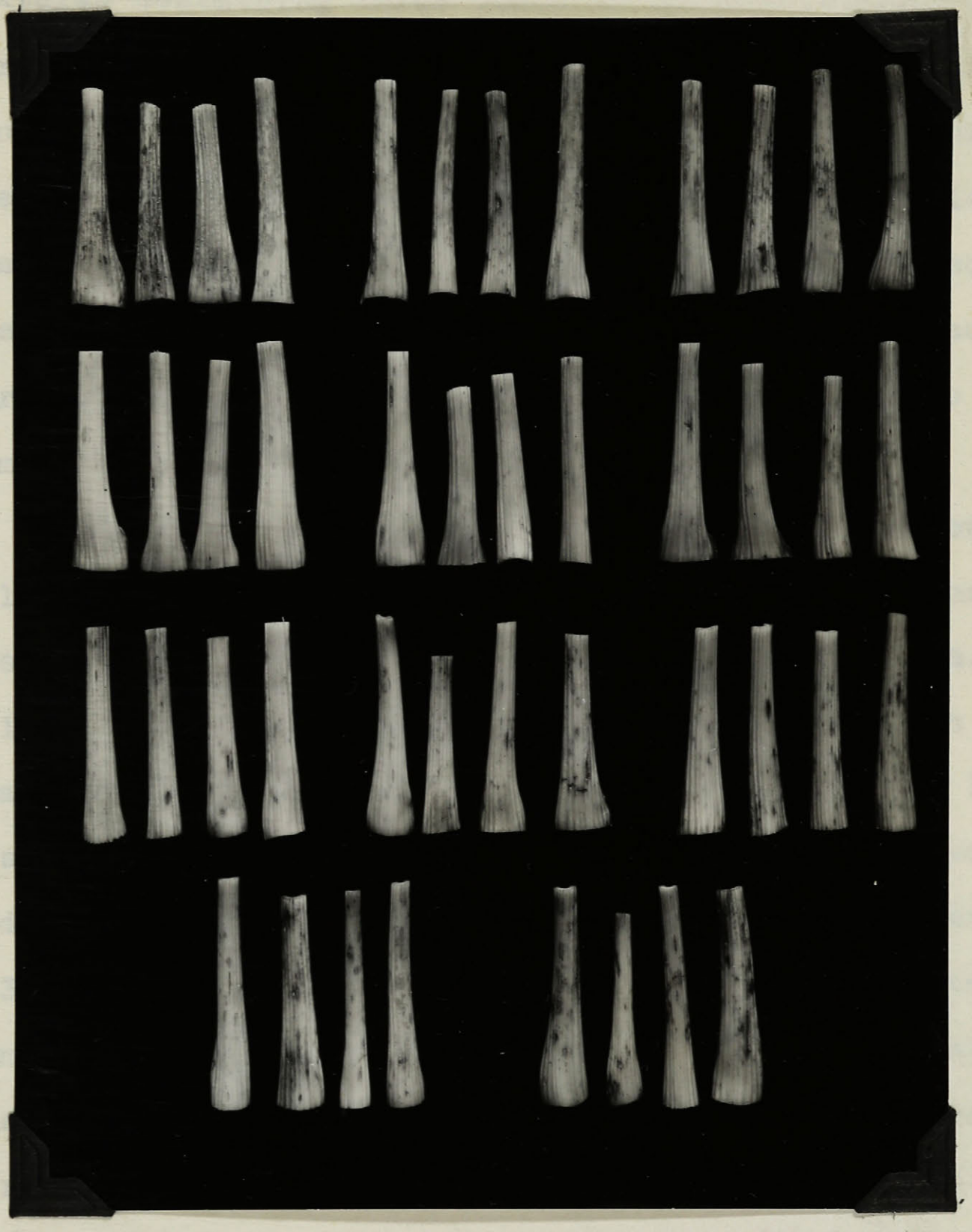

Plate VI. - Collapsed lesions induced by a series of calcium Bordeaux mixtures. Reading from left to right, and from top to bottom, the groups of four petioles each, received the spray treatments listed in Table XV.

The results of this experiment show that Bordeaux mixtures containing three parts of calcium hydrate to one part by weight of magnesium oxide are not as effective in causing collapsed lesions on celery as are those containing chemically equivalent amounts of calcium hydrate alone. Also, in both series, the greatest development of collapsed lesions was observed where the Bordeaux mixtures had the largest amounts of copper sulphate in proportion to the lime.

An unsuccessful attempt was made to induce collapsed lesions on growing plants in the greenhouse with the same series of Bordeaux mixtures. Thus it seems, that collapsed lesions develop more readily on celery in storage than on celery growing in the field or greenhouse. It might also be mentioned here, that careful observations were made in the field on sprayed plots during the summer of 1938, and no collapsed lesions were recorded. To the present time, they have never been observed on growing plants in the field. However, they were induced in the greenhouse with certain chemicals other than Bordeaux mixtures. Within 24 hours, collapsed lesions developed on the petioles of the plants sprayed with $Cu(NO_3)_2$ and also on the leaf blades, injury occurred in the form of small necrotic areas and this very much resembled the foliage rot observed in the early

part of the storage period. Within 48 hours, collapsed lesions had developed on the petioles of plants sprayed with 1% $CuSO_4$ solution, but in this case no injury was observed on the leaves. At the end of 10 days, the plants sprayed with $CuSO_4$ had completely recovered, except for the older petioles, while the plants sprayed with $Cu(NO_3)_2$ solution did not recover.

Experiment IV.

Object: To determine the effects of temperature and humidity on the development of collapsed lesions. The experiment was divided into three phases, each having a different procedure.

Materials and Methods

This experiment is subdivided into three different procedures, which are outlined below, with the results and observations obtained from each. The data, tabulated in this experiment, are quite accurate; because each part of the experiment was carried out fourteen times, and the results of the fourteen trials were averaged to give the figures recorded.

Procedure A: -

This procedure was followed to determine the effect of a range of temperatures on collapsed lesion development, with the atmospheric humidity at the saturation point in each chamber. A differential thermostat was used to maintain seven different temperatures as follows:- 32° F., 41° F., 44.6° F., 52.7° F., 55.9° F., 58.1° F. and 71.6° F. Corresponding readings are:- 0° C., 5° C., 7° C., 11.5° C., 13.25° C., 14.5° C. and 22° C. It was impossible to establish a uniform temperature difference between the various chambers of the differential thermostat because of the inefficiency of this method of establishing a temperature range. Saturated atmospheres were maintained by putting wet sacks in the chambers and watering them at intervals. Three lots of celery, treated as follows, were placed at each temperature: -

(1) Sprayed with 1% $CuSO_4$.

(2) Sprayed with 4-4-40 Bordeaux.

(3) Check - no treatment.

The intensity of collapsed lesion development was recorded as percentage of the total petiole area covered with lesions (Plate V, page 61).

The results are recorded in Table XVI.

Table XVI - Intensity of development of collapsed lesions at the end of seven days, at various temperatures, and saturated, atmospheric humidities.

Temperature	Percentage of total petiole area covered with lesions		
	1% $CuSO_4$	4-4-40 Bordeaux	Check
32° F.	10.2	0.0	0.0
41° F.	13.0	0.4	0.0
44.6° F.	15.0	0.6	0.0
52.7° F.	16.0	1.0	0.0
55.9° F.	16.0	1.8	0.0
58.1° F.	16.0	2.4	0.0
71.6° F.	16.0	2.8	0.0

Procedure B: -

In Procedure B, an attempt was made to determine the effect of high and low humidities on collapsed lesion development at storage temperature (32° F.). Saturated solutions of KNO_3 and NaCl maintained constant humidities of 98% r.h. and 78% r.h. at 32° F. Metal flower boxes, tightly covered with rubber sheeting, were used as constant humidity chambers.

The results are recorded in Table XVII.

Table XVII - Intensity of collapsed lesion development at the end of seven days, with high and low humidities, at a constant temperature of 32° F.

Humidity	Percentage of total petiole area covered with lesions		
	1% $CuSO_4$	4-6-40 Bordeaux	Check
78% r.h.	4.8	0.0	0.0
98% r.h.	11.8	0.0	0.0

Procedure C: -

The third phase of this experiment is a study of the interaction of temperature and humidity on the development of collapsed lesions. Celery petioles, treated as outlined above, were placed in high and low humidity chambers; and these were allotted so that one high, and one low humidity chamber were placed in each of the constant

temperature chambers. The following range of temperatures was used:- 32°F., 45°F., 55°F., 60°F. and 68°F. Corresponding readings would be:0°C., 7.2°C., 10°C., 12.8°C., 15.5°C. and 20°C. A large cold chamber, cooled by an ammonia system, maintained a constant temperature of 32°F. The chambers at temperatures of 45°F., 50°F., 55°F., and 60°F. were located in a corridor, which was kept at 35°F. by ventilation with cold air from outside. Resistance coils and light bulbs were placed in each chamber and thermoregulators governed the heating so that the desired temperatures could be maintained. The highest temperature of the range (68°F.) was room temperature.

Metal flower boxes, tightly covered with rubber sheeting, were again used as constant humidity chambers, and saturated solutions of NaCl and KNO_3 maintained humidities over the range of temperatures as follows:

Temperature	Humidities maintained with saturated solutions of	
	NaCl	KNO3
32°F.	78.0% r.h.	98.0% r.h.
45°F.	77.3% r.h.	96.6% r.h.
50°F.	77.0% r.h.	96.0% r.h.
55°F.	76.8% r.h.	95.4% r.h.
60°F.	76.5% r.h.	95.0% r.h.
68°F.	76.0% r.h.	94.0% r.h.

Due to the changing of the saturation points of the salt solutions over the range of temperatures, the humidities maintained are not the same for different temperatures. However, they are near enough for the purposes of this experiment. The humidities maintained by saturated solutions of NaCl may be considered as low, while those maintained by saturated solutions of KNO_3 may be considered as high.

The results are recorded in Table XVIII.

Table XVIII - Intensity of collapsed lesion development at the end of seven days, at various temperatures and humidities.

Temperature	Percentage of total petiole area covered with lesions					
	Low humidity			High humidity		
	1% $CuSO_4$	4-6-40 Bordeaux	Check	1% $CuSO_4$	4-6-40 Bordeaux	Check
32° F.	4.8	0.00	0.00	12.0	0.00	0.00
45° F.	6.4	0.00	0.00	13.0	0.4	0.00
50° F.	10.0	0.2	0.00	13.2	0.8	0.00
55° F.	10.0	0.6	0.00	14.8	1.9	0.00
60° F.	11.0	1.0	0.00	16.0	3.2	0.00
68° F.	12.2	1.8	0.00	16.0	4.6	0.00

Observations and Discussion

From the results of this experiment, it appears that humidity is not as important as temperature in affecting the development of collapsed lesions. However, it cannot be as important as high temperature, because heat is always applied to hasten chemical reactions. According to Van't Hoff's Rule, the velocity of a chemical reaction is doubled or tripled for every ten degrees rise in temperature, within certain limits. Humidity might be a very important factor and yet not be as important as temperature. When the effect of high humidity can be recorded in a laboratory experiment of one week duration, it can be easily understood that humidity might be a very important factor in a cold storage room over a period of 90 to 110 days, which is the length of the storage life of celery.

It was also observed, that raising the humidity caused greater increase in collapsed lesion development at low temperatures, than at high temperatures. This is because high humidity with low temperature, brings about condensation of water on the celery, which seems to be very important in collapsed lesion development. As pointed out earlier in this paper, the toxicity of Bordeaux residues may be due to ions in solution. Therefore, it seems feasible, that free

moisture might favour collapsed lesion development, by serving as a medium for hydrolysis of the copper salts of Bordeaux residues.

Experiment V.

Object: To determine the effect of free moisture on the development of collapsed lesions.

Materials and Methods

Celery sprayed with 1% $CuSO_4$ and 4-6-40 Bordeaux was stored at 32° F. and 98% r.h. (1) while still wet from the spray, and (2) after it was allowed to dry.

Observations and Discussion

The results of this experiment are recorded in Table XIX

Table XIX - Collapsed lesion development at the end of seven days on celery receiving two spray treatments and stored while still wet, and after being allowed to dry.

Percentage of total petiole area covered with lesions					
1% $CuSO_4$		4-6-40 Bordeaux		Check	
Wet	Dry	Wet	Dry	Wet	Dry
12.4	5.0	0.6	0.00	0.00	0.00

Development of collapsed lesions was much more severe on the celery which was stored while still wet. As usual, a 1% solution of $CuSO_4$ caused greater intensity of collapsed lesion development than 4-6-40 Bordeaux. No collapsed lesions were observed on the Bordeaux-sprayed celery, which was allowed to dry before storing, or on the checks. Collapsed lesions, on the celery sprayed with 1% $CuSO_4$ and stored while wet, covered a larger area, but were much smaller and therefore more numerous than on celery receiving the same spray treatment, but stored after being allowed to dry. An explanation of this might be, that in the drying, small globules of solution accumulated, so that the $CuSO_4$ was deposited in definitely localized areas, and collapsed lesion development was confined to these areas. Where there was no drying or evaporation of water, the deposition and penetration of the copper was more uniform, and hence the smaller but more numerous collapsed lesions.

Experiment VI.

Object: To determine if the removal of the 1% $CuSO_4$ and 4-6-40 Bordeaux sprays, at various intervals after their application, would affect subsequent collapsed lesion development.

Materials and Methods

Celery petioles were sprayed with 0.25%, 0.50%, 0.75%, 1% $CuSO_4$ and 4-6-40 Bordeaux, and then stored at 32° F. and 98% r.h. At the end of various time intervals, the sprays were removed by washing, first with water, and then the final traces of $CuSO_4$ were removed with 1% H_2O_2, and the final traces of Bordeaux were removed by washing with 0.2% HNO_3 (21). The celery was washed at the following time intervals after spraying:- 15 minutes, 30 minutes, 1 hour, 2 hours, and 3 hours. Records were taken on the intensity of development of collapsed lesions, on the $CuSO_4$ sprays at the end of seven days, and on the Bordeaux sprays at the end of twenty days.

Observations and Discussion

The results are recorded in Table XX.

Table XX - Collapsed lesion development on celery sprayed with various $CuSO_4$ and 4-6-40 Bordeaux sprays at the end of seven and twenty days, when the spray residues were removed at various time intervals.

Spray removed at the end of	Percentage of total petiole area covered with lesions				
	At the end of 7 days				At the end of 20 days
	0.25% $CuSO_4$	0.50% $CuSO_4$	0.75% $CuSO_4$	1% $CuSO_4$	4-6-40 Bordeaux
15 minutes	0.0	0.3	0.5	0.7	0.9
30 minutes	0.3	0.6	0.92	1.4	1.5
1 hour	0.6	1.1	2.2	4.2	1.7
2 hours	0.8	1.7	4.6	7.6	2.4
3 hours	0.8	2.4	5.6	9.0	3.0

From these results a correlation can be observed between the time of exposure to the spray and the subsequent development of collapsed lesions. Also, the more concentrated the spray solution, the more intense is the resulting collapsed lesion development. Time of exposure to the spray treatment does not influence collapsed lesion development, as much with 4-6-40 Bordeaux over a twenty-day period, as with the $CuSO_4$ solution over a seven-day period. Thus, the effect of time of exposure to the spray on collapsed lesion development is partly obscured by increasing the length of the storage period.

Experiment VII.

Object: To show that collapsed lesions are induced by actual penetration of the tissues by copper from the spray residues.

Materials and Methods

An attempt was made to measure the copper content of celery tissue by the spectrographic method. A Littrow spectrograph, with a linear dispersion of about 30 inches between wave lengths 4650 (Angstrom units) and 2850 (Angstrom units), was used. Two prisms were used with this instrument; a glass prism for lines of wave lengths shorter than 3800 Angstrom units, and a quartz prism for shorter wave lengths. The slit aperture was 6.0 and the distance from the slit to the sample was 20 inches.

Determinations were made with celery taken directly from the Harbour Cold Storage in Montreal, and with celery on which collapsed lesions were induced in the laboratory. The celery was first carefully washed with water and 0.2% HNO_3 to remove all traces of spray residue. Thin sections of celery tissue of uniform size

were made from the following locations: (1) the epidermis of a collapsed lesion, (2) the tissue beneath the epidermis of a collapsed lesion, and (3) healthy tissue from corresponding locations on the same petiole.

These sections were placed on aluminum electrodes, which had been previously boiled in dilute nitric acid and washed in redistilled water. One drop of 10% lithium citrate was used as a sticker, for attaching the sections to the electrodes. Throughout the procedure, all precautions were taken to avoid contamination with copper from outside sources. The small aliquots of celery tissue were volatilized in a 220 volt arc, using a current of 9 to 10 amperes. In taking the spectrum of a sample, the arc was maintained until the sample was completely volatilized. Incomplete volatilization would permit fractionation, involving a possible retention of the higher boiling elements in the residue. Quantitative estimations of the amounts of copper present were then made by comparing visually, the intensities of the spectral lines from the healthy and diseased tissue. Duplicate determinations were made on each sample, and a blank spectrum of the aluminum electrodes and

the lithium citrate was made to insure a control of electrode impurities. Another blank determination was made using copper electrodes. The purpose of this was to bring out the spectrum lines of copper.

Results

It was found impossible in the time allotted, to perfect the spectrographic method so that quantitative determinations of copper content could be made. For this reason, the copper contents of the various aliquots of celery tissue can be compared only by studying visually, the intensities of the copper lines of the various spectra shown in Plate VII, page 84.

The spectral lines with which we are concerned and which are lettered on the plate, may be defined as follows: -

a and b - aluminum lines

c and d - calcium lines

e, f and g - copper lines

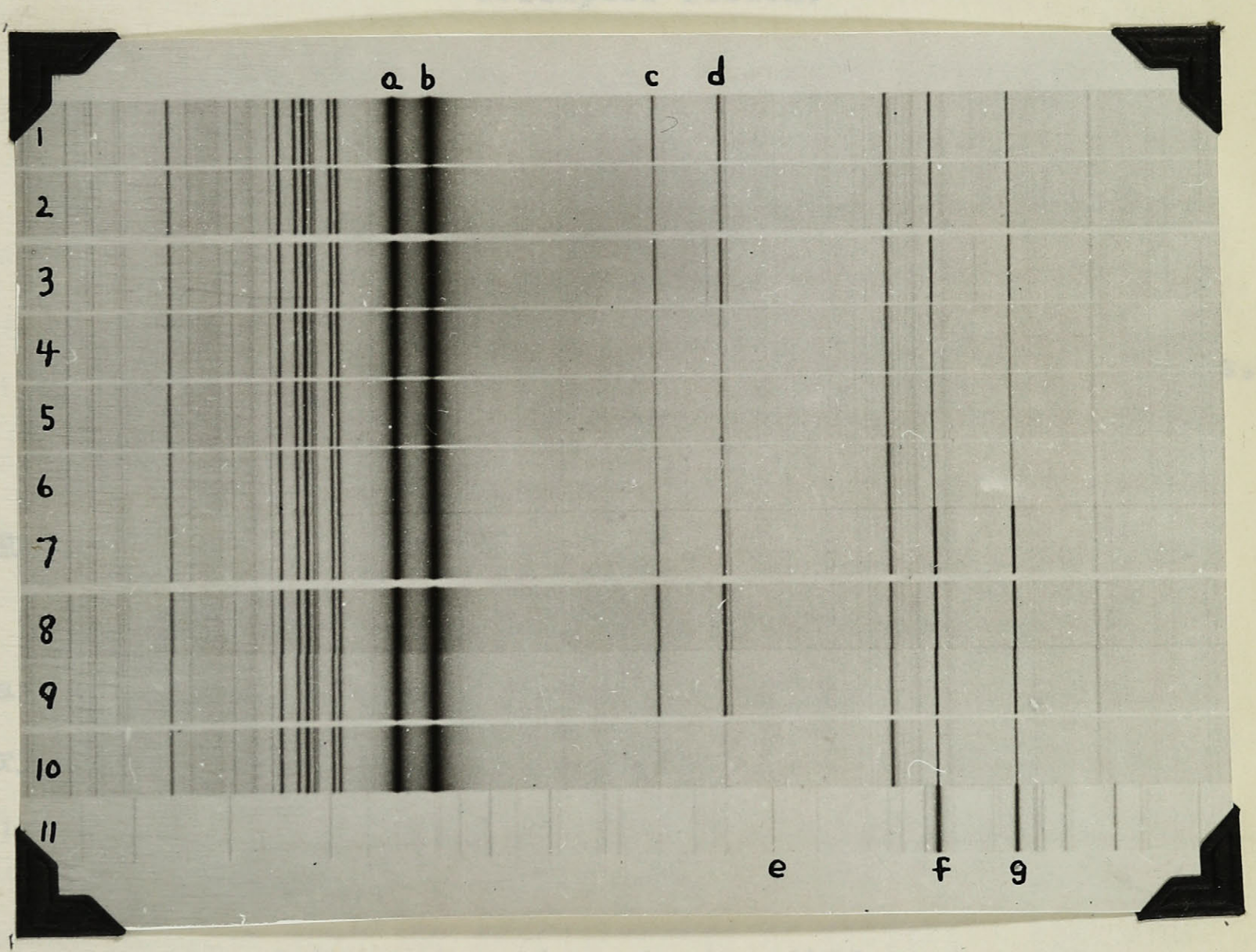

Plate VII. - Spectra emitted by various aliquots of celery tissue on volatilization.

The spectra emitted by the various aliquots of celery tissue on volatilization, are numbered on Plate VII. Reading from top to bottom, they are in the following order: -

1, 2 and 3 - Tissue under the epidermis of a collapsed lesion.

4, 5 and 6 - Healthy tissue from corresponding locations on the same petiole.

7, 8 and 9 - Epidermis of a collapsed lesion.

10 - Blank spectrum of the aluminum electrodes and the one drop of lithium citrate.

11 - Blank spectrum using copper electrodes.

Observations and Discussion

The most intense copper lines are observed in the blank spectrum of the copper electrodes. Copper line (e) is very weak and shows up only in this spectrum. Copper lines (f) and (g) are more intense, and are found at wave lengths of 3247 and 3274 Angstrom units, respectively. By comparing the intensity of these two lines in the different spectra, we can quantitatively compare the copper content of diseased and healthy celery tissue. It is evident that the copper content of the epidermis of a collapsed lesion is higher than healthy tissue from corresponding locations on the same petiole. Furthermore, the copper content of the epidermis of a collapsed

lesion is higher than that of the underlying tissue. These results show that the copper actually penetrates the celery tissue in bringing about collapsed lesion development.

Spectral lines (a) and (b), the aluminum lines, are very intense in all spectra except spectrum (13). This is because aluminum electrodes were used in making the determinations.

Spectral lines (c) and (d) are calcium lines. These lines, like the copper lines, are more intense in collapsed lesion tissue than in healthy tissue. Also, they are more intense in the epidermis of collapsed lesions than in the underlying tissue. This suggests that calcium as well as copper, in some way, penetrates celery tissue from Bordeaux residues, in the formation of collapsed lesions.

Experiment VIII.

Object: Since collapsed lesions in storage can be caused by penetration of copper from Bordeaux residues, it seemed feasible that the effectiveness of different Bordeaux mixtures in causing this disorder might be correlated with their content of copper ion, soluble copper, or free copper as it is termed by different investigators. With this in mind, an attempt was made to determine the copper ion content of the various Bordeaux mixtures used in the field and storage experiments of the 1938-39 term.

Review of the Literature

At the present time, the chemistry of Bordeaux mixture is not well understood. Goldsworthy and Green (16) claim that copper in Bordeaux mixture is in the form of basic sulphates, which become slightly soluble when acted upon by carbon dioxide of the air, or by spore and host excretions. Thus, the toxicity is due to the liberation of soluble copper after the materials have been deposited upon the plant, and not to the soluble ions and soluble complexes inherent in the deposits themselves. However, they found that by some means copper in Bordeaux mixtures, dissolves in water at the rate of 4 p.p.m. Crandall (13) states that the Bordeaux precipitates consist mainly of a chemical complex known as cupric trioxy-sulphate, in various stages of hydration: -

$$(CuO)_4SO_3 . \quad H_2O$$

Bordeaux precipitates have been shown to contain no copper hydroxide. He expresses the opinion that these complex chemicals may react with more lime to form compounds of an even more basic nature. Crandall's finding that soluble copper does not exist in well made Bordeaux mixtures, has the greatest bearing on this investigation. He agrees with Goldsworthy and Green (16) and McCallan and Wilcoxon (23)

in believing that the copper becomes soluble after the Bordeaux mixtures have been applied. McCallan and Wilcoxon (22) and (24) thought that spore excretions such as carbon dioxide and the weak organic acids; malic and acetic, were mostly responsible for putting the copper in the soluble, toxic form. However, they state that atmospheric agencies; especially carbon dioxide and meteoric water, may be secondary factors. Finally, they express the opinion that some copper would go into solution, even from the most insoluble copper protectants. Bordeaux has been shown by Hockenyos (20) to contain considerable amounts of soluble copper. He claims that insoluble copper will not cause serious injury to the plant, and that it was the dissolved copper which caused most of the injury. Hockenyos found that the supernatant liquid of 4-4-40 Bordeaux contained 1.4 p.p.m. of soluble copper, and that the precipitate after filtering and washing, gave up approximately the same amount of copper to distilled water in which it was suspended. McCallan and Wilcoxon (24) poured off the supernatant liquid, washed the precipitate ten times with distilled water; and found that on standing, the supernatant liquid still contained 1 p.p.m. of soluble copper. Therefore, it seems that a constant amount of soluble copper is always found in equilibrium with the insoluble Bordeaux precipitates.

Most of the work on Bordeaux injury has been done on fruit trees, particularly the apple tree. Crandall (13) points out that the formation of brownish spots on apples has usually been attributed to the penetration of copper, in the soluble form, at points where the epidermis had been punctured by an insect or fungus. However, Barker and Gimingham (4) claim that the extent of Bordeaux injury on apples is not entirely governed by the amount of mechanical, insect and fungous leaf injury, because of the increased susceptibility of certain varieties. Clark (12), who also considered that spray injury is the result of absorption of soluble copper by the plant, concluded that the extent of injury would be dependent upon the following factors: - (1) the specific susceptibility of the protoplasm of the plant, (2) the solvent properties of the cell sap and (3) the permeability of the epidermis or cuticle. He was therefore, of the opinion shared by Bain (3) that the copper could penetrate the plant tissue directly. The latter investigator attributed the more pronounced susceptibility of the peach and Japanese plum to a greater solvent action of leaf exudations upon the insoluble Bordeaux precipitate. Martin in his book "The Scientific Principles of Plant Protection" points out that the question of leaf excretions is often met with in

studying the action of spray materials on the plant. He states that in many cases, notably the bean (Phaesolus spp.) and certain Malvaceae, the existence of alkaline exudations has been established; in other cases, e.g. the Fuchsia and Oenthera, the excretions are acidic.

The question of just how direct penetration of soluble copper takes place is a difficult one. Hendrick (19) found that the preliminary stage of Bordeaux injury on apple fruits arose around the stomata and at the basal cells of the plant hairs, but he could reach no conclusion as to the mode of entry of the soluble copper into the cellular tissue of the leaf. As was pointed out earlier, this investigation revealed that penetration of copper into celery was by way of the stomata.

Since it was well known that solutions of copper sulphate produced severe injury when applied to the plant tissue, it was but natural that the addition of more lime was recommended as a protective against Bordeaux injury. It was considered that a large excess of lime would retard the formation of soluble copper. We therefore find, that for the most susceptible plants, Bordeaux mixtures containing larger proportions of lime have been recommended. For example, in England (47), it was suggested that upon

those varieties of apple to which the 4-4-50 mixture caused injury, an excess lime Bordeaux mixture (4-12.5-50) should be employed. To avoid spray injury, Saunders and Brittain (33) recommended the use, not only of even more alkaline mixtures; but also a reduction of the amount of copper present, employing for the first spraying 3-10-40, and for subsequent sprayings 2-10-40 Bordeaux mixtures. Hockenyos also found that Bordeaux mixtures of various formulae caused different amounts of injury, because of varying content of free copper or soluble copper.

On the other hand, some investigators have found that spray injury is not prevented by the addition of more lime. Hendrick (19) concluded that in general, the more copper the greater the injury; but that excess of lime neither prevented nor greatly reduced the amount of injury. Later, Adams (1) stated recent work had indicated that excess lime did not counteract the tendency of Bordeaux mixtures to cause spray injury.

Materials and Methods

The following Bordeaux mixtures, which were used in the field and storage experiments of the 1938-39 term, were made up: 2-6-50, 4-6-50, 8-6-50, 4-3-50, and 4-12-50. Two series of these formulae were used: in the first only hydrated calcium lime was used, and in the

second magnesium oxide was used as a partial substitute for the hydrated calcium lime (three parts of hydrated calcium lime to one part by weight of magnesium oxide). The Bordeaux mixtures were allowed to stand for two days, and then filtered through Jena glass sintered filters. The determinations were made on this clear filtrate. At first, the clear supernatant liquid was taken for the determinations without filtering; but it seemed that varying amounts of colloidal material (probably copper hydroxide, or calcium carbonate, to which had been adsorbed a certain amount of copper ion) were being taken up by the pipette, and so filtration of the supernatant liquid was begun. Jena glass sintered filters were used because it was found in the present investigation, and also by Hockenyos (20), that filtering through ordinary filter paper altered the results of the copper ion determinations. Two procedures were used in making the determinations.

Procedure A: -

The first method is a modification of the sodium diethyldithiocarbamate method as used by Parker and Griffin (28). The following procedure was used: 5 ccs. of the clear filtrate were pipetted into a colorimeter tube, 1 cc. of glycine solution and 1 cc. of α^1-α^1-dipyridyl were

added. This was to prevent the interference of iron. If these reagents were not added, the iron would give a brown colour which would interfere with light transmission and hence the readings of the photoelectric colorimeter. 3 ccs. of distilled water were added and then 0.1 gms. of solid sodium diethyldithiocarbamate. This gives a definite golden-brown colour. The test is very sensitive; as little as one part of copper in 100,000,000 parts of water gives quite a definite colour, which is the same in acid, neutral or alkaline solutions. One precaution must be taken, and it is that the solution to be tested should not exceed 0.0001 gms. of copper per 100 ccs. (10^{-12} y per cc.) as above this concentration the colour becomes too deep to measure. Finally, 5 ml. of iso-amyl alcohol were added, and the colorimeter tubes were then tightly stoppered and shaken for 30 seconds. These were finally centrifuged at 1500 r.p.m. to separate the iso-amyl alcohol, which then bore the colour, from the lower colourless layer. Readings were made immediately in the colorimeter, using a 420 M filter and a special sleeve so that a uniform ray of light passed through the coloured layer. The colorimeter was set so that the blank (pure amyl alcohol) gave 100% light transmission. The percentage light transmission of the various test solutions was then recorded, and the copper ion content was determined from a

calibration curve. The calibration curve was made by plotting percentage light transmission as ordinates against copper ion content (in γ per cc.) as abscissae.

Procedure B: -

Because, as will be shown later, no definite variations in copper ion content of the different Bordeaux mixtures could be recorded by the first method; a second method was introduced. This method is a modification of that employed by Hockenyos (20) in determining the soluble copper content of Bordeaux mixtures.

To a 15 cc. sample of the filtrate, 0.5 ccs. of concentrated hydrochloric acid were added. (This is to remove cloudiness caused by calcium salts). 1.5 ccs. of concentrated NH_4OH and 1 cc. of a 1% solution of saponin were then added. The saponin was added to peptize the colloids formed. Finally, 2 ccs. of a 1% solution of the colour reagent (sodium diethyldithiocarbamate) were added.

A suitable standard was made as follows: -
To 2 ccs. of a 1/5000 N solution of copper sulphate were added 10 ccs. of distilled water. Then, were added 0.5 ccs. of concentrated HCl, 1.5 ccs. of concentrated NH_4OH, 1 cc. of a 1% solution of saponin and 2 ccs. of a 1% solution of the carbamate reagent. This brought the volume of the standard to 17 ccs.

The colour intensities of the solutions to be determined and the standard were compared in a Klett colorimeter and the soluble copper content of the Bordeaux filtrates was determined from the following formula: -

$$\text{Conc. of } Cu^{++} \text{ in filtrate} = \frac{S}{R} \times 72.71$$

where S = depth of the standard in mm.

R = " " " unknown in mm.

and 72.71 = conc. of Cu^{++} in standard (γ per 100 cc.)

Observations and Discussion

The results of this experiment are recorded in Table XXI.

Table XXI - Copper-ion contents of various Bordeaux mixtures, as determined by two different methods.

Formula	Gammas per 100 ccs. of the filtrate	
	Procedure A	Procedure B
Ca 2-6-50	69.8	53.2
Ca 4-6-50	73.5	66.5
Ca 8-6-50	74.2	165.5
Ca 4-3-50	48.0	158.9
Ca 4-12-50	70.6	48.3
Mg 2-6-50	49.3	51.3
Mg 4-6-50	32.1	51.7
Mg 8-6-50	49.4	102.1
Mg 4-3-50	60.1	87.4
Mg 4-12-50	36.5	45.3

The results tabulated on the preceding page are the averages of six determinations in duplicate so that in each case twelve figures were averaged to give the final, recorded figure.

There is great variation in results obtained by the two different methods. The second method shows a high content of soluble copper in both Ca and Mg sprays of the formulae 8-6-50 and 4-3-50. The first method shows only slightly higher soluble copper content in these sprays. It is worthy of note at this point that celery; receiving the two sprays, 8-6-50 and 4-3-50, throughout the storage period; showed the greatest development of collapsed lesions in storage (pages 40 and 41). Throughout the whole series in general, a higher soluble copper content is recorded by Procedure I than by Procedure II. Furthermore, with the exception of the Bordeaux mixtures mentioned above, Procedure II shows less fluctuation throughout the whole series.

However, the results obtained by the two procedures, are consonant in some respects. They both show higher content of soluble copper in the calcium sprays than in the magnesium sprays. It should be noted that the calcium sprays caused more serious development of collapsed lesions in storage than the magnesium sprays (pages 40 and 41). Thus, we can conclude that there is a correlation between the soluble copper content of the Bordeaux sprays used in the field, and the subsequent development of collapsed lesions in storage. Also,

it may be observed that neither the amount of copper sulphate used in making the spray, nor the proportions of hydrated lime and copper sulphate, greatly influence the soluble copper content of the resultant Bordeaux mixture. The latter observation may be explained by the fact that in all Bordeaux mixtures used, the lime is very greatly in excess of the copper sulphate equivalent. Pickering (29) found that slightly more than one part by weight of hydrated lime, unites with five parts of copper sulphate in the formation of Bordeaux mixtures. Thus, in all the Bordeaux mixtures under consideration, the hydrated lime is very greatly in excess of the amount necessary to unite with the copper sulphate, and consequently, similar amounts of soluble copper would be found in the different Bordeaux mixtures.

It is the opinion of the writer, that the principal cause of inaccuracy in this experiment has been the difficulty of getting filtrates free of colloidal material. Just as the various basic salts formed as Bordeaux precipitates vary in colour, so do they vary in rate of settling and ability to remain in colloidal suspension. For this reason, varying amounts of the colloidal material pass through even the sintered filters. Consequently, the concentrated hydrochloric acid used to remove cloudiness caused by calcium salts, also disperses the colloidal copper material and increased the soluble copper content of the test solution.

Another observation is that the calcium carbonate scum, which forms on the surface of Bordeaux mixtures on exposure to air, has the property of adsorbing some of the copper ions in solution. Therefore, when the sintered filters remove the calcium carbonate, they take with it some of the soluble copper which should pass on in the filtrate. The Bordeaux mixtures, with the highest hydrated lime content, show the greatest tendency to form the calcium carbonate scum. Consequently, on filtration, they would lose more soluble copper per unit volume, than would sprays with a lower content of hydrated lime.

From the results of this experiment, it would seem that a means of preventing Bordeaux injury on stored celery might be to spray in the field with Bordeaux mixtures containing magnesium oxide as a partial substitute for hydrated calcium lime. Probably burnt dolomitic limestone would be more easily obtained and would be more satisfactory. This material was tried by Bonde on potatoes in Maine, and found to be very satisfactory.

Experiment IX.

Object: To determine if there is any correlation between the pH values of the various Bordeaux mixtures and the development of collapsed lesions in storage, on celery which was sprayed with these mixtures in the field.

Results

Wendt's electro-titration apparatus, set up with hydrogen and calomel electrodes was used in making the pH determinations, and the results were recorded as follows:

Bordeaux mixtures containing calcium lime		Bordeaux mixtures containing equivalent amounts of magnesium lime	
Formula	pH	Formula	pH
Ca 2-6-50	12.00	Mg 2-6-50	12.00
Ca 4-6-50	12.00	Mg 4-6-50	12.00
Ca 8-6-50	11.62	Mg 8-6-50	11.50
Ca 4-3-50	11.85	Mg 4-3-50	11.85
Ca 4-12-50	12.00	Mg 4-12-50	12.00

Observations and Conclusions

It was observed that the two Bordeaux mixtures which caused the most severe development of collapsed lesions in storage; namely, 8-6-50 and 4-3-50 (pages 40 and 41) had the lowest pH values. However, the difference was not very significant and there seemed to be little change in pH value throughout this series of Bordeaux mixtures. The great excess of lime used in all of the mixtures, was thought to be the cause of this uniformity. It was also interesting to note that the kind of lime used had little effect on the pH of the mixtures.

Stirring or shaking the mixtures, or allowing them to stand exposed to the air for several days, did not change the pH readings.

Experiment X.

Object: To determine the effect of atmospheres containing various CO_2 concentrations on the development of collapsed lesions.

Review of Literature

Brooks and McColloch (7) found that two days exposure to atmospheres containing 20 to 45% CO_2, greatly reduced the amount of pitting that developed in grapefruit during 12 weeks subsequent storage. Brooks *et al.* (8) found that celery was injured by three days exposure to atmospheres, containing 50% CO_2, and showed a slight browning of the vascular tissue after a similar exposure to atmospheres containing 20% CO_2.

Materials and Methods

The celery used in this experiment received no spray treatment in the field or at the time of storing. Large mouthed, four-liter bottles were used for storing the celery in atmospheres containing various carbon dioxide concentrations. The desired concentration of CO_2 was

attained by inverting the bottle filled with water over a pneumatic trough, and running in CO_2 from a pressure tank. The volume of water displaced was taken as a measure of the volume of CO_2 in the bottle. Three series of atmospheres containing the following concentrations of CO_2 were made up in this manner:- 0%, 2%, 4%, 8%, 12% and 20%. In each bottle of the first series, was placed six petioles sprayed with a 1% solution of $CuSO_4$. In each bottle of the second series, was placed six petioles sprayed with 4-6-50 Bordeaux. In each bottle of the third series, was placed six petioles of unsprayed celery. The bottles were then tightly stoppered and stored at 32° F. The intensity of collapsed lesion development was recorded at the end of seven days as the percentage of the total petiole area covered with lesions, using the chart shown in Plate V, page 61. To eliminate error as much as possible, the experiment was repeated three times and the results were averaged to give the final figures.

Observations

The results of this experiment are recorded in Table XXII.

Table XXII - Collapsed lesion development at the end of seven days in atmospheres containing various carbon dioxide concentrations.

CO_2 concentration	Percentage of total petiole area covered with lesions		
	Spray treatment		Unsprayed
	1% $CuSO_4$	4-6-50 Bordeaux	
0%	10.4	0.1	0.00
2%	10.6	0.3	0.00
4%	10.4	0.6	0.00
8%	10.2	1.32	0.00
12%	10.6	2.85	0.00
20%	10.6	3.19	0.00

From these results, it is first of all very evident that the copper sulphate and Bordeaux sprays were directly responsible for collapsed lesion development. In no case did unsprayed celery show collapsed lesion development, even when stored in atmospheres containing high concentrations of carbon dioxide. Therefore, the effect of atmospheric carbon dioxide was secondary and its effect was observed only with the Bordeaux spray and not with the copper sulphate spray. A feasible explanation of this might be that the action of atmospheric carbon dioxide in some way increased the dissolution of the Bordeaux precipitates. The carbon dioxide concentration in the atmosphere would not affect the penetration of copper from copper sulphate, because much of it is already in the soluble or ionized form. McCallan and Wilcoxon (22) and (24) state that atmospheric

carbon dioxide may be partly responsible for the dissolving of copper from insoluble Bordeaux precipitates.

It was observed in all cases that, at the end of the storage period, celery stored at the higher carbon dioxide concentrations (8% to 20%) presented a better general appearance, than celery stored at low carbon dioxide concentrations (0% to 4%). There was less bending of the petioles, less wrinkling of the epidermis, and less browning of the vascular tissue at the higher carbon dioxide concentrations.

Discussion

It must not be overlooked, that as the carbon dioxide content of the air was increased, the oxygen content was decreased. Brooks and McColloch (7) point out that when the carbon dioxide content of normal air is increased to 10%, the oxygen content is therefore reduced from 21% to 18.9%; and with 25% carbon dioxide added, the oxygen content is reduced to 15.75%. Consequently, there is a possibility that some of the effects observed in this experiment, may be due to lowering of the oxygen content of the air. However, since only six petioles were placed in each four-liter bottle, and since the storage period was limited to seven days; one would think that there would be ample oxygen for respiration.

Experiment XI.

Object: To determine whether or not collapsed lesions on celery could be induced by chilling.

Review of Literature

Mulder and Sprenger (26) and Wardlaw (38) found pitting in tomatoes due to chilling. Bondartzeff (5) states that defective storage of fruits during the winter, may result in a considerable percentage of the fruits showing the types of physiological (chilling) injury known as "peteca" and "red blotch". With these investigations in mind, an experiment was conducted to determine if chilling would induce pitting on celery.

Method and Observations

Celery was subjected to a temperature of 26° F. for varying lengths of time, from 15 minutes to 2 hours; and then stored at 32° F. and 98% r.h. No pits, such as occur on tomatoes, stone fruits and grapefruits, as a result of chilling, were observed.

Experiment XII

Object: The eleven laboratory experiments outlined previously were a study of the cause and nature of collapsed lesions. The final experiment of this series, was carried on to determine whether or not a correlation could be made between chilling effects and the development of collapsed lesions. The present experiment may be considered as an extension of Experiment XI and constitutes a more thorough study of the relation of low temperatures to the storage of celery, and has been mostly concerned with the following lines of investigation: -

(1) Symptoms of freezing injury.

(2) Variations in the length of time required to freeze different parts of the celery plant at a room temperature of 26° F.

(3) The actual freezing temperature of celery, and how it varies for different parts of the plant.

(4) The keeping qualities of celery after it has been subjected to freezing temperatures.

Review of the Literature

Platenius *et al.* (30) recommend 32° F. as the storage temperature of celery. They reported that celery stored at approximately 30° F., with a fluctuation of 1° F. on either side, froze; and when subsequently stored at 40° F., it began to rot in a month or six weeks. Thompson (36) has shown that

the temperature in the centre of the crate is likely to be 2 or 3° higher than that of the air surrounding the crate. As pointed out previously, this variation in temperature could not be recorded in the present investigation. Thompson also showed that the air temperature is likely to be 2 to 4° F. higher at the top of the storage room, than at the floor, unless provision is made to keep the air in circulation.

With regard to pre-cooling and post-cooling of celery, very little work has been done. Williams (44) states that fruits and vegetables should not be taken from a low to a high temperature, without being kept for a time, in a room which is in closer proximity to the outside temperature. Rapid heating and condensation of water on their surfaces, produce harmful effects and shorten the life of the produce. He recommends a post-cooling temperature of 55 - 60° F. for a few hours before the vegetables are placed on the market.

Materials and Methods

In this experiment constant temperatures were maintained in a large cold-chamber, cooled by an ammonia system. A thermoregulator was attached to the chamber so that the temperature could be maintained at any desired point. Temperature determinations were made by the use of thermocouples (10). The cold point was kept at a temperature

of 0° C. and the differences in temperature were measured by the deflection of a galvanometer which was set up outside the cold chamber. Thus, it was possible to record accurately the temperatures in various parts of the celery plant without having to open the cold chamber, which would have affected its temperature. The thermocouples were inserted without removing the petioles from the celery plants, and the points of attachment of the thermocouples were sealed tightly with wax so that the conduction of heat through the wires would be at a minimum.

The time required for freezing was recorded as the time necessary for formation of ice crystals in the intercellular spaces. It was observed that when formation of ice crystals began the temperature was usually 1 to 2 degrees below the actual freezing temperature. While freezing progressed, the temperature was observed to rise several degrees over a period of 1 to 1½ hours. As all the tissue surrounding the point of attachment of the thermocouple became frozen, the temperature suddenly became constant and remained at this level until freezing was complete. This final constant temperature was recorded as the actual freezing temperature.

In order to determine accurately the length of time required for freezing, and the freezing temperature; all experiments were repeated ten times and the results were averaged to give the final determinations.

Observations

A. Symptoms of Freezing Injury.

Celery plants that have been frozen for several hours after ice formation, and then placed in storage, were recognized without difficulty. The leaves were limp, water-soaked and appeared as though they had been boiled. Even the odour resembled that of cooked celery. While in the frozen state, the rigid leaves showed the first stages of freezing injury in the form of dark green spots, slightly water-soaked in appearance. The dots enlarged as freezing progressed and intumescences similar to those observed by other investigators (18) on cabbage and lettuce, were observed. These intumescences disappeared on thawing.

Frozen leaves were rigid, because of the formation of ice masses in the tissue; and the intumescences were small. On the petioles, the intumescences were much larger, and a few remained several days after thawing. When the plants had been frozen for an hour or less, no trace of freezing was observed except on the outermost petioles and on old bruised leaves. This hardly affected the general condition of the product; but, even when the tissue seemed to be normal, the epidermis was loosened so that a slight twisting of the stem showed corrugations, resulting from separation and wrinkling of the epidermis. In some cases, only proper light conditions made the corrugations visible.

B. Variations in the Length of Time Required to Freeze Different Parts of the Celery Plant at a Room Temperature of 26° F.

In general, celery leaf blades froze sooner than other parts of the plant. The freezing temperature curves for different parts of the celery plant are presented on Plate VIII, page 110.

The following are the observed time intervals required to freeze different parts of the celery plant at a room temperature of 26° F.

Part of celery plant	Time required for formation of ice crystals
(1) Leaf blades	2 hrs. 40 mins.
(2) Upper portion of outer petiole	4 hrs. 45 mins.
(3) Lower portion of outer petiole	4 hrs. 50 mins.
(4) Crown	7 hrs. 15 mins.
(5) Lower portion of inner petiole	8 hrs. 50 mins.
(6) Upper portion of inner petiole	12 hrs. 0 mins.

These results show that the leaf blades froze much more quickly than any other part of the plant. The outer petioles froze more quickly than the inner petioles. There was very little difference in the length of time required to freeze the upper and lower portions of the outer petiole.

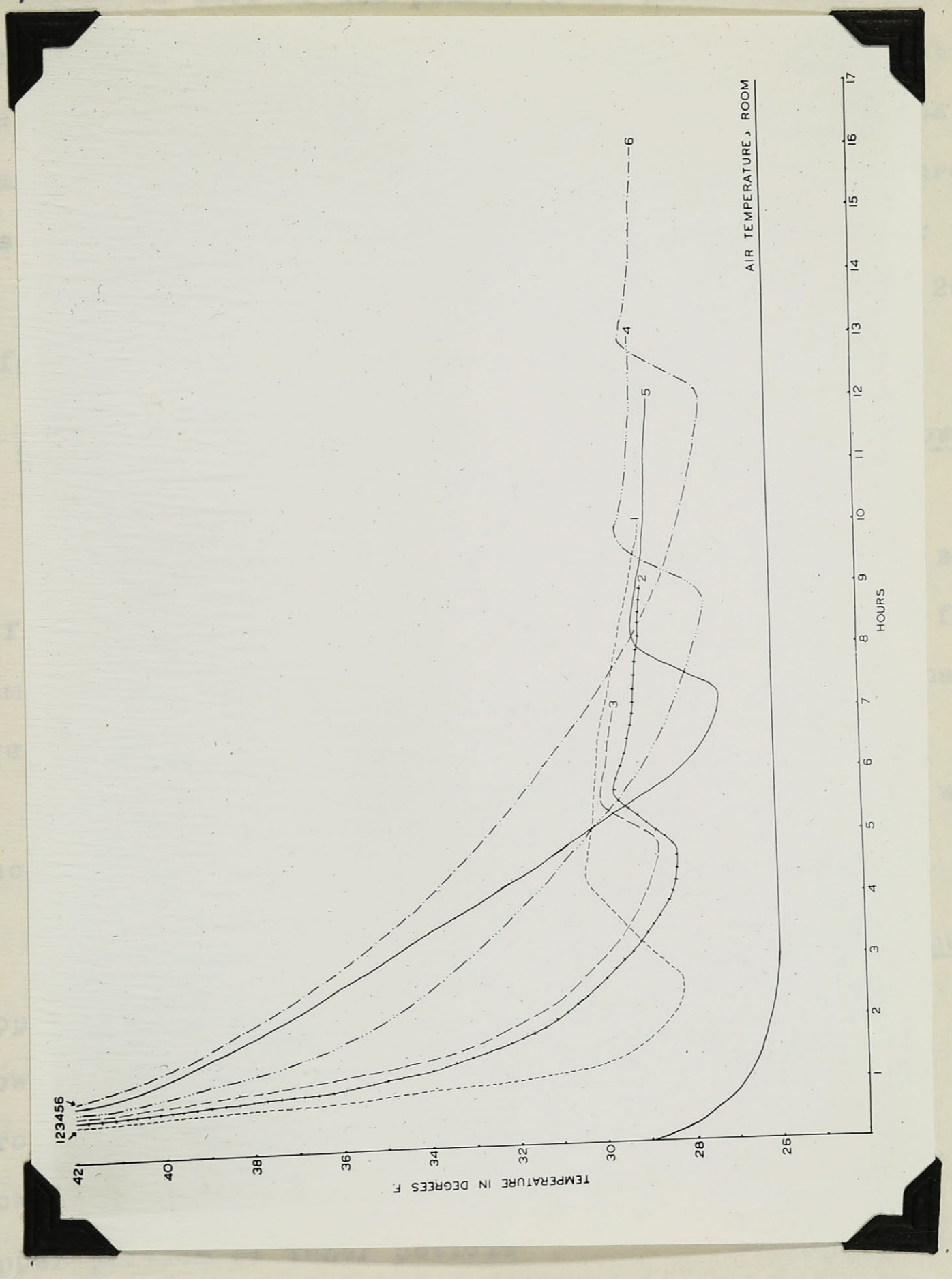

Plate VIII.- Freezing temperature curves for various parts of the celery plant, as listed on page 109.

The crown or base of the plant, froze more rapidly than the inner petioles, but not as rapidly as the outer petioles. The lower portion of the inner petiole froze more quickly than the upper portion; probably because its temperature was influenced by its nearness to the crown. The upper portion of the inner petiole required the longest exposure to 26° F. before freezing began.

C. The Actual Freezing Temperature of Celery and How it Varies for Different Parts of the Plant.

The average of 10 determinations on each of six different parts of the plant, showed that the average freezing temperature of celery tissue was 29.79° F., and this had an average deviation of 1.15° F.

The following average freezing temperatures were recorded for different parts of the celery plant.

Part of plant	Average freezing temperature
Upper portion of outer petiole	30.42
Lower portion of outer petiole	30.08
Crown	29.35
Lower portion of inner petiole	29.65
Upper portion of inner petiole	29.51

From these results which are graphically presented in Plate VIII, page 110, it appears that the parts of the celery plant in which the food is stored, have the lowest freezing

temperatures. The crown, although it froze more quickly than some other parts of the plant, reached the lowest temperature before there was actual formation of ice in the tissue. This is what might be expected, because substances in solution in the cell sap would lower the freezing point of the tissue. Each gram molecular weight of an inorganic salt in solution depresses the freezing point 1.86°C., and organic solutes cause depression of the freezing point in varying proportions.

D. The Keeping Qualities of Celery After it has been Subjected to Freezing Temperatures.

The keeping quality of frozen celery plants was much altered. Those in which signs of freezing could be determined only with difficulty, kept well for five to eight days at 50° F. After this time, the crown was generally attacked by soft rot organisms, although the leaves and petioles of the plant were still in good condition. The celery plants used in this experiment had been prepared for market, and part of the crowns had been removed. The results might have been different with the roots remaining on the plants. At laboratory temperatures, these slightly frozen plants began to wilt after two days; although they were moistened from time to time. It was concluded that slight freezing (exposure to 26° F. for one hour after ice formation) halved the storage life of the product.

Severely frozen celery decayed soon after thawing. Its marketability was very severely reduced at the end of three days, even when kept at storage temperature (32°F.). This reduction in marketability was caused mostly by loss of crispness and flavour. In some cases, the natural sweetness was impaired and the tissue became more fibrous and stringy. Also, in some cases, air spaces of considerable size formed between the collenchyma strands of the petiole. This is apparently caused by killing and disintegration of cells, and much resembles the storage disorder known as pithiness. However, following severe freezing; the emission of the volatile esters, which give celery its characteristic odour, seemed to continue. A secondary effect of freezing injury was that it afforded favourable courts for the inroads of saprophytic organisms, which soon very rapidly increased the rate of decay.

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Special mention is due Professor J.G. Coulson, Macdonald College, for supervising the spraying of field plots and taking records on stored celery in the experiments conducted during the 1938-39 term, and for valuable advice and criticism given at all times.

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SUMMARY AND CONCLUSIONS

Translocation of sugars and their breakdown in the process of respiration are among the most important factors influencing the gradual lowering in quality and deterioration of celery in storage.

Besides the natural physiological breakdown of celery, due to respiration; certain definite disorders in storage must also be classed as physiological breakdowns, because they are not caused by pathogenic organisms. These disorders have been termed "collapsed lesions" and "basal rot" in this investigation. A series of experiments and many observations, have shown these disorders to be due to penetration of copper from Bordeaux residues. Foliage rot, a third disorder, is due in the early part of the storage period entirely to toxicity of Bordeaux residues and the injury so induced affords courts for the inroads of micro-organisms. However, the action of micro-organisms on stored celery is not entirely secondary because after six weeks of storage the organisms cause primary infection. This is probably due to natural lowering of the resistance of the plants as the time in storage increases. After this time, foliage rot develops rapidly. The organisms most responsible for foliage rot are three species of bacteria

of the Bacillus caratovorus type, three species of Botrytis, an Alternaria sp. and Sclerotinia sclerotiorum.

There was no difference in the keeping qualities of celery grown on mineral and muck soils.

Foliage rot, collapsed lesions and basal rot were more prevalent on celery which received a complete fertilizer mixture, than on celery which received a fertilizer lacking one or two of the three essential components (nitrogen, phosphorus and potash).

There was no correlation between the prevalence of stem cracking in the field, and the development of collapsed lesions in storage.

Celery exposed to frosts before harvest, suffered more severely from foliage rot and collapsed lesions than did celery stored before frosts occurred. Also, basal rot development was slightly more severe in the frosted celery.

Delaying storage for three days after harvest, shortened the time of profitable storage.

Cutting back of the foliage at the time of storing, had no effect upon the storage life of the plants, and so was not found to be a worth while practice.

Fungicidal treatments at the time of storage, did not satisfactorily reduce the amount of rot caused by pathogenic organisms; nor did they decrease the severity of development of other storage disorders.

Celery sprayed in the field, with Bordeaux mixtures containing the largest amounts of copper sulphate in proportion to the lime content showed the most severe development of collapsed lesions in storage. Determinations in the laboratory, showed these Bordeaux mixtures to contain the largest amounts of soluble copper. Thus, the proportion of the copper sulphate to the lime in a Bordeaux mixture, determined its content of soluble copper; and this in turn, determined the extent of collapsed lesion development in storage. It is therefore suggested that the development of collapsed lesions in storage could be decreased by modifying the composition of the Bordeaux mixture used in the field, so that the content of copper sulphate would be reduced in proportion to the lime content.

Celery sprayed in the field or at the time of storing, with Bordeaux mixtures containing hydrated calcium exhibited greater development of collapsed lesions in storage than did celery sprayed with Bordeaux mixtures containing a chemically equivalent amount of magnesium lime composed of $\frac{1}{4}$ magnesium oxide and $\frac{3}{4}$ calcium hydrate. Laboratory determinations showed the calcium Bordeaux mixtures to have the highest content of soluble copper. Consequently, it is further suggested, that burnt dolomitic limestone could be used to advantage in Bordeaux mixtures as a partial substitute for calcium hydrate.

The field, storage and laboratory experiments showed a direct correlation between the soluble copper content of Bordeaux mixtures used in the field, and the subsequent development of collapsed lesions in storage.

Collapsed lesion development was much more severe on celery, which was sprayed at the time of storing; than on celery which was sprayed, only in the field. Thus, the more Bordeaux residue on the celery at the time of storing, the greater the severity of collapsed lesion development in storage. Furthermore, a laboratory experiment showed that removing of copper sulphate and Bordeaux spray residues with 1% H_2O_2 and 0.2% HNO_3 respectively, decreased the severity of collapsed lesion development in storage. It is therefore recommended, that celery growers refrain from spraying late in the growing season, and further, that wherever possible, the celery should be washed before it is placed in storage.

Collapsed lesions developed in storage on celery which received no spray treatment in the field or at the time of storing. The severity was very much less than on the copper sulphate and Bordeaux spray treatments,but nevertheless there was some development. This would indicate that collapsed lesions may be caused in some other way than by the toxicity of chemicals. This investigation affords no explanation of

this seemingly spontaneous development of collapsed lesions.

Throughout the course of this investigation, collapsed lesions and basal rot were not observed in the field. It was observed, that the low temperatures and high humidities of storage rooms, brought about condensation of water on the celery plants in storage. A series of laboratory experiments showed that high humidity favoured the development of collapsed lesions. From these observations and experiments, it was concluded that the high humidity of the storage room favours collapsed lesion development. Condensation of water on plants in storage may be responsible for hydrolysis of copper salts of Bordeaux residues and thus the setting free of soluble copper.

The storage records in this investigation showed that the three disorders, namely; foliage rot (chemically induced), collapsed lesions and basal rot, develop simultaneously in storage. However, it was difficult to follow the progress of the chemically induced foliage rot; because it was made more severe by the secondary effects of bacterial and fungal pathogens. Collapsed lesion development began about three weeks after the celery was stored, and continued until a maximum was reached about ten weeks after storing. After that time, no further development was recorded. Basal rot developed more slowly

than either foliage rot or collapsed lesions, but its development continued until the end of the storage period.

Spectrographic analysis showed that the copper content of collapsed lesion tissue was higher than that of healthy tissue, and microscopic examination showed the epidermis to be intact, so it was concluded, that penetration of copper takes place by way of the stomata. This was further proven by staining reactions with methylene blue.

Laboratory experiments showed that atmospheres containing high concentrations of carbon dioxide, favoured the development of collapsed lesions only in the presence of copper. Furthermore, it was observed that the higher carbon dioxide concentrations (8% to 20%) were more effective in preserving the general appearance of the celery than the lower carbon dioxide concentrations (o% to 4%).

Collapsed lesions developed in twelve hours on celery which was sprayed with 1% $CuSO_4$ and then stored at 32° F. and 98% r.h. The Bordeaux spray commonly used in the field, produced collapsed lesions at the end of twenty days, under similar conditions.

Many chemicals, other than Bordeaux and copper sulphate, induced collapsed lesions in laboratory experiments. Among these are included the chloride, nitrate and sulphate salts of Cu, Zn, Hg, and Ag. It was also observed that the strong acids; nitric, hydrochloric and sulphuric, in very dilute solutions, induced collapsed lesions. In general, it was concluded, that the elements with the highest atomic weights, were the most effective in producing collapsed lesions. Also, elements near the centre of the periodic table were more effective than those at either end.

Collapsed lesions were not observed on growing plants in the field, but they were induced on growing plants in the greenhouse, by 1% solutions of copper nitrate, and copper sulphate. They were not induced on growing plants in the greenhouse by any of a series of Bordeaux mixtures.

A laboratory experiment showed, that collapsed lesions could not be induced on celery by chilling.

A more thorough laboratory study of the effect of low temperatures on the storage of celery was conducted, and the following observations were made: -

(1) The symptoms of freezing injury were studied and described.

(2) There was a difference in the length of time required to freeze different parts of the celery plant. The leaves froze more quickly than any other part of the plant,

while the upper parts of the inner petioles required the longest time for freezing.

(3) The average freezing temperature of celery tissue was 29.79° F., taking all parts of the plant into consideration. However, it was observed that all parts of the plant did not freeze at the same temperature. The leaves froze at 30.42° F., while the crown did not freeze until a temperature of 29.35° F. was reached. It was concluded, that the parts of the plant in which the food is stored, had the lowest freezing temperatures.

(4) The keeping qualities of celery were impaired by freezing. It was shown that slight freezing (exposure to 26° F. for one hour after ice crystal formation) halved the storage life of the product. Severely frozen celery was completely unmarketable at the end of three days subsequent storage, at 32° F. and 98% r.h. A secondary effect of freezing injury was that it afforded favourable courts for the inroads of saprophytic organisms, which very rapidly increased the rate of decay.

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