STUDIES ON FOLIAR ABSCISSION OF CAULIFLOWER AND CABBAGE IN STORAGE, WITH SPECIAL REFERENCE TO THE EFFECTS OF CERTAIN GROWTH REGULATING SUBSTANCES

By

SHU-HSIEN LEE

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY

Department of Horticulture

1948

ACKNOWLEDGMENTS

The writer wishes to express his appreciation to Dr. R. L. Carolus for his effective guidance throughout this investigation. He also wishes to express appreciation to Dr. H. B. Tukey, Dr. R. E. Marshall, Dr. E. H. Lucas, Dr. S. H. Wittwer, and other members of the Department of Horticulture, and to Dr. F. L. Wynd, of the Department of Botany and Plant Pathology, for their advice and cooperation.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

During storage, premature leaf fall in cauliflower, and to some extent in cabbage, will frequently reduce the maketability of these crops. In connection with a storage study with cauliflower, some preliminary work conducted by Carolus, et al. (1947) indicated that the abscission of the jacket leaves surrounding the head could be delayed by treatment with methyl ester of α-naphthaleneacetic acid.

It is the purpose of this study to discover the extent to which foliar abscission in cabbage and cauliflower can be delayed, and to study the effectiveness of several substances applied in different ways, in various concentrations, at successive stages in the life of the plant in altering this phenomenon. The anatomical nature of abscission, as well as the effect of abscission on the biochemical and physiological conditions of the plant, were observed in order better to understand the nature and control of this process.

REVIEW OF LITERATURE

Anatomical Nature of the Abscission Formation

The formation of an abscission layer, in most mature deciduous leaves, is usually characterized by a narrow tranverse zone which differs anatomically from the adjacent cells. After the formation of the abscission zone is complete, in most leaves, as well as flowers and fruits, a separation layer is developed which causes the abscissed structure to fall.

Sampson (1918) reported that the dissolution of the middle lamella in the leaves of Coleus Blumei, is the structural cause of leaf fall. Fehr (1925) found that in many woody plants the abscission of the mature fruits is developed by means of a separation layer through the activity of secondary cell division. It is generally known that leaf fall of most flowering plants is due to the dissolution of the middle lamellae and outer walls of cells of the separation layer, and is not produced by a separation resulting from the complete dissolution and destruction of a layer of tissue.

The meristematic layer is not necessary differentiated before the development of a separation layer in all abscissed organs. Kendall (1918) reported that no cell divisions or elongations were observed to accompany

abscission in many species of Solanaceae. McCown (1939, 1943) found in apple that the abscission of the flowers and of immature fruits was preceded by differentiation of an abscission layer, whereas the abscission of mature pedicels was initiated independently in the pith and cortex, and not preceded by cell division.

In the leaf abscission of Valencia orange, Scott, et al. (1947) found that the middle lamellae break down between the new thin cellulose and the old thick ligno-suberised walls. Deposition of suberin occurs not only on the abscission zones but also throughout the leaf. In mature leaves, a continuous suberin network outlines intercellular spaces and middle lamellae partially or completely.

This chemical deposition might not be the only factor in the mechanism of the abscission formation. The turgor pressure of the cells in the abscission zone might have influence. Kendall (1918) reported that an increase in cell turgor frequently occured during abscission, but probably served merely to hasten and facilitate the process. Recently, Livingston (1947) claimed that in the foliar abscission of Citrus, the peripheral pressure and the resulting tension at the center of the abscission zone might have an active part in foliar abscission.

Relation of Chemical Changes to Abscission Formation

As with other chemical changes in a plant tissue, the dissolution of the cell wall in the abscission zone followed by the separation of the whole petiole, is the result of physiological activity. In Coleus Blumei, Sampson (1918), using microchemical analysis, showed that the chemical processes of the leaf abscission are a result of the conversion of cellulose into pectose, which is further transformed to pectin and pectic acid. This leads to the formation of an excess amount of pectic acid over that of the available calcium sufficient to maintain the solidity of the middle lamellae of the cell walls of the abscission layer.

Kendall (1918) reported that in the course of flower abscission of many species of Solanaceae, cell separation is brought about by the hydrolysis and consequent dissolution of the middle lamella or perhaps both the primary and in part, secondary cell membranes. The agency active in the hydrolysis of the cell membranes is probably an enzyme. McCown (1943) found almost the same chemical change in the course of cell separation in the abscission zone of apple fruit.

In addition, Sampson (1918) also found in Coleus leaves that nitrates, reducing sugars, and oxidases all

increase. Oxalates remain fairly constant and soluble calcium disappears during the formation of abscission. These changes were possibly initiated and probably accelerated by the presence of oxidases and ferric ions, both of which accumulated in the abscission layer.

Lloyd (1916) found that in the leaf abscission of Mirabilis jalapa the starch in the abscission cells decreases as abscission progresses, until it is very materially reduced or entirely disappears. It was considered to be used as a source of energy for the separation of cells during their growth. In a study of leaf abscission of Valencia orange, Scott, et al. (1947) found that the nodal zone is roughly indicated by starch accumulation, and that intercellular space distribution is not clearly defined until the imminence of leaf fall.

This chemical changes of cell wall in the abscission zone might result from or cause the physiological change of other parts of the cells. Cytoplasm, nuclei, and nucleoli according to Lloyd (1916) bear evidence of greater physiological activity, and are alive and normal when separation is achieved. He also claimed that there is meanwhile no loss of turgor; however, Livingston (1947) points out in citrus leaves that pressure and tension of the abscission zone might be involved in this process.

External Factors Associated with Abscission Formation

As mentioned before, the formation of abscission layers of leaves as well as of flowers and fruits is not merely a mechanical rupture, but is a result of physiological activities which might cause the chemical change of the cell walls in the abscission layer. Any condition, internal or external, which might retard or accelerate the rate of physiological activity, would naturally affect the abscission formation.

Studying the effect of growth substances on the abscission layer in Coleus leaves, Myers (1940) reported that abscission could be accelerated by a number of external factors such as high or low light intensity, high or low water supply, high or low temperature, low concentrations of anesthetics, toxic concentrations of acids and salts, ethylene gas, and wounding or complete removal of the blades. Sampson (1918) in Coleus Blumei, found that leaf fall was accelerated by treatment with ethylene, amputation of the blade, and by allowing the soil to become dry and then suddenly applying an excess of water. Kendall (1918) claimed that the abscission of flowers and fruits in Solanaceae could be accelerated by nicotine vapors, injury of floral organs, sudden rise in temperature, and even changes in soil conditions.

In addition, Hoffman (1940) indicated that an application of nitrogen-carrying fertilizer made under conditions which would permit the trees to obtain excessive amounts of nitrates during the latter half of the growing season increases the pre-harvest drop of McIntosh apples. Heinicke, Reuther, and Cain (1942) found that an excessive pre-harvest drop of McIntosh apple was associated with incipient stages of boron deficiency which may not be severe enough to cause cork or drought spot. In a study of apple flower and fruit abscission, MacDaniels (1936) suggested that the rate at which the abscission zone is cut across is probably influenced by the carbohydrate-nitrogen relationship within the tissue. When there is excessive amount of carbohydrate as compared to nitrogen, more woody tissues are formed so that the abscission zone is not so easily cut across.

All these external factors, which are able to influence the abscission formation, possibly could affect the synthesis of the growth substances being associated with this process. Since growth substances are believed to be synthesized in the leaf blade (Avery, 1935), its removal should hasten leaf drop. LaRue (1936) pointed out that the petiole of Coleus from which the blade has been removed will fall within a few days. He also found that the leaf blade is necessary to prevent abscission

formation in the leaves of Betula alba var. papyrifera Spach., and Alnus incana Moench. If the leaf blade was entirely removed, leaving only the petioles, the latter fall in most plants within a few days.

Furthermore, in Morus bombycis Koidz., Cudrania triloba Hce., Ficus carica L., Thea sinensis L., Salix viminalis L., and Ginkgo biloba L., Okabe (1940a) reported that if the leaf blades are removed it appears that the growth promoting substance in the petioles of the leaves goes on decreasing and that the growth-inhibiting substance increases day by day until the petioles fall off. Myers (1940) mentioned in Coleus leaves that partially debladed petioles do not fall as soon as petioles from which the entire blade has been removed. Actively expanding portions of the blade are more effective in checking abscission than the mature parts.

The Effect of Growth Regulating Substances in Modifying Abscission Formation

From the standpoint of the phenomenon of abscission, the physiological activity of the organ that will subsequently fall is of greater significance than that of the remaining parts of the plant. It has long been known that the premature drop of fruit is due to the lacking of certain growth hormones which are produced by the fertilized ovule and translocated downward to the abscission

zone of the pedicel. Similarly, the falling of a bladeless leaf is considered to be due to the absence of certain growth hormones which are synthesized in the leaf blade and transported downward to the abscission zone of the petiole. As concluded by LaRue (1936), the abscission always resulted when the development of the abscission layers was not inhibited by some substances produced in the leaf blades, and the abscission layers did not need any special stimulus for their development.

Based on this consideration, many synthetic growth regulating substances have been recognized to replace the function of the naturally produced phytohormones. Some of them have been used commercially to prevent the pre-harvest drop of fruits by great number of workers. Gardner, et al. (1939), Hitchcock and Zimmerman (1941), Tukey and Mamner (1945), used naphthaleneacetic acid; and Batjer and Thompson (1946), Harley, et al. (1946) and many others used 2,4-dichlorophenoxyacetic acid to prevent the preharvest drop of apples. Davey (1942) reported that spraying with naphthaleneacetic acid, and naphthaleneacetamide reduced the fruit drop of Bartlett pears 50 per cent. Hasse and Davey (1942) also used naphthaleneacetic acid and naphthaleneacetamide to prevent the fruit drop of apricot and peach. Recently, Stewart and Klotz (1947) used 2,4-dichlorophenoxyacetic acid to prevent the fruit drop of oranges.

Beal and Whiting (1945) reported that the plants of Mirabilis jalapa L. decapitated and treated with a 2 per cent indoleacetic lanolin mixture on the cut surface of the stem showed continued growth of the internodes, and the entire absence of abscission or of an abscission zone at the bases of the internodes. LaRue (1936) and Myers (1940) found that auxin-containing substances, when applied to the cut end of the petiole, have the ability to delay abscission in Coleus. Gardner and Cooper (1943) have tested 156 organic compounds for the activity to delay petiole drop. None of them, however, showed sufficient activity for practical usage. The prevention of leaf abscission appears to require a much higher concentration of applied growth substance than the prevention of mature fruit drop. This result, according to Gardner and Cooper (1943), may be related to the differences in the types of abscission involved.

The effectiveness of the growth regulating substances on the retardation of abscission formation varies with the substances used and the plant organs treated. It also depends on the maturity of the leaves and fruits (Batjer and Thompson, 1946), (Hesse and Davey, 1942), (Batjer and Moon, 1945), the temperature at which the treatment was made (Batjer and Moon, 1945), the formulations and methods of application (Hoffman, et al., 1942),

(Southwick, 1942), (Tukey and Hamner, 1945), (Ennis and Boyd, 1946), and even varies with different trees of the same variety and with different branches of the same tree (Hitchcock and Zimmerman, 1941).

Generally, any condition that influences the phytohormone production in the plants, might influence the effectiveness of the growth regulating substances used. Mineral nutrition, light, temperature, age of the plant, position of the plant organ, as pointed out by Gustafson (1946), should be considered in connection with the application of the growth regulating substances for this purpose. Externally, man can probably do nothing to modify the position or the structure of abscission, but he has done much to retard or accelerate this process.

EXPERIMENTATION

The Effect of Post Harvest Treatment with Growth Regulating Substances on Foliar Abscission

General Methods

The cauliflower and cabbage used in testing the effects of time of treatment, method of application, and the formulation of the growth regulating substances on abscission were procured from the local market; while the cauliflower (Snowball X) and cabbage (Resistant Detroit) used for the test of the effect of trimming and temperature on abscission were grown on the college farm in 1947, under ordinary culture practices.

The cabbage and cauliflower were treated immediately after harvest or after shipment, and then stored at a temperature of approximately 32° F. at a relative humidity of 85 per cent. Each head was stored in an open kraft paper bag.

The methyl ester of both 2,4-dichlorophenoxyacetic acid (2,4-D), and a-naphthaleneacetic acid (NA) were first sprayed on shredded paper which was then placed loosely around the cabbage or cauliflower heads. The concentration of the methyl ester of either 2,4-D or NA is expressed as milligram of the liquid per head contained in the shredded paper. The sodium salt of either 2,4-D or NA was sprayed

with a hand sprayer directly on the leaves of the plants to be treated. The concentration of the sodium salt is expressed as parts per million (p.p.m.) in aqueous solution and the amount of the solution used is expressed as milliliters for each head. In certain experiments a soluble plastic material (Geon X-31),* capable of filming, was mixed with the growth regulating substance in order to localize and concentrate the effect of treatment. The sodium salts of NA and 2,4-D were used in this way mixed with 50 per cent Geon containing 56% solids.

Fresh weight loss was determined at various storage intervals. The falling leaves were counted periodically during the storage period. The per cent of leaf drop per head was calculated on the basis of the total number of leaves per head. Usually a cabbage head contained 40 to 50 leaves (excluding those shorter than three inches), and a cauliflower head contained 25 to 30 leaves.

---

* Obtained from Goodrich Chemical Co., Cleveland, Ohio.

Effect of Different Times of Treatment

Fresh cabbage and cauliflower shipped from Arizona and procured on the local market were used in this experiment. The cabbage and cauliflower were treated four heads for each treatment with three different concentrations of the methyl ester of NA, at 50, 100, and 200 mgs. per head. In order to determine the effect of time of treatment on abscission, cauliflower heads were also treated in the same manner immediately after harvest in Texas and then shipped to East Lansing.* The fresh weight and number of dropped leaves were recorded weekly.

Results and Discussion

Time required for abscission---Fifty per cent of the leaves dropped in untreated cauliflower head in 30 days, while in the treated heads 80 days storage were required to cause the same percentage of leaves to fall. The shredded paper placed either on top or under the heads was equally effective in delaying abscission. Observation indicated that abscission progressed from the inner (younger) to the outer (older) leaves of the treated cauliflower, and from the outer to the inner leaves in the untreated heads.

---

* Heads were collected and treated by Mr. S.B.Apple, Extension Horticulturist, Weslaco, Texas.

In order to study the effect of high humidity on abscission, a few heads treated with 100 mgs. of NA were loosely wrapped with pliofilm. Wrapping of treated heads delayed abscission of 50 per cent of the leaves for 150 days, or 60 days longer than the unwrapped heads.

It was observed that the retarding effect of the methyl ester of NA on the abscission of cabbage was not as pronounced as in cauliflower. Unlike cauliflower, which usually held its leaves for only a few weeks, the untreated cabbage held their leaves for more than three months without any dropped leaf.

Loss in weight---Before the formation of abscission, only slight differences in the rate of weight loss were found between the treated and untreated heads. After leaf fall, loss of weight was higher in the untreated than in the treated lots (Table 2). This was due to the fact that the fallen leaves withered rapidly. The weight loss in cabbage was similar to that of cauliflower (Table 3).

Since the loss in fresh weight during storage is mainly due to transpiration, the rate of loss, therefore, varied with the exposed surface area on the one hand, and the permeability of the epidermis on the other. In cabbage where the leaves fold closely on one another and were not trimmed, the rate of fresh weight loss was less than that

of cauliflower, where the leaves were more exposed and usually trimmed. This higher rate of weight loss in cauliflower might accelerate abscission formation, and is probably one reason why cabbage can be stored for a longer time than cauliflower.

TABLE I. Effect of the Methyl Ester of Naphthaleneacetic Acid on Foliar Abscission in Cauliflower (Average of five heads).

Treatment*	Days required for 50 per cent of leaves to drop	
	Treatment after shipment	Treatment before shipment
50 mg. NA per head	80	--
100 mg. NA per head	84	95
200 mg. NA per head	92	103
Check	32	45

* In storage at 32°F.

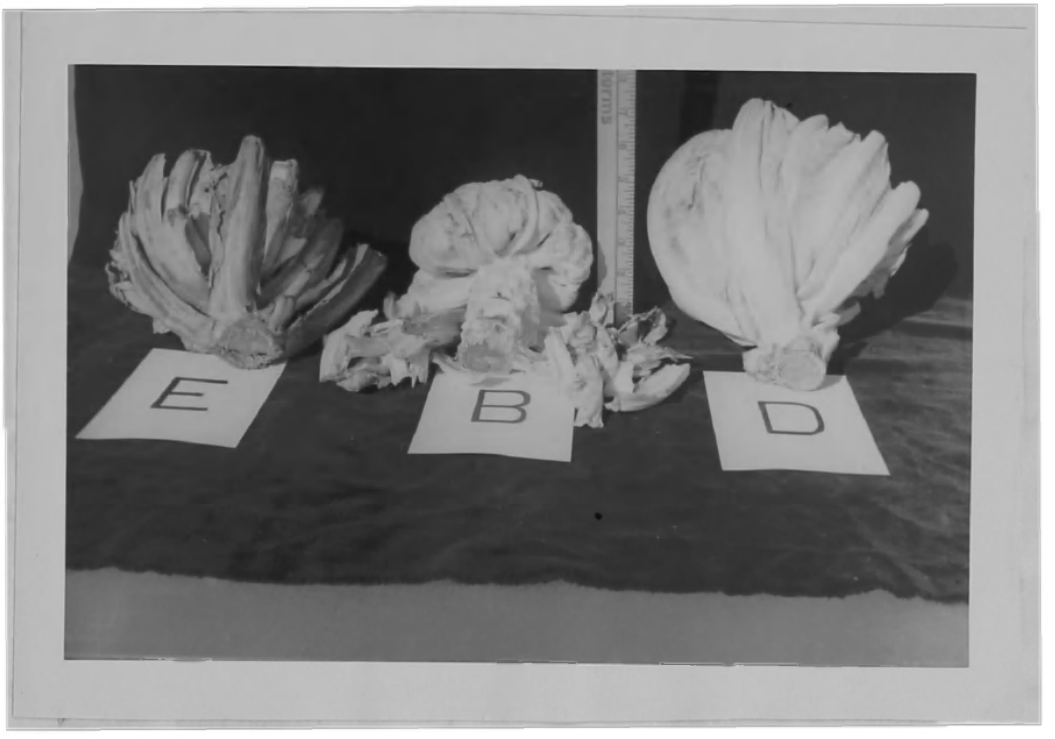

Figure 1. Effect of methyl ester of naphthalene-acetic acid on the abscission of cauliflower, 33 days after treatment. E, directly sprayed with NA; B, check; D, treated with 100 mg. of methyl ester of NA.

TABLE II. Per Cent Loss in Weight of Cauliflower in Storage, Following Treatment with Different Concentrations of the Methyl Ester of Naphthaleneacetic Acid (Average of four heads).

After Shipment				Before Shipment			
Days after Treatment	Per Cent Fresh Weight Loss			Days after Treatment	Per Cent Fresh Weight Loss		
	NA 100 mg.	NA 200 mg.	Check		NA 100 mg.	NA** 200 mg.	Check
16	1.9	2.4	3.3	10	4.1	0.0	5.4
31	7.1	9.2	10.5	19	6.2	0.5	7.7
44	11.5	13.2	18.9	38	11.8	1.3	14.9
61	16.3	17.4	24.9	59	18.1	3.0	23.8
80	20.1	20.8	31.6	95	26.3	5.4	34.3
93	23.4	22.7	--	113	34.1	8.9	44.4

** Wrapped in pliofilm.

TABLE III. Per Cent Loss in Weight of Cabbage in Storage Following Treatment with Different Concentrations of the Methyl Ester of Naphthaleneacetic Acid (Average of four heads).

After Shipment				Before Shipment			
Days after Treatment	Per Cent Fresh Weight Loss			Days after Treatment	Per Cent Fresh Weight Loss		
	NA 100 mg.	NA 200 mg.	Check		NA 100 mg.	NA* 200 mg.	Check
16	1.1	1.5	2.8	10	2.9	0.2	2.4
31	6.6	7.4	9.8	19	4.4	0.3	3.6
44	10.2	11.3	16.3	38	8.3	0.6	7.5
61	13.8	15.0	21.6	59	11.6	2.3	11.1
80	16.6	17.6	25.9	95	15.8	4.8	15.9
93	18.7	19.9	28.7	113	19.4	7.7	20.4

* Wrapped in pliofilm.

Effect of Different Formulations of the Growth Regulating Substances

Both the methyl ester and the sodium salt of both 2,4-D and NA were used to determine their effectiveness in retarding abscission formation, inflorescence elongation, and leaf color change. Also, the sodium salt of 2,4-D mixed with a 50 per cent solution of Geon (X-31) was used. Five heads of locally purchased cauliflower were used for each treatment.

Results and Discussion

From the data in Table 4, it appears that both the methyl ester and the sodium salt of 2,4-D delayed the drop of 50 per cent of the leaves for longer than 100 days. On the other hand, the sodium salt of NA at the concentration used had little effect on leaf fall.

Elongation of the cauliflower inflorescence was induced by all treatments. There was no elongation of the inflorescence in the untreated cauliflower, even after all the leaves had fallen. Apparently, there was little relationship between the abscission formation and the elongation of the inflorescence.

The methyl ester forms of either NA or 2,4-D were found to be more effective in inducing elongation of the inflorescence than their sodium salts, and 2,4-D was more

effective than NA. The color of the leaves was also influenced by the treatment. With either NA or 2,4-D, the sodium salt was more effective in lessening the chlorophyll content in the leaves than the methyl ester forms. The leaves of the untreated cauliflower did not lose color up to the time of leaf fall. The presence of chlorophyll seemed to have little relation to abscission formation.

In both cabbage and cauliflower, after treatment with methyl ester of NA, tumor tissue was formed on the cut surface of the stem after a few weeks storage (Figure 2). These tumor tissues were usually found on the exposed surface near the cambium and pericycle regions, and occasionally on the pith.

Anatomically, the cells of this tumor tissue were particularly larger and the cell walls were weaker than that in normal tissue. This tissue originated from the reversion of the subepidermis of the exposed surface to a meristematic state, from which the tumor tissue was differentiated. However, it has also been observed that the reversion of a secondary tissue into a meristematic state could result from high humidity.

TABLE IV. Effect of Different Formulations of Naphthaleneacetic Acid and 2,4-Dichlorophenoxyacetic Acid on the Color, Abscission, and Elongation of Inflorescence of Cauliflower.

Treatment (amount per head)	Color of Outer Leaves	Days until 50% Leaf Drop	Days for the Initiation of Elongation after Treatment
ME*of NA 100 mg. on paper	Green	> 100	56
SS**of NA 5 mg. on leaf	Greenish yellow	55	No elongation
ME of 2,4-D 50 mg. on paper	Green	> 100	48
SS of 2,4-D 5 mg. on leaf	Yellow	> 100	95
SS of 2,4-D in Geon, 5 mg. on leaf	Yellow	> 100	95
Check	Green	40	No elongation

*ME, methyl ester

**SS, Sodium Salt

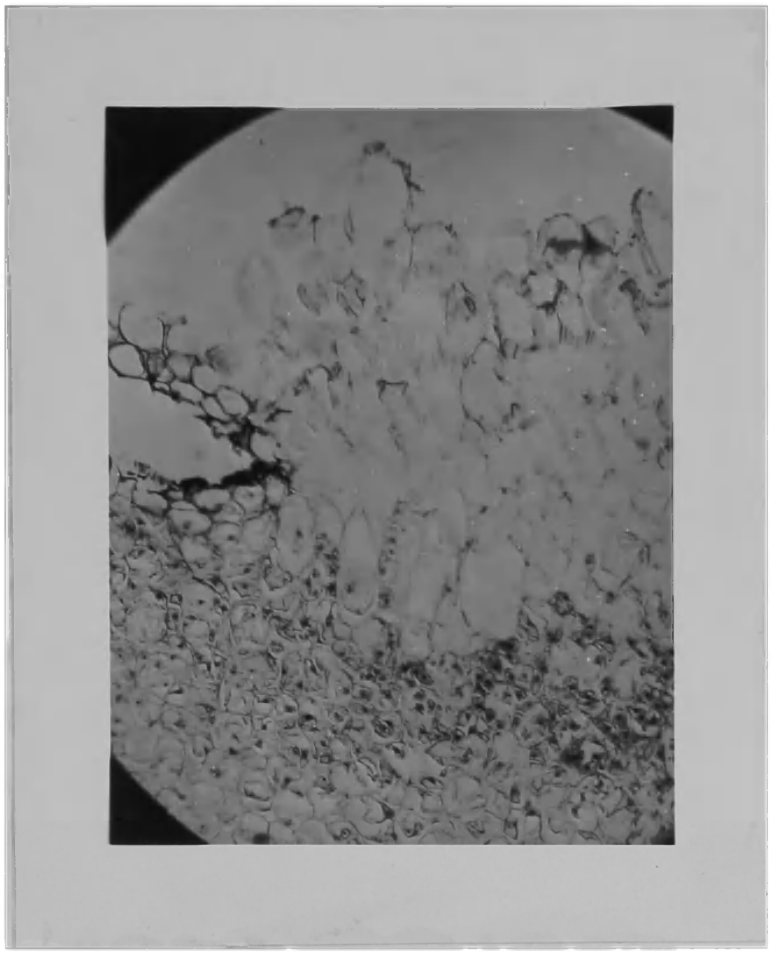

Figure 2. Tumor tissue on the cut surface of the stem of cabbage, three weeks after treatment with methyl ester of naphthalene-acetic acid; x ca. 100.

Effect of Different Methods of Application

This experiment was conducted to study the direction of transport of growth substances in cauliflower tissue. The sodium salts of either 2,4-D or NA at 500 p.p.m. in Geon mixture were used to retard the abscission formation.

Five different treatments were set up as follows:

1) NA was applied on the cut ends of all the midribs,
2) NA on the cut end of the stem,
3) 2,4-D on the cut ends of all the midribs,
4) 2,4-D on the cut end of the stem,
5) 2,4-D applied only on the cut ends of the midribs on one side of a cauliflower head.

Four heads were selected for each treatment, and the leaf fall was recorded at ten day intervals.

Results and Discussion

The Sodium salt of NA mixed with Geon, when applied either on the cut ends of the midribs or the stems, was found to have little effect on the retardation of abscission formation. However, the application of 2,4-D at the same concentration and under the same conditions was very effective. It is interesting that the retarding effect on the foliar abscission of cauliflower was obtained only when 2,4-D in Geon was applied on the cut ends of the midribs.

When it was applied on the cut ends of the stems, only the outer most whorl of the leaves was affected.

It appears that the transport of 2,4-D in stored cauliflower tissue is polar, that is, its predominant movement is downward. This conclusion differs from the observations of Hitchcock and Zimmerman (1935) and Ferri (1945) who reported that growth substances move both upward and downward in the transpiration stream, and is probably due to the difference in the physiological activity of intact and excised plants.

Not only did the 2,4-D move downward vertically, but it also moved horizontally in the stem. When the 2,4-D mixed with Geon was applied on the cut ends of the trimmed leaves on one side of a cauliflower, the abscission of the leaves on the other side were retarded (Figure 3).

TABLE V. The Effect of Point of Application of Growth Regulating Substances on Leaf Fall of Stored Cauliflower (Average of five heads).

Days after Treatment	Per Cent Leaf Fall				
	Check	NA on Cut End of Midrib	NA on Cut End of Stem	2,4-D on Cut End of Midrib	2,4-D on Cut End of Stem
23	7.2	10.8	9.1	0	0
30	29.7	35.1	46.6	0	0
40	73.8	59.4	73.9	0	0
48	92.8	79.5	83.6	0	6.9
56	100.0	89.2	90.9	0	20.4
69	--	100.0	100.0	0	53.0
82	--	--	--	0	64.1
99	--	--	--	0	79.5

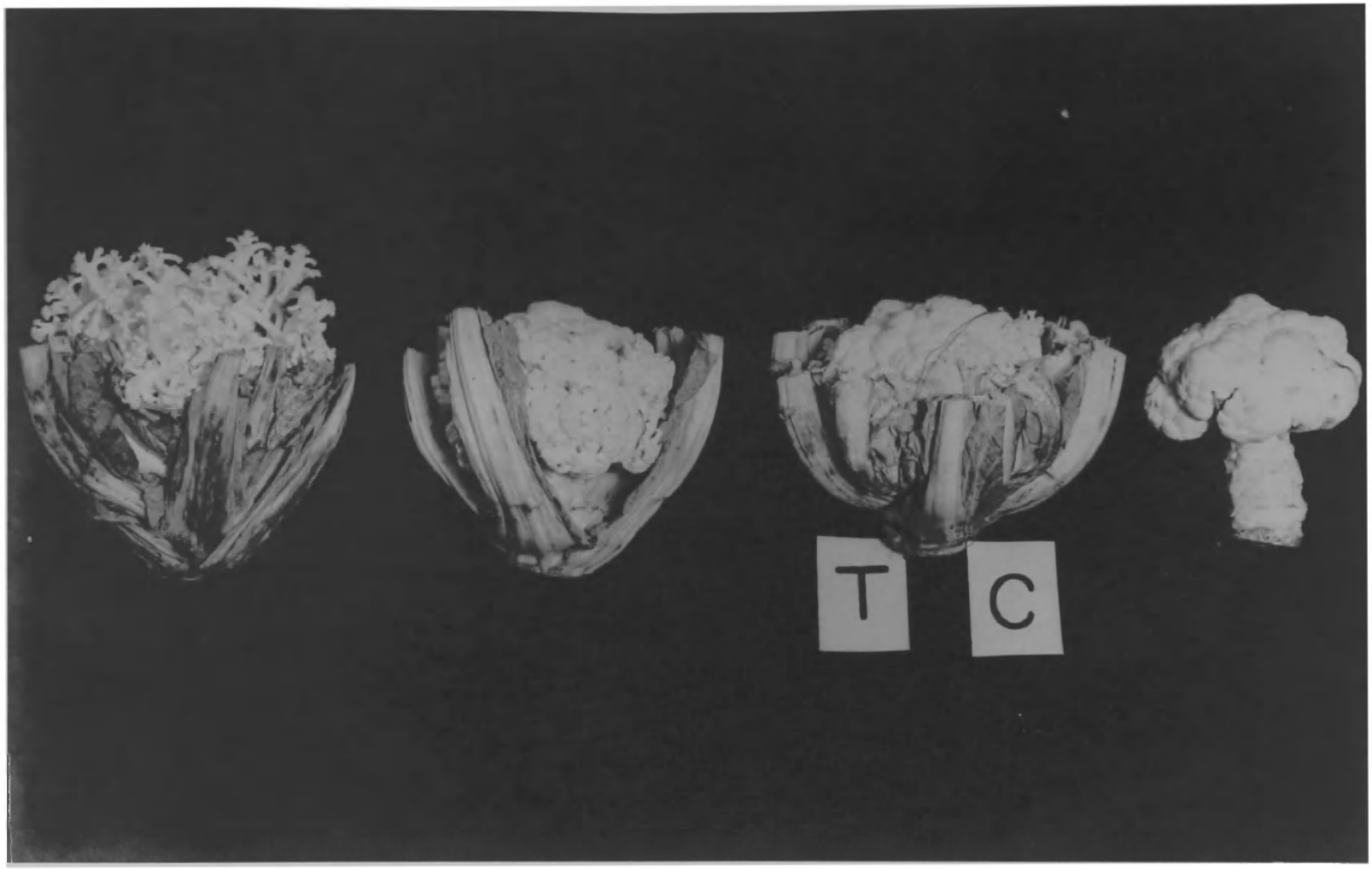

Figure 3. Effect of method of application and formulation of 2,4-dichlorophenoxy--acetic acid on foliar abscission of cauliflower, 55 days after treatment. Left to right, 1) methyl ester of 2,4-D, 100 mg. per head; 2) sodium salt of 2,4-D in Geon solution, on cut end of stem only; 3) sodium salt of 2,4-D in Geon solution, on cut ends of midribs on only one side of a head(T); 4) check.

Effect of Temperature

Five heads of freshly harvested cabbage, Resistant Detroit, were treated with the methyl ester of NA (100 mg. per head), and five left untreated as check. The cabbage was then held at approximately 32° F. (cold storage), and 70° F. (room temperature). The number of dropped leaves was recorded every 48 hours until all the leaves had fallen.

Results and Discussion

The methyl ester of NA retarded the leaf drop at both room temperature (70° F.) and cold temperature (32° F.) (Table 6). Since the higher temperature would increase the rate of transpiration, respiration, enzymic activity and probably many other metabolic functions, the higher temperature would be expected to accelerate the abscission formation.

The data in Table 5, at room temperatures, indicates that the abscission of the untreated cabbage occurred within one week, while treatment with NA delayed the abscission from three to four weeks. Under low temperatures, the treated cabbage could be stored for two to three months without any leaf fall. Similar results were also obtained with cauliflower, however, the time required for abscission was much shorter than that in cabbage at both room and cold temperatures.

TABLE VI. Time Required for the Initiation of Abscission in Cabbage Stored at Various Temperatures (Average of five heads).

Temperature	Treatment (Sept.27,1947)	Days required for initiation of abscission	Difference in days between treated and check
32°F	NA, 100 mg. per head	63	42
	Check	21	
70°F	NA, 100 mg. per head	25	19
	Check	6	

Effect of Trimming

In order to study the effect of the leaf blade on the formation of foliar abscission, cauliflower (Snowball X) was used. There were four treatments:

1) leaves trimmed blade attached,
2) leaves untrimmed blade attached.
3) leaves trimmed blade removed,
4) leaves untrimmed blade removed.

Each treatment comprised ten uniform heads treated with methyl ester of NA (100 mgs. per head), and ten controls. The number of dropped leaves was recorded weekly.

Results and Discussion

Regardless of trimming treatment, the leaves of the untreated lots began to drop after two weeks storage. Treatment with NA delayed abscission in all tests. Leaf blades were found to have little effect on the leaf fall in both trimmed and untrimmed cauliflower.

One hundred days after harvest, only ten per cent of the leaves in treated lots had dropped. In the untreated lots, all the leaves had completely fallen after 40 day storage. Although LaRue (1916), Hitchcock and Zimmerman (1935), and Myers (1940) considered that the growth hormone were synthesized in the leaf blade, in this study with

stored cauliflower the blade had little inhibiting effect on abscission.

Since the loss of weight is mainly due to the loss of water through the transpiration process, the larger the exposed surface area, the more water will be transpired. Consequently, the cauliflower with leaf blade attached lost weight faster than heads in which the blade had been removed. Generally, the fresh weight loss was found to be higher in the treated, and somewhat lower in the untreated lots.

TABLE VII. The Effect of Leaf Blade Tissue on Abscission Formation of Cauliflower (Expressed as the per cent of leaves that dropped per head).

Treatment	Days after harvest	Leaf trimmed		Leaf untrimmed	
		Blade attached	Blade removed	Blade attached	Blade removed
Check	18	4.4	1.0	8.9	10.0
	25	38.2	25.7	41.0	50.0
	33	67.2	56.7	73.5	72.6
	39	89.4	81.5	89.0	84.9
	46	96.2	95.2	96.8	92.4
	55	100.0	100.0	100.0	100.0
Treated NA 100 mg. per head	89	--	7.4	7.7	6.1
	102	--	18.5	16.5	10.7
	116*	--	27.7	38.4	20.4

* The cauliflower was not markable after 116 days storage.

TABLE VIII. The Influence of Methods of Trimming on Weight Loss of Cauliflower (Average of five heads).

Condition	Treatment	Original Weight per Head (gm.)	Weight per Head 31 Days after Harvest (gm.)	Per Cent Loss of Weight
Untrimmed Blade attached	NA 100 mg. per head	1676	1216	27.5
	Check	1745	1151	34.0
Untrimmed Blade removed	NA 100 mg. per head	1277	1101	13.8
	Check	1197	1034	13.6
Trimmed Blade Attached	NA 100 mg. per head	1756	1649	6.1
	Check	1732	1636	5.5
Trimmed Blade removed	NA 100 mg. per head	1413	1352	4.32
	Check	1478	1415	4.2

Figure 4. Effect of the methyl ester of naphthaleneacetic acid on retarding foliar abscission of cauliflower with leaf blade removed, 45 days after treatment. 5) treated, and 6) check.

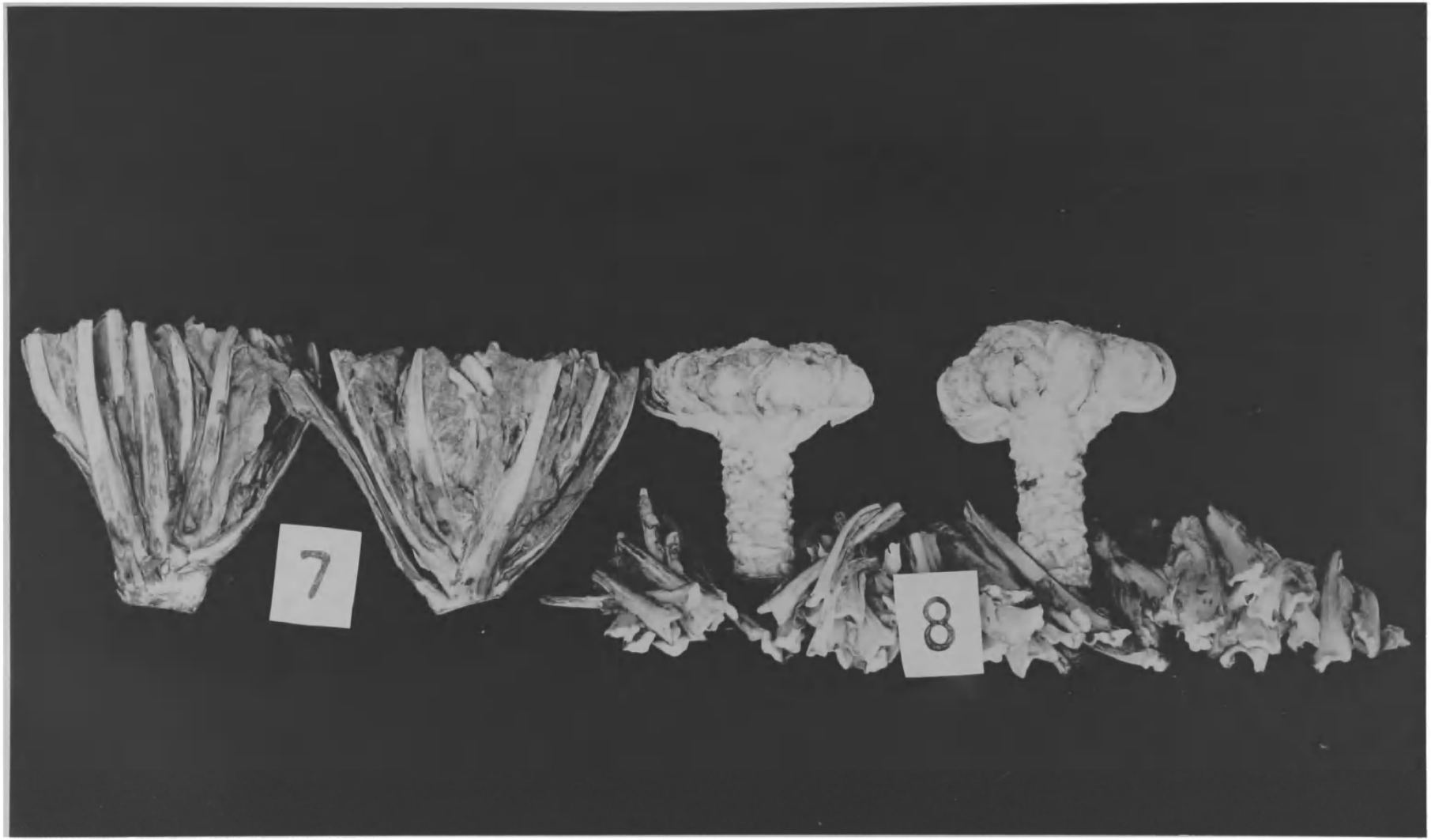

Figure 5. Effect of the methyl ester of naphthaleneacetic acid on retarding foliar abscission of cauliflower with leaf blade attached, 45 days after treatment. 7) treated, and 8) check.

The Effect of Field Treatment with Growth Regulating Substances on Foliar Abscission

Material and Methods

In this experiment, cabbage, Wisconsin Hollander No. 8, and cauliflower, Snowball A, grown under ordinary cultural practices were used.

Treatment of cabbage--The sodium salt of a-naphthaleneacetic acid and 2,4-dichlorophenoxyacetic acid were used at three different concentrations: 100, 250 and 500 p.p.m. Each plant was sprayed with approximately 10 ml. of the solution with a hand sprayer. In order to determine the most effective interval between application and harvest with respect to the retardation of abscission formation, two dates of harvest, one day and seven days after treatment, were chosen. There were twelve treatments and two checks of five plants each arranged in randomized blocks, with four replications. After harvest, the cabbage was stored at 34° F. and one hundred days after harvest all the heads were examined and the number and percentages of leaf fall were recorded.

Treatment of cauliflower--As in the case of cabbage, the sodium salt of NA and 2,4-D in aqueous solution were applied in three different concentrations: 100, 250 and 500 p.p.m. In addition, the combination

of sodium salt of NA and 2,4-D (both in 250 p.p.m. and with 1:1 ratio) was used. An average of 15 ml. of each solution was sprayed on each of ten plants.

Results and Discussion

Cabbage--Treatment with the sodium salt of 2,4-D before harvest was found to have an effect in retarding the leaf fall of the stored product. The 2,4-D treatments within the limit of concentrations used were more effective than the NA treatments. One hundred days after harvest, about one half of the leaves had fallen from the heads that were left untreated or treated with NA. On the other hand, those treated with 2,4-D on an average, had lost approximately only one leaf per head.

From the data in Table 10, it appears that there were great differences in the retarding effect of the sodium salt of NA and 2,4-D on abscission formation. However, the methyl ester of both NA and 2,4-D, as found in previous experiments, had almost comparable effects.

Cauliflower--The effects of growth regulating substances on foliar abscission of cauliflower were more pronounced than in cabbage. After 80 days in storage, 100 per cent of the leaves of the untreated lots had fallen. The sodium salt of 2,4-D was found to have a much greater retarding effect on foliar abscission than the sodium salt of NA.

There was little difference between the untreated and those treated with NA in all concentrations used; fifty per cent of the leaves had fallen after fifty days storage. No difference was found between the effectiveness of the three concentrations used. From the standpoint of marketability, ordinary cauliflower could be stored for about two weeks, while those sprayed with sodium salt of 2,4-D might be stored up to 100 days without any leaf drop. The treatment with the combination of 2,4-D and NA had the same effect as using 2,4-D alone.

When sprayed with the sodium salt of 2,4-D at 100 p.p.m. one to seven days before harvest, cauliflower as well as cabbage can be stored without leaf fall for at least three times as long as untreated lots. Another advantage of field spraying which does not obtain with post harvest treatment is the uniform effect on the retardation of abscission in all the leaves. This uniform effect is probably due to the transport of the growth regulating substances within the tissues of the growing plants.

Leaves of growing cauliflower that had been sprayed on the upper surfaces with NA or 2,4-D were found to bend downward; that is, the chemicals induced a negative curvature of the leaves. The degree of curvature was found to be more pronounced in those treated with 2,4-D than in those treated with NA, and also generally proportional

to the concentration of the growth substances used. At the same time, if the growth substances were sprayed on the lower side of the leaves, the leaves turned upward due to the same type of effect.

From an anatomical point of view, this effect was caused by an increase in the size of the epidermal and subepidermal cells of the midribs and not to an increase in cell number (Table IX). The ratio between the diameters of the upper and lower epidermal cells was found to be higher in the treated midribs than in the untreated ones. In this experiment, the young undeveloped leaves were more sensitive to treatment than older leaves. It was probably due to the fact that their cells had not obtained the maximum size at the time of treatment.

TABLE IX. Cell Size in Midrib Tissue of Cauliflower, Taken One Week after Field Treatment with 2,4-Dichlorophenoxyacetic Acid at 250 p.p.m. (Average of five midribs).

Treatment	Average Length of Epidermal Cells			Average Diameter of Subepidermal Cells*		
	Upper (μ)	Lower (μ)	Upper / Lower	Upper (μ)	Lower (μ)	Upper / Lower
Treated	23.1	21.1	1.09	34.7	39.5	0.88
Check	22.7	27.3	0.83	36.5	42.8	0.85

* The term, subepidermal cells, used here to indicate the five tiers of cells next to the epidermis; most of them are collenchyma cells.

Figure 6. Cauliflower (Snowball A) one week after treatment; left, 250 p.p.m. NA; right, 250 p.p.m. 2,4-D; the leaves were sprayed on the under surface and bend upward after treatment.

Figure 7. Cauliflower (Snowball A) one week after treatment; the 2,4-D, 250 p.p.m. was sprayed on the upper surface of the leaves which bend downward after treatment.

TABLE X. Number of Fallen Leaves Per Head in Cabbage Following Field Spray Application with 2,4-Dichlorophenoxyacetic Acid and Naphthaleneacetic Acid, After 106 Days Storage. (Each replication on average of five heads).

Treatment	Replication				Average *
	I	II	III	IV	
Harvested One Day after Treatment					
NA,100 p.p.m.	18.0	20.1	18.2	21.2	19.3
NA,250 p.p.m.	20.6	20.2	21.6	16.4	19.7
NA,500 p.p.m.	25.6	21.4	19.4	21.2	21.9
2,4-D,100 p.p.m.	0	3.6	2.0	0	1.4
2,4-D,250 p.p.m.	0	2.8	0	0	0.7
2,4-D,500 p.p.m.	1.8	2.6	3.0	1.4	2.2
Check	20.5	21.4	21.1	23.6	21.7
Harvest Seven Days after Treatment					
NA,100 p.p.m.	10.2	13.4	15.6	11.8	12.8
NA,250 p.p.m.	10.8	10.0	11.7	16.0	12.1
NA,500 p.p.m.	12.0	11.8	13.2	14.5	12.9
2,4-D,100 p.p.m.	0	0	0	0	0
2,4-D,250 p.p.m.	0	1.2	0	0	0.3
2,4-D,500 p.p.m.	3.0	4.5	0	0	1.9
Check	19.6	25.9	20.2	21.7	21.8

* The total number of leaves per head is 47.6 ± 4.4, excluding the leaves which are shorter than three inches.

TABLE XI. The Effect of Field Treatment with Naphthaleneacetic Acid and 2,4-Dichlorophenoxyacetic Acid on the Per Cent of Leaf Fall of Cauliflower in Storage. (Average of five heads for each treatment).

Days in storage	NA, 500 p.p.m.		NA, 250 p.p.m.		NA, 100 p.p.m.		2,4-D, at either 500, 250 or 100 p.p.m.		Check	
	A†	B*	A	B	A	B	A	B	A	B
24	10.0	1.8	6.4	4.3	5.5	4.5	0	0	5.7	22.7
33	15.8	17.1	18.0	15.5	14.4	16.8	0	0	20.6	57.5
39	26.6	25.4	29.0	24.1	24.5	26.9	0	0	36.3	70.5
51	38.0	45.6	46.3	46.2	43.3	45.5	0	0	54.9	82.8
61	76.4	64.9	78.8	66.0	73.9	71.6	0	0	79.4	91.6
80	94.2	82.5	96.2	82.5	93.3	86.6	0	0	100.0	100.0
93	100.0	100.0	100.0	100.0	100.0	100.0	0	0	—	—

The plants were sprayed on Oct. 14, 1947. The cauliflower heads treated with 2,4-D turned yellow 93 days after harvest, and had no market value, however, the leaves were still attached.

† A, harvested on Oct. 21; 7 days after treatment.
* B, harvested on Oct. 16; 2 days after treatment.

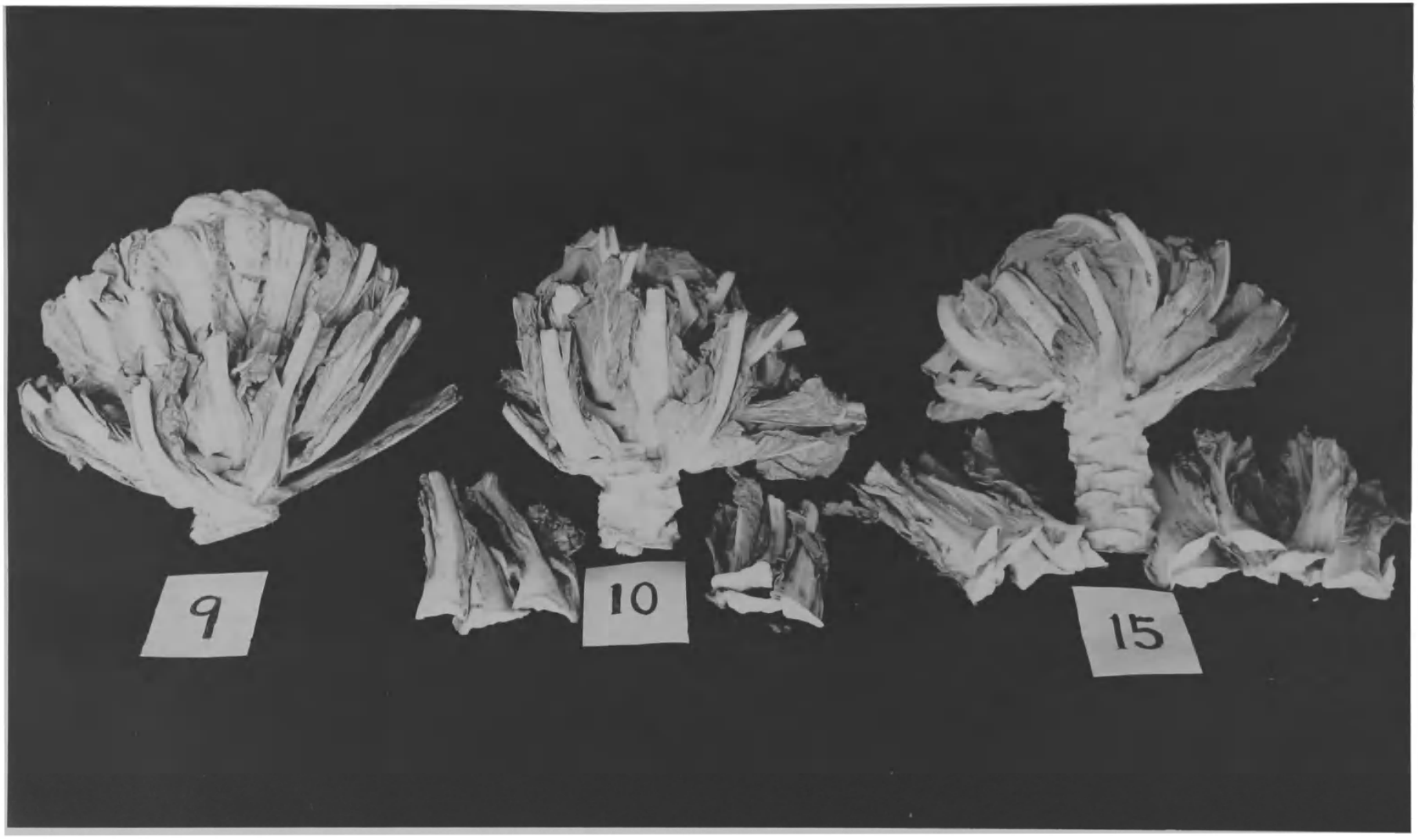

Figure 8. Retarding effect of field treatment on foliar abscission of cauliflower, 45 days after treatment. 9) sprayed with sodium salt of 2,4-D, 100 p.p.m.; 10) sodium salt of NA, 100 p.p.m.; 15) check.

Anatomical Changes in Relation to Foliar Abscission

Material and Methods

The plant material for sectioning was taken from cabbage and cauliflower which had been treated with the methyl ester of NA,100 mg. per head, and from untreated lots. Samples were obtained from the second outer whorl of leaves of both cabbage and cauliflower. The tissues were first fixed in FAA (Formalin, acetic acid and alcohol) killing solution. Dehydration was carried out in an alcohol series, and finally the tissue was embedded in paraffin. Sections were made with a microtome at 20 u thickness and stained with safraine O (aqueous solution) and light green (alcoholic solution).

Since the abscission formation varies greatly with the position of the stem on which the leaves are borne, the abscission structure of every fifth leaf taken from outer (older) to inner (younger) whorls was also studied, in order to find the relationship between the leaf order and the development of the abscission. Tissues for this purpose were prepared by free hand section. The length of the separation layer of the abscission zone was measured by a micrometer.

Anatomy of the Abscission Zone

The abscission of both cabbage and cauliflower leaves resulted from two processes, growth of a

meristematic layer, followed by dissolution of certain lamellae in the separation layer. However, in the upper half of the petiole, the meristematic layer was normally found preceding separation, whereas in the lower half of the petiole, especially in cauliflower, this was not the case.

The meristematic layer was differentiated at a very early stage after harvest. Two weeks after storage five to ten layers of meristematic cells could be seen in a meristematic layer at the base of the petiole (Figures 10 and 12). These cells were characterized by small size, well defined protoplasts and embryonic nature. In cabbage these cells were oblong in shape, arranged in parallel, and normally they were well developed, especially near the upper epidermis; in the petiole of cauliflower, on the other hand, the cells were isodiametric in shape and were not so well developed.

In both cabbage and cauliflower, the differentiation of the meristematic layer generally was initiated near the upper epidermis of the petiole and became less well developed toward the lower epidermis. This was followed by the formation of the separation layer, which also began from the upper epidermis and progressed toward the lower epidermis. However, the separation layer in the lower side started to form after the separation layer

in the upper side had developed about half way across the petiole, even though the meristematic layer was lacking in the lower side (Figure 9).

From an anatomical point of view, the difference in foliar abscission between cabbage and cauliflower was found to be the position of the separation layer in relation to the meristematic layer. In cabbage, the separation layer always occurred five to ten tiers of parenchymatous cells apart from the outer side of the meristematic layer. In other words, it was formed from the secondary meristem (Figures 9 and 11). On the other hand, the separation layer in the petiole of cauliflower always occurred within the meristematic layer itself; that is, it developed from one or two tiers of the meristematic layer or it was formed from the primary meristem (Figures 9, 13 and 14).

The separation layer of cabbage and cauliflower was not formed smoothly across the petiole. The direction of this layer was sometimes modified by the vascular bundles or other mechanical elements and left a sunken leaf scar after leaf fall. After the formation of the separation layer was complete, the whole leaf was supported only by the vascular bundles which were then ruptured mechanically (Figures 14 and 15). So the separation of this tissue was not the result of the dissolution of the middle lamellae or a physiological disintegration but was due to a mechanical force.

After the falling of the leaf, a protective layer was differentiated on the exposed surface of the leaf scar. In the case of cabbage, the cells of the meristematic layer could still be seen near the exposed surface after leaf fall (Figure 16).

Delay of abscission formation by growth regulating substances may be due to retardation of either the differentiation of the meristematic layer or the weakening of cell walls in the separation layer. The meristematic layer was apparently more clearly defined and well developed in leaf petioles from treated heads than in the petioles from untreated heads.

Leaf Order in Relation to Abscission Formation

Under natural conditions, in both cabbage and cauliflower, the outer (older) leaves drop first and, subsequently the inner (younger) leaves fall. In many instances, the abscission of the outer leaves had been completely formed and the leaves had dropped, while the inner leaves were still attached. In mature cauliflower with few leaves (usually less than 30) and no very young leaves, the abscission was found even in the innermost leaves after one and a half months of storage. However, in cabbage, which contained many whorls of younger leaves, usually abscission of the outer twenty leaves occurred after three months of storage, and the inner leaves remained intact for a considerably longer length of time.

The above relation between leaf order and abscission was drastically altered by post harvest treatment with growth regulating substances. With treatment, the leaves of the intermediate whorls in the case of cabbage, and of the innermost whorls in the case of cauliflower, dropped first and the outer whorls last. This inverted relationship possibly is both of theoretical interest and of practical importance. This phenomenon may be caused by the lack of an appreciable upward transport of the growth regulating substances in the plant tissues. Treatment with 2,4-D only on the cut surface of the stem, which did not delay the abscission formation of the inner younger leaves, indicated that this is probably the case.

With treated cabbage, the abscission of the intermediate whorls of leaves were always formed first. Treatment with the growth regulating substances could only delay the abscission in outer leaves which came in contact with the chemicals, but apparently did not affect the inner younger leaves. From the data in Table XII, the "percentage of separation"* of the outer five leaves of the untreated cabbage heads was much higher than that of the 25th leaf. On the other hand, in the treated heads, the

---

* The term, "percentage of separation", is used here to indicate the depth of the separation layer of the abscission zone of the petiole (in longisection) in relation to the thickness of the petiole; i.e.,

$$\text{Percentage of separation} = \frac{\text{Depth of the separation layer}}{\text{Total thickness of the petiole}} \times 100$$

"percentage of separation" of the 15th leaf was higher than both the first and the 25th leaves. The time required for abscission of the 30th leaf was approximately the same in both treated and untreated lots.

In treated cauliflower, the relationship between the abscission formation and leaf order was basically the same as that in cabbage. The outer five leaves of a treated cauliflower, 67 days after harvest, showed no separation on the abscission zone, while the leaves in the 20th position indicated 80 per cent separation.

It was also observed that with the pre-harvest field treatment with 2,4-D, there was no abscission to be noted during a storage period of more than 100 days. This was probably due to a more uniform and rapid transport of the applied chemicals into every leaf of growing plants in comparison with stored plants.

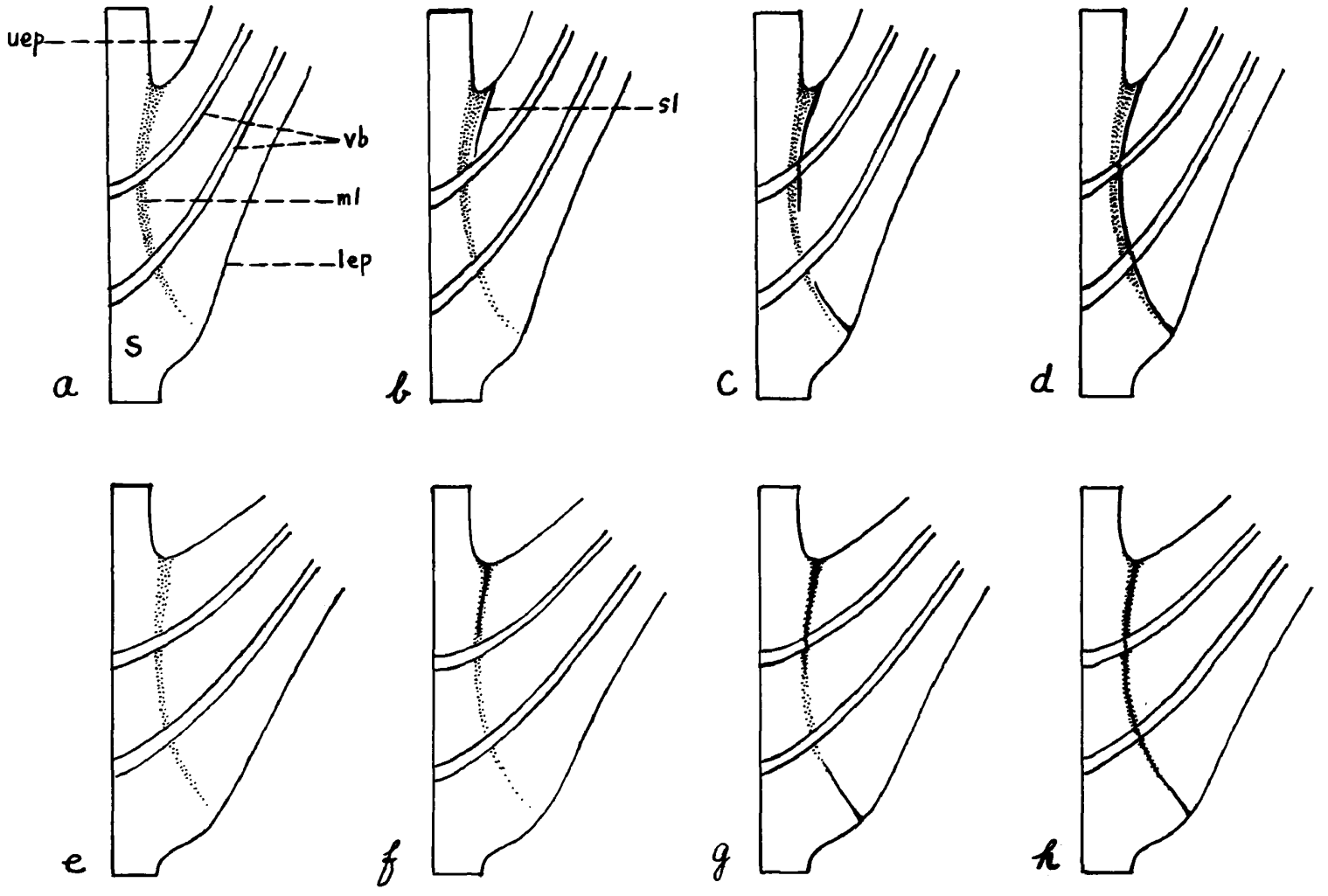

Figure 9. Diagrams of the formation of foliar abscission at various stages of development in cabbage , a, b, c, d; and in cauliflower, e, f, g, h. Key: lep, lower epidermis; ml, meristmatic layer; sl, separation layer; uep, upper epidermis; vb, vascular bundle; s, stem.

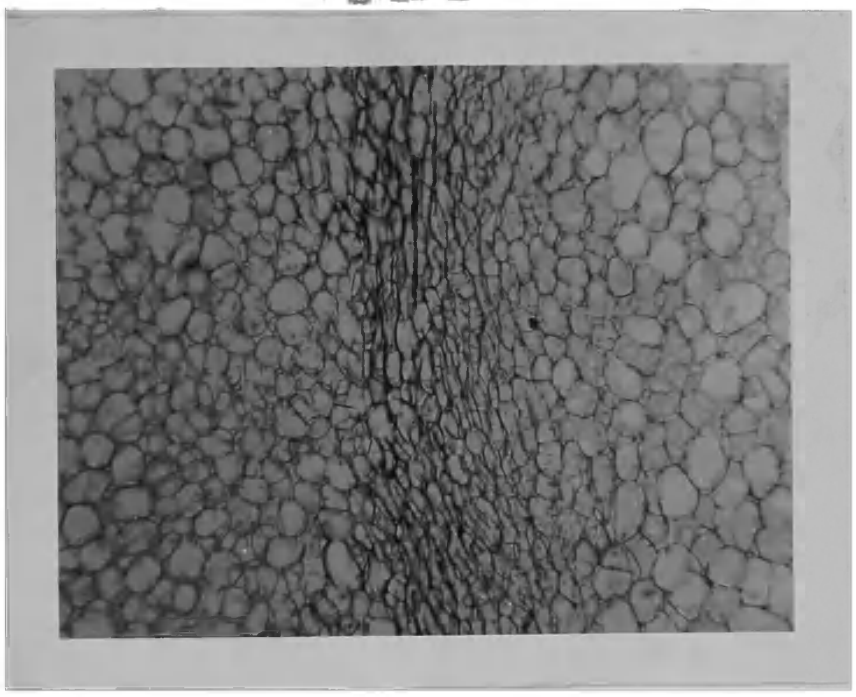

Figure 10. Foliar abscission of cabbage, showing the meristematic layer near the upper side of the midrib; x ca. 100.

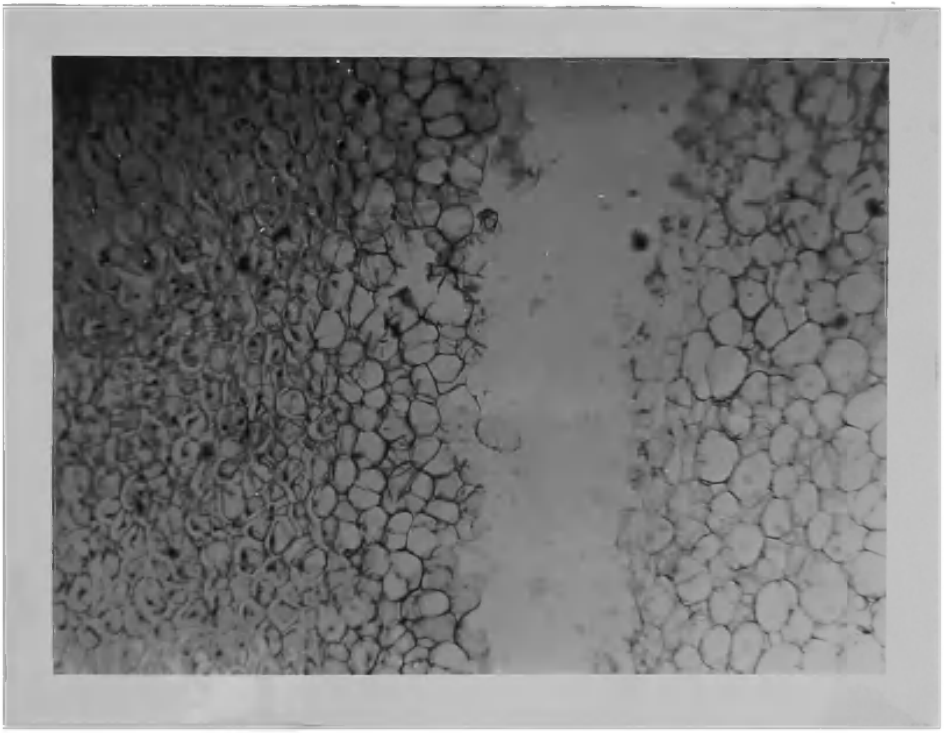

Figure 11. Foliar abscission of cabbage, showing the separation layer; x ca. 100.

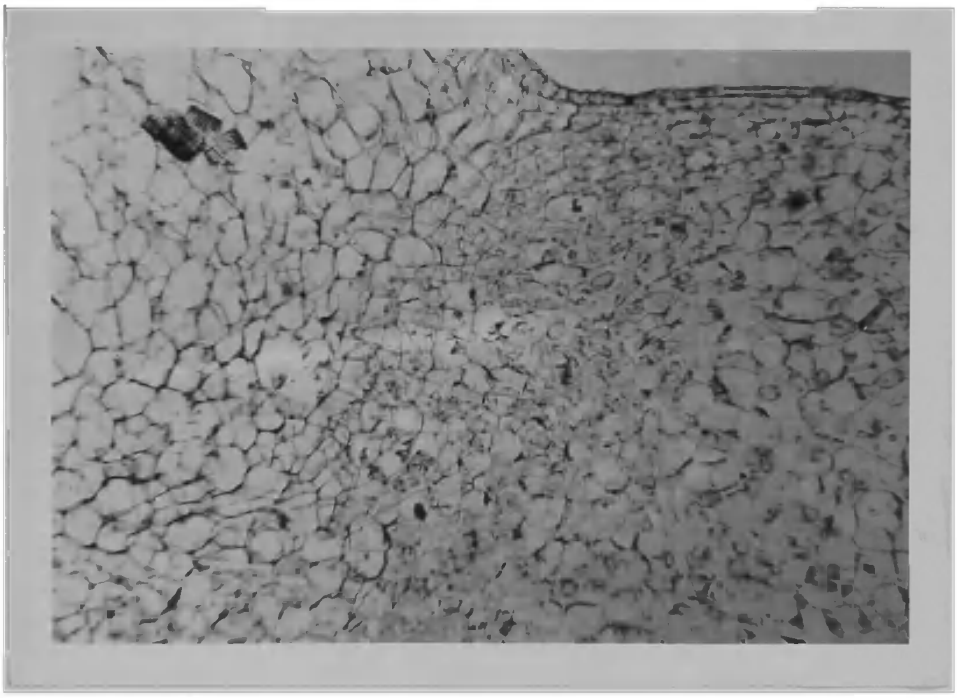

Figure 12. Foliar abscission of cauliflower, showing the meristematic layer near the upper side of the midrib; x ca. 100.

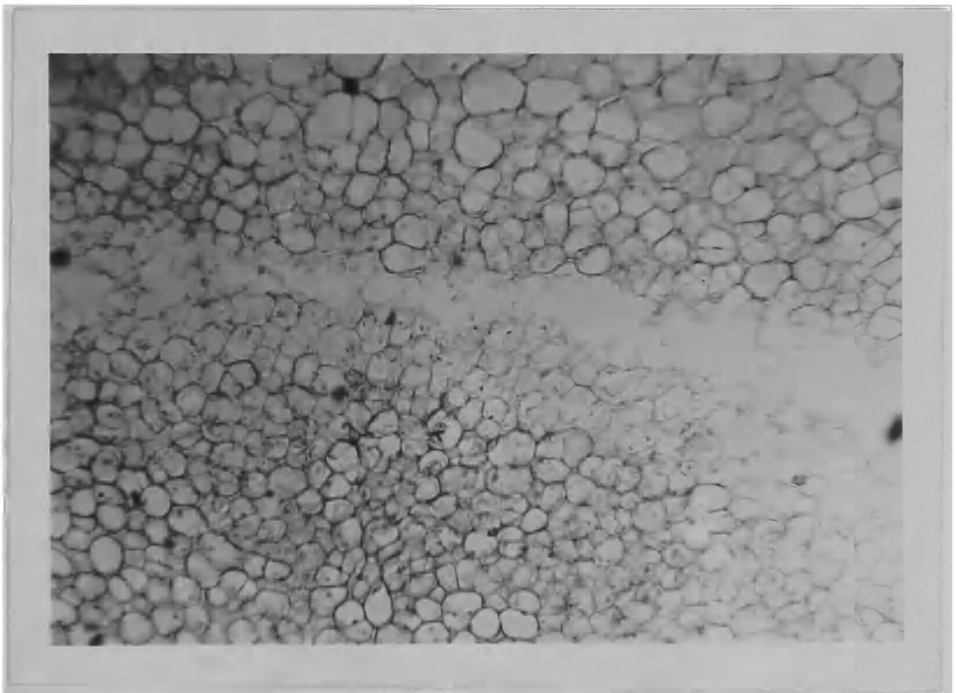

Figure 13. Foliar abscission of cauliflower, showing the separation layer; x ca. 100.

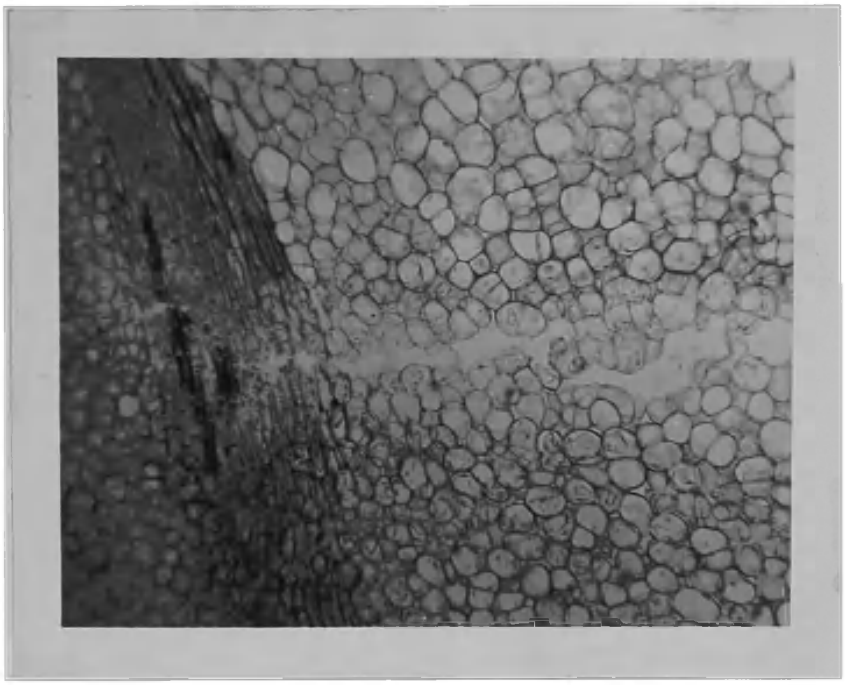

Figure 14. Foliar abscission of cauliflower, showing the separation layer near a vascular bundle; x ca. 100.

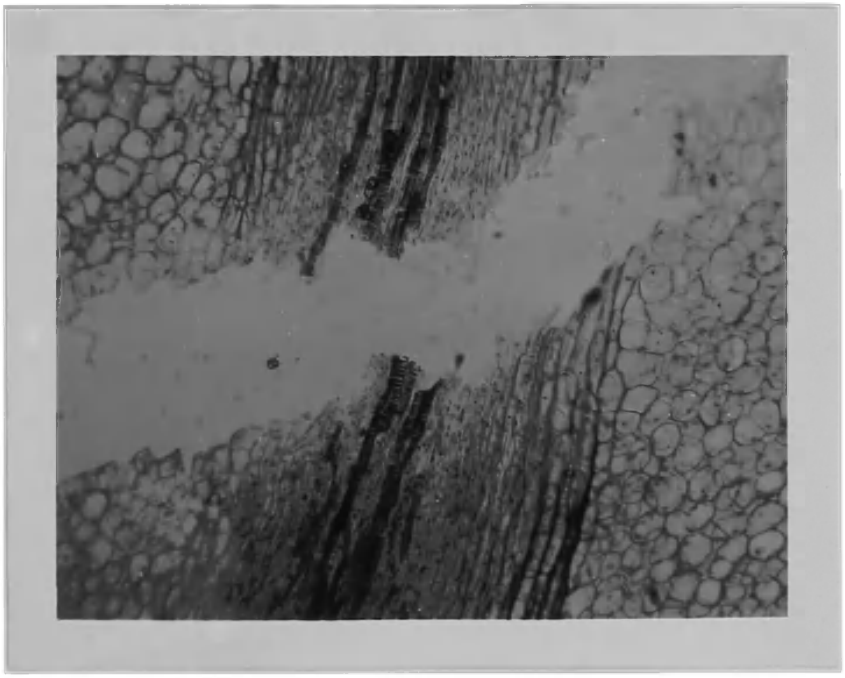

Figure 15. Foliar abscission of cauliflower, showing the breaking down of a vascular bundle; x ca. 100.

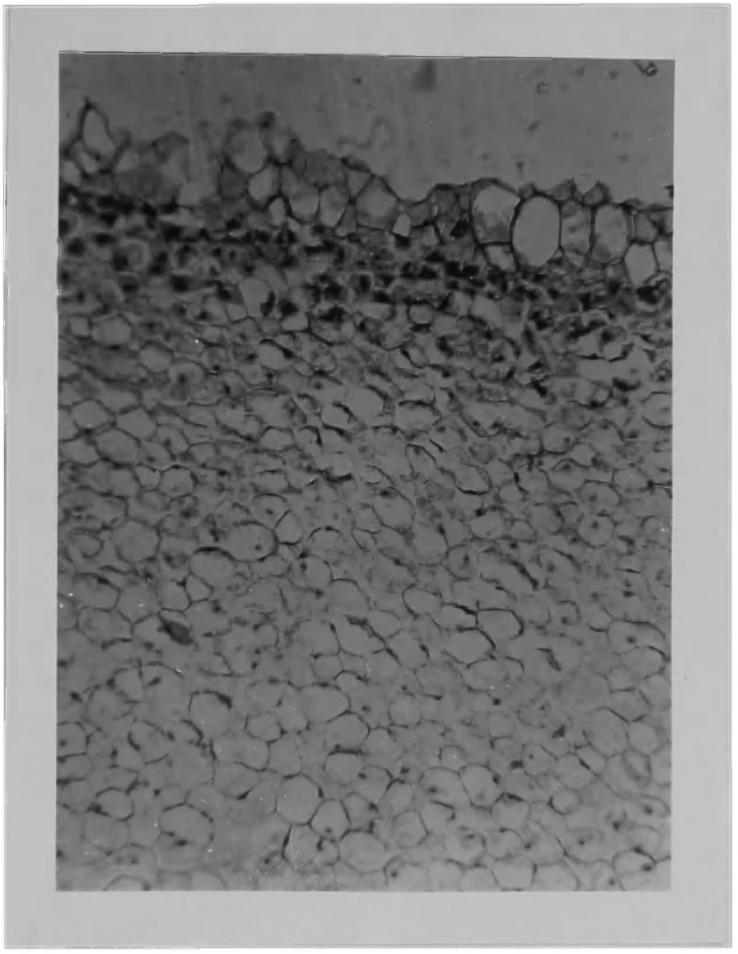

Figure 16. Foliar abscission of cabbage, showing the protective layer on the exposed surface of the leaf scar after abscission formation.

TABLE XII. Leaf Order in Relation to Percentage of Separation of Leaf Abscission in Cabbage, 80 days after Treatment (Average of five heads).

Leaf Order	Treated with ME of NA, 100 mg./head	Check
	(per cent)	(per cent)
1	16.9	100.0
5	45.9	100.0
10	46.7	83.2
15	65.9	66.4
20	22.1	18.7
25	10.5	5.2
30	0.0	0.0

TABLE XIII. Leaf Order in Relation to Percentage of Separation of Leaf Abscission in Cauliflower, 67 Days after Storage for Treated, and 32 Days after Storage for Check (Average of five heads).

Leaf Order	Treated with ME of NA, 100 mg./head	Check
	(per cent)	(per cent)
1	0.0	100.0
5	0.0	82.5
10	4.0	37.3
15	63.2	26.8
20	77.3	12.3
25	--	0.0

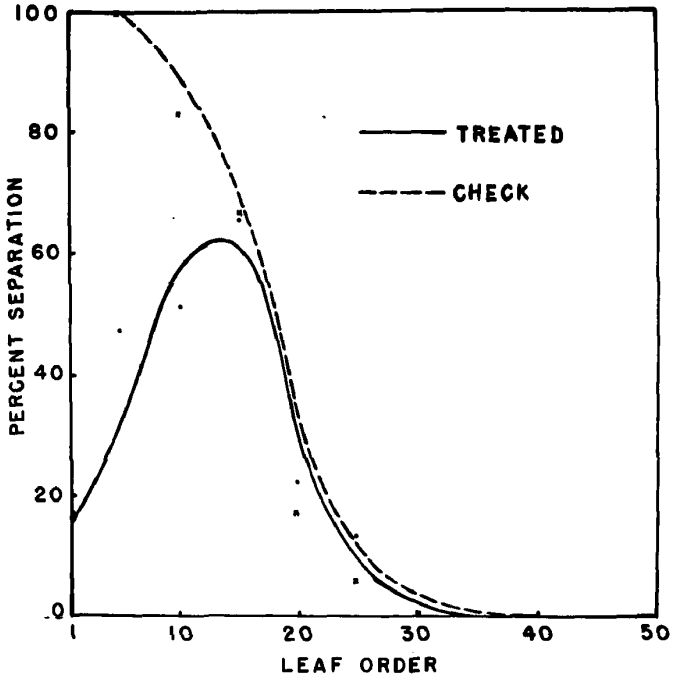

Figure 17. Leaf order in relation to percentage of separation of leaf abscission in cabbage, 80 days after treatment with methyl ester of naphthaleneacetic acid.

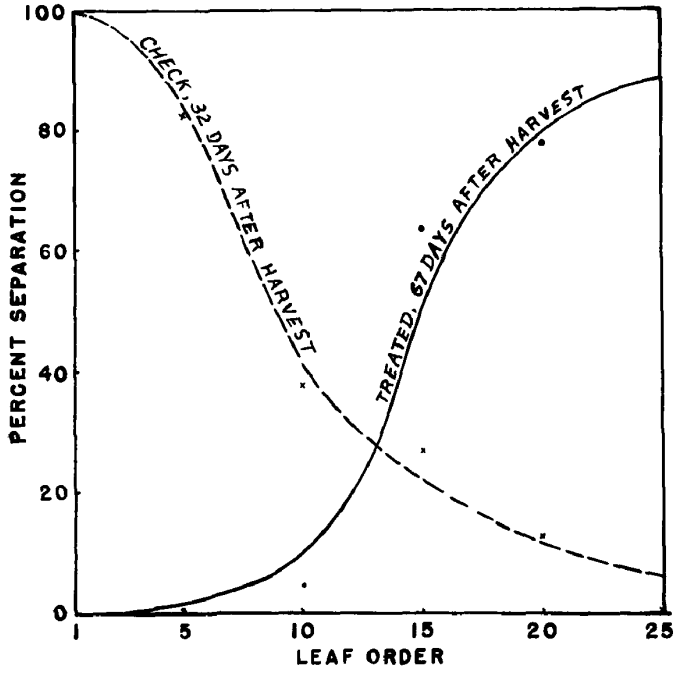

Figure 18. Leaf order in relation to percentage of separation of leaf abscission in cauliflower, treated with methyl ester of naphthaleneacetic acid.

Biochemical Changes in Relation to Foliar Abscission

Sugars

Material and Methods

Cauliflower plants, variety Snowball X, were harvested on Oct. 28, 1947, and placed in cold storage immediately. Forty heads of uniform size and good quality were selected. Twenty of them were treated on Oct. 29 with 100 mg. of the methyl ester of NA. The other twenty heads served as controls.

Analyses of total sugars, reducing sugar, and dry matter were made at weekly intervals from Nov. 3 to Dec. 22, 1947, at which time nearly all the leaves of the untreated lots had fallen. The method of sugar analysis followed was the one that is used in the Agricultural Chemistry Laboratory, Michigan Agriculture Experiment Station.* The procedure is as follows:

Each cauliflower sample was separated into three portions, the "head"**, the leaf blade, and the midrib. Twenty grams samples of each portion were weighed out for

---

* Mrs. Dorothy R. Waldron assisted in carrying out the analysis.

** The word "head" is used here, for the convenience of description, to indicate the edible part of the cauliflower from which the inflorescence develops; and the compact edible radical leaves as a whole of cabbage. It is not a morphological term, however.

each determination and then transferred to a blender receptacle to which 0.5 gram of $CaCO_3$ and 100 ml. of 95 per cent ethyl alcohol were added. The blender was operated for three minutes. The blended material was then filtered and made up to 200 ml. with 80 per cent alcohol, in a volumetric flask. Fifty ml. aliquots were transferred to beakers and condensed to a small volume on a steam bath. Lead acetate solution was added to the evaporated material, which after precipitation was filtered and the excess lead was removed with potassium oxalate. The filtrate was made up to 50 ml.; a 25 ml. aliquot was measured for the estimation of reducing sugar, the other 25 ml. were hydrolysed with HCl for the determination of total sugars. Reducing and total sugars were determined by the precipitation of cuprous oxide according to the official A.O.A.C. method (1940).

Results and Discussion

The amount of both reducing and total sugars content varied not only with the length of the storage period, but also with the different tissues (Table 15 and Figure 20).

In the "head" tissue, total sugars were much higher than reducing sugar in both the treated and untreated cauliflower. The "heads" which had been treated with the methyl ester of NA contained less sugars than the untreated ones. The formation of foliar abscission had little influence on

the change of sugars, especially the total sugars. Fifty five days after harvest, the reducing sugar content of the untreated heads was over twice as high as that found in the treated heads and total sugars were about 20 per cent higher.

The decrease in the sugar content in leaf blade tissue was most rapid in the early stage of storage just after harvest and before abscission formation. The difference between total and reducing sugars in the leaf blade was much less than that in the "head" tissue, indicating the presence of very little invert sugar. After three weeks storage, 50 per cent of the leaves had dropped in the untreated lots, whereas no leaf fall occurred in the treated lots. The leaf blade of both treated and untreated cauliflower at that time contained less than 0.2 per cent of either total or reducing sugars.

The sugar content in the midrib was more closely related to abscission formation. The difference between total and reducing sugars was quite uniform throughout the stored period. Treatment with NA retarded the loss of both total and reducing sugars, especially after 50 per cent of the leaves of the untreated lots had fallen. When all the leaves of both treated and untreated heads had fallen, there was only a slight difference in sugar content between the treated and the untreated lots.

During storage, sugars decreased more rapidly in the midrib and blade tissues than in the "head" tissue. Treatment with the methyl ester of NA retarded the loss of sugars in the midrib for a time, and finally had little effect.

TABLE XIV. Leaf Drop in Cauliflower used for Sugars and Dry Matter Analyses (Average of five heads).

Date	Days after Harvest	Per Cent Leaf Drop	
		Untreated	Treated (NA, 100 mg./head)
Nov. 18	21	10.0	0
Nov. 24	27	22.6	0
Dec. 1	34	32.1	0
Dec. 8	41	71.8	0
Dec. 15	48	92.6	0
Dec. 22	55	100.0	8.5
Jan. 4	68	--	31.4
Jan. 17	81	--	77.3
Jan. 24	88	--	96.9
Jan. 31	95	--	100.0

TABLE XV. The Effect of Methyl Ester of Naphthaleneacetic Acid on the Sugar Content of Stored Cauliflower. (Expressed on fresh weight basis, average of duplicate determinations).

Date Sampled	"Head"		Midrib		Leaf Blade	
	Treated	Check	Treated	Check	Treated	Check
			Reducing Sugar (%)			
Nov. 3	1.13	1.53	2.74	2.94	1.14	1.39
Nov. 12	1.06	1.34	2.10	1.76	0.43	0.52
Nov. 19	1.03	1.18	1.31	1.12	0.01	0.01
Nov. 26	0.91	1.14	1.75	0.54	0.09	0.01
Dec. 10	0.67	1.42	0.94	0.65	0.25	0.23
Dec. 22	0.58	1.43	0.55	0.72	--	--
			Total Sugar (%)			
Nov. 3	3.04	2.58	3.22	3.36	1.52	1.49
Nov. 12	2.29	2.64	2.25	1.94	0.52	0.59
Nov. 19	2.30	2.45	1.42	1.28	0.08	0.08
Nov. 26	2.33	2.62	1.92	0.64	0.41	0.06
Dec. 10	2.30	2.71	1.11	0.73	0.32	0.30
Dec. 22	2.32	2.76	0.62	.86	--	--

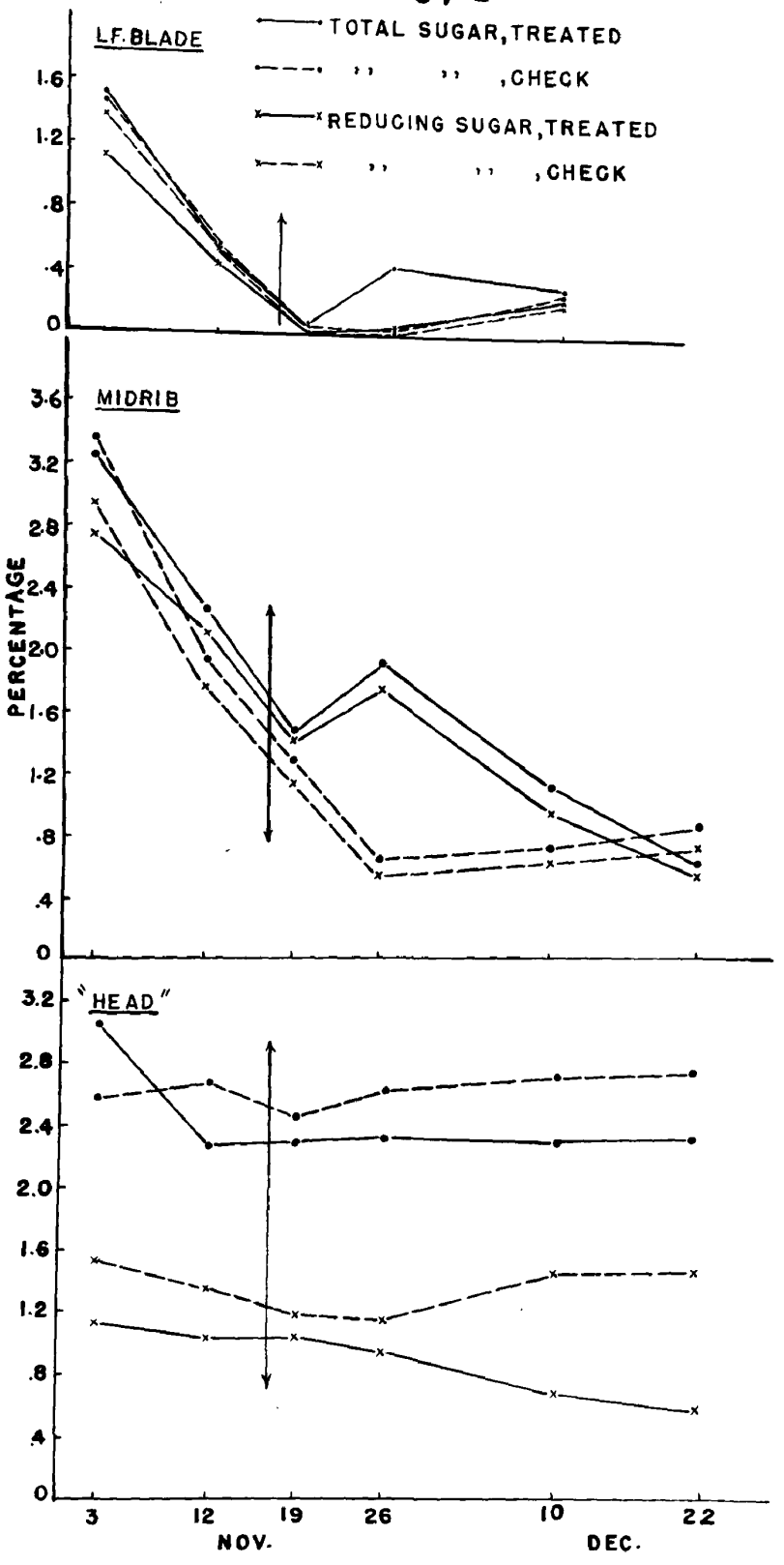

Figure 19: Effect of treatment with methyl ester of naphthalene-acetic acid on the sugar content of cauliflower in storage. The vertical line bisects the graph at the time when the untreated plants had dropped 10% of their leaves, whereas the treated lots had dropped none.

Dry Matter

Per cent dry matter was determined on ten grams samples from the same cauliflower tissues used in the sugar analysis. The samples were oven dried at 100° C. for 24 hours, and the results were expressed on the fresh weight basis.

Results and Discussion

The dry matter in both leaf blade and midrib was somewhat higher in the untreated cauliflower than in the treated lots, and was only slightly different in the "head" tissue (Table 16). It varied more in the leaf blade than in the other two tissues, especially after the formation of abscission. Probably the increase in dry matter resulting from storage that was found in both treated and untreated leaf blade tissue was due to the loss of water through transpiration, while the slight decrease in dry matter in both "head" and midrib tissues was due to a decrease in sugar from respiration.

With the formation of abscission, the dry matter content of the leaf blade increased, whereas there was no appreciable effect on the "head" tissue.

TABLE XVI. The Effect of Methyl Ester of Naphthaleneacetic Acid on the Dry Matter Content of Stored Cauliflower (Expressed on fresh weight basis, average of duplicate determinations).

Date Sampled	"Head"		Midrib		Leaf Blade	
	Treated	Check	Treated	Check	Treated	Check
	%	%	%	%	%	%
Nov. 3	8.39	9.20	9.27	8.48	13.65	13.07
Nov. 12	9.02	9.20	6.91	7.23	12.01	13.60
Nov. 19	8.84	8.45	6.66	6.88	13.06	13.53
Nov. 26	8.81	8.45	6.31	6.79	14.89	16.17
Dec. 10	9.15	8.77	6.33	7.19	14.59	16.45
Dec. 22	7.26	7.90	5.11	6.47	--	--

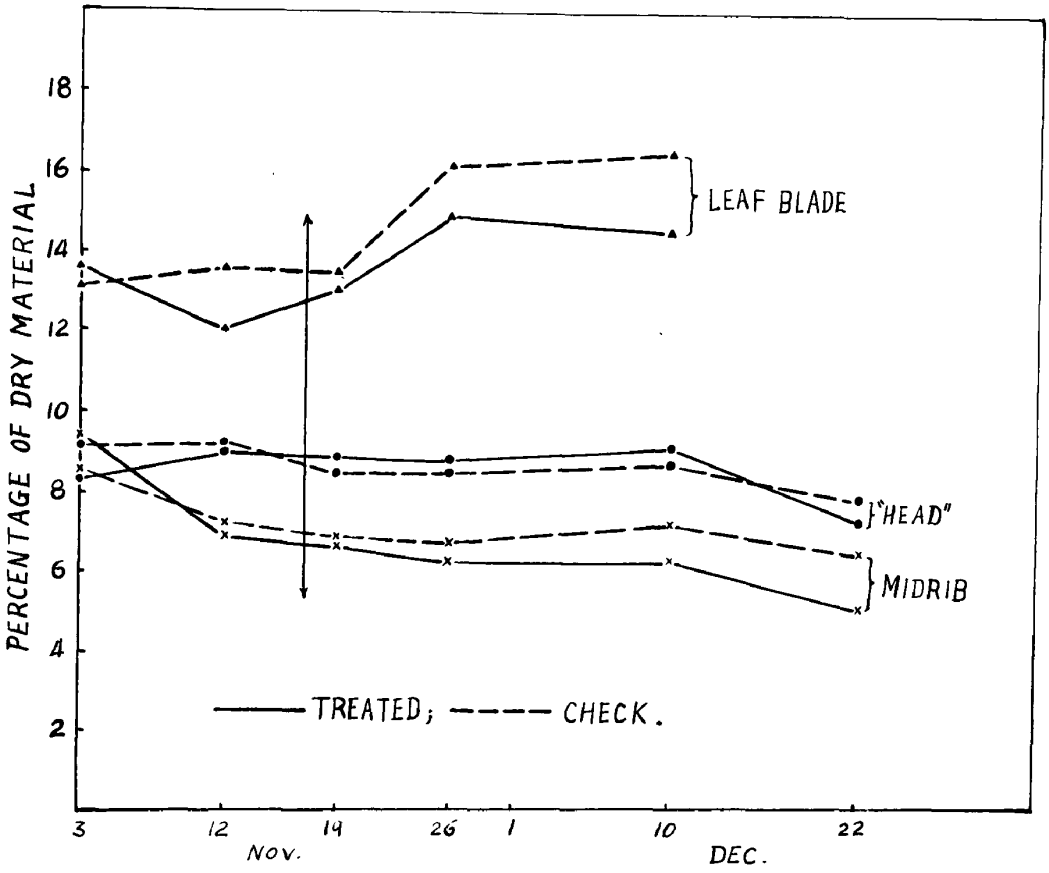

Figure 20. Effect of treatment with methyl ester of naphthaleneacetic acid on the dry matter content of cauliflower in storage. Vertical line indicates the time when the untreated plants had dropped 10% of their leaves, whereas the treated lots had dropped none.

Ascorbic Acid

Material and Methods

Forty heads of cauliflower were selected on Jan. 16; twenty were treated with methyl ester of NA in order to retard the abscission formation, and the other twenty served as controls. Analyses were made at weekly intervals from Jan. 27 until practically all the leaves had fallen in the untreated lots on Feb. 26. The method of analysis used by Lucas (1944) was followed.

Three different sections, the "head", the leaf blade, and the midrib, of the cauliflower were sampled. The tissues were collected from four heads for each determination. 200 ml. of 2% metaphosphoric acid were placed in blender container with 40 gr. of tissue. The blender was operated for 3 minutes. The extractant was first filtered through cheese cloth into a suction flask and then transferred to a centrifuge tube and centrifuger for 10 minutes. The supernatant aliquot was used for titration. Two ml. of the extractant were titrated with a 0.02 per cent solution of sodium 2,6-dichlorobenzenoneindophenol to a faint pink end point.

Results and Discussion

The ascorbic acid content was found to be the highest in the leaf blade, low in the "head", and still

lower in the midrib (Table 17). After the first month of storage the ascorbic acid content declined in all tissues. The rate of loss in the ascorbic acid was lower in both "head" and midrib tissues than in the leaf blade. On Feb. 26, after 51 days in storage, both "head" and midrib tissues contained about 40 mg. per 100 grams of fresh weight, indicating a loss of two thirds of the ascorbic acid in the "head", and about one half in the midrib. There was no appreciable difference between treated and untreated tissues of head and midrib. However, in the leaf blade, the loss of ascorbic acid occurred early in the storage period, with the untreated lots showing the greater loss. Since the treatment with NA retarded the foliar abscission, the leaf blade of the treated heads lost less ascorbic acid than that of the untreated cauliflower (Table 17).

TABLE XVII. The Effect of Methyl Ester of Naphthaleneacetic Acid on the Ascorbic Acid Content of Stored Cauliflower (Average of duplicate determinations).

Date Sampled	Ascorbic Acid, mgs. per 100 gms. Fresh Weight					
	"Head"		Midrib		Leaf Blade	
	Treated	Check	Treated	Check	Treated	Check
Jan. 27	115.8	106.1	70.8	68.4	159.6	158.6
Feb. 3	113.7	111.1	70.7	67.2	147.3	124.8
Feb. 12	100.4	107.4	72.8	67.8	143.6	129.8
Feb. 19	81.6	78.3	69.7	62.7	105.6	96.0
Feb. 26	46.2	42.0	41.7	37.8	101.7	69.6

Respiration

Material and Methods

Two uniform cauliflower heads, one treated with methyl ester of NA (100 mg. per head), and the other left untreated were placed into two desiccators which were set up as respiratory chambers, at a temperature of approximately 70° F. The respiratory rate was determined by the method of Haller and Rose (1932) before and after treatment at varying intervals. The sealed desiccators which served as respiratory chambers, were connected by tubing to two closed liter-graduated cylinders containing O_2. The CO_2 given off by the respiration of the cauliflower heads was absorbed by KOH solution, and the O_2 used was replaced by the O_2 in the cylinder. At the end of a run, 24 hours after operation, the KOH solution in the funnel on the bottom of the desiccators was drained into a flask for titration. Fresh KOH was then added again for another determination. The KOH was titrated against 2N H_2SO_4 to the phenothalein end point and then to the methyl orange end point and the amount of CO_2 computed from the difference between the two end points.

In one experiment, two smaller heads (average 790 grams per head) were used, while in a second experiment, two larger heads (average 1500 grams per head) were used to compare the difference of respiratory activity between various sizes of heads as well as the effect of the methyl ester of NA.

Results and Discussion

The rate of respiration of cauliflower was found to be slightly increased by the treatment with methyl ester of NA. Ten days after completion of the respiration determination, all the leaves of the untreated heads dropped, whereas the leaves of the treated heads remained intact. The fluctuation of the respiratory rate during the course of this experiment was considered to be more closely related to environmental temperature change rather than the effect of treatment. The smaller cauliflower heads had higher rate of respiration per unit of weight than the larger heads. The heads used in experiment I were about one half the weight of those used in experiment II, whereas the rate of respiration of the heads in experiment I was about twice as that found in experiment II.

TABLE XVIII. The Effect of Methyl Ester of Naphthaleneacetic Acid on the Rate of Respiration of Cauliflower.

Experiment I			Experiment II		
Days after Treatment	Mg. CO_2 per Kg. Plant Meterial per Hour		Days after Treatment	Mg. CO_2 per Kg. Plant Material per Hour	
	Treated	Check		Treated	Check
1	58.5	52.2	1	88.3	90.6
2	54.9	44.2	2	102.1	108.9
5	41.5	41.9	10	75.5	68.2
7	43.9	32.5	12	105.9	90.2
10	42.3	37.4	15	73.3	65.3

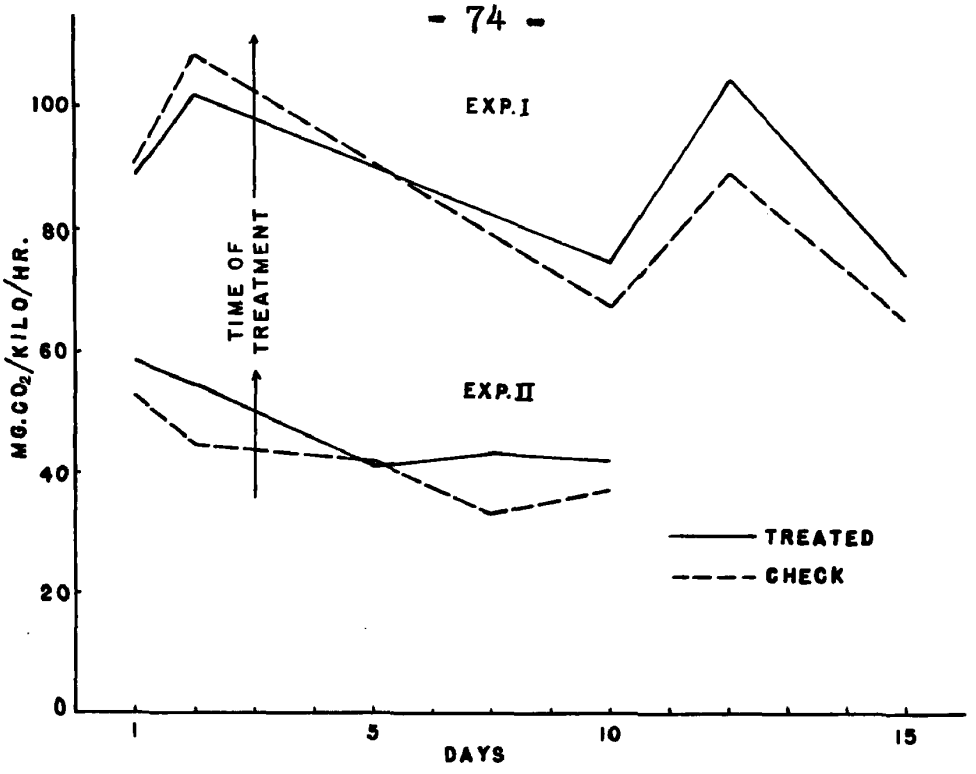

Figure 21. Effect of methyl ester of naphthaleneacetic acid on the rate of respiration in cauliflower.

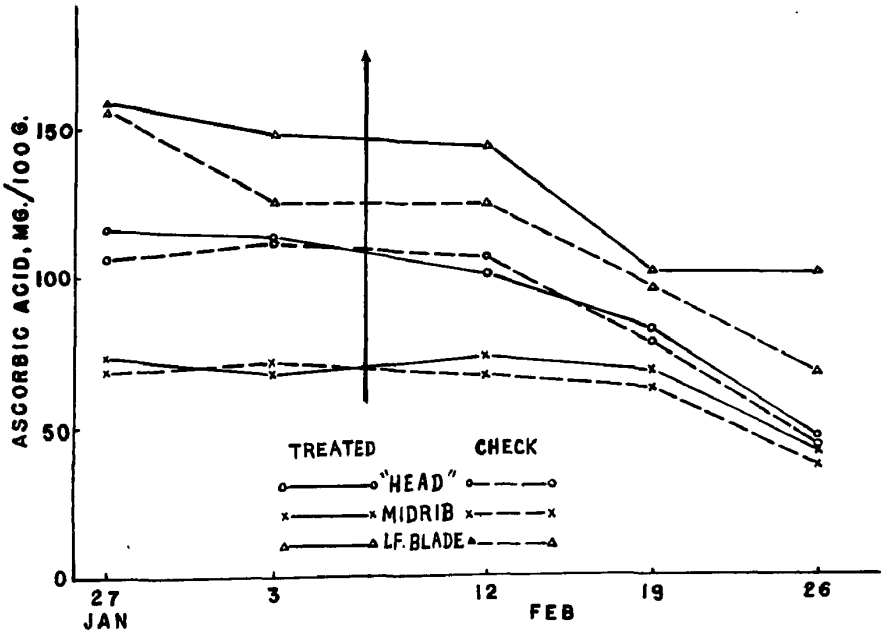

Figure 22. Effect of methyl ester of naphthaleneacetic acid on the ascorbic acid content of stored cauliflower. The vertical line indicates the time when the untreated heads had dropped 30% of their leaves, whereas the treated lots had dropped none.

Catalase Activity

Material and Methods

Samples were taken from the cauliflower used in ascorbic acid determination, and the catalase determinations were made at the same time intervals.

The method of determination generally followed that used by Heinicke (1924) and Knott (1927), with the following variation. A constant temperature water bath was used in order to maintain a more uniform environment. Forty grams of each of the "head", midrib, and leaf blade tissues were sampled separately from four heads. One hundred millliliters of cold water and 0.5 gram of calcium carbonate were added into the blender with the plant tissue, which was operated for three minutes. The mixture was filtered, then centrifuged and the supernatant liquid was used for the determination of catalase activity.

Two millliliters of 3 per cent hydrogen peroxide were placed in one arm of the shaking tube and an equal amount of the extract in the other. The whole tube was immersed into the water bath at 25° C. for one minute before shaking. The activity was measured as the number of millliliters of oxygen released per second of shaking. Measurements in duplicate were made at intervals from ten seconds up to 300 seconds.

Results and Discussion

The catalase activity was found to be highest in the "head", and intermediate in the leaf blade, and lowest in the midrib tissue (Table 19).

The catalase activity in the "head" was less variable than that of the two other tissues, and the oxygen was released at a relatively uniform rate during the course of the observation. There was no apparent difference between the treated and the untreated lots. Within the time limits of the experiment (i.e., 300 seconds), the rate of oxygen released plotted against time was almost a straight line relationship. Other tests indicated that the oxygen released continued at a diminishing rate for another 350 seconds. In the midrib and leaf blade tissues catalase activity was negligible after 200 seconds of shaking.

In the leaf blade, the catalase activity was found to be more variable than that of either the "head" or midrib tissues. Like sugars and ascorbic acid, the catalase activity decreased during the storage period. On Jan. 27, about 6 ml. of oxygen were released after the 300 seconds of shaking, while on Feb. 26, only 2 ml. were released. The catalase activity was found to be a little higher in the untreated cauliflower, especially during early storage, when the abscission had not yet been formed. Apparently,

the methyl ester of NA retarded the catalase activity in the beginning of treatment. Perhaps the application of the growth regulating substance is a possible cause of the delay in abscission formation.

Leaf Order and Catalase Activity--The leaves of each of four heads of cauliflower were separated into five groups according to the arrangement of the leaves from outside (older) to inside (younger). Every five contiguous leaves were treated as a group and all five groups were analyzed separately for their catalase activity.

The catalase activity was found to vary from group to group, not only in the total amount of oxygen released, but also in the rate at which it was released (Figure 24). In the blade tissue, it was much higher in the outer leaves and lower in the inner leaves. This result was similar to that found by Knott (1927) in spinach and celery leaves, in which he reported that the younger and the older leaves are usually low in catalase activity, while those intermediate in age have higher or approximately equal activity. In cauliflower, the oldest leaves are removed in harvest, and so the outer and older leaves of the stored cauliflower are actually "intermediate" in age.

The catalase activity in the midrib of leaves of different ages did not exhibit any significant differences.

Repeated determination from another variety of cauliflower indicated the same general relationship.

From the data in Table 21, it is suggested that the formation of foliar abscission of cauliflower might be caused by an enzymic activity existing in the leaf tissues. The higher the enzymic activity, the earlier the abscission would be formed. Any environmental condition or chemical treatment which might retard the enzymic action would delay the abscission formation.

Rate of Catalase Reaction--The rate of catalase activity in the "head" and the leaf differed markedly. In the leaf tissue, the rate of oxygen released was higher during the first 100 seconds of shaking and decreased subsequently. While in the "head" tissue, the release of oxygen increased gradually up to 600 seconds.

These two types of reactions found in different tissues of the same cauliflower are of interest from a theoretical point of view. The relationship between time of shaking and the amount of oxygen released in the "head" tissue was practically a straight line with a constant slope up to 300 seconds of shaking. However, the relationship in the leaf tissues, especially in the leaf blades, when plotted, became a parabola over the 300 seconds time interval.

If the volume of oxygen (v) released is plotted against the logarithm of the time of shaking (log t), the curves become straight lines. By this method, the equations of all the straight lines could be found, so that any number of milliliters of oxygen released can be predicted by the logarithm of the seconds of shaking.

TABLE XIX. The Relation Between the Volume of Oxygen Released and the Time of Mixing the Extract of the Leaf Blade of Cauliflower with Hydrogen Peroxide.

Date	Equations	
	Treated	Untreated
Jan. 27	$v = 3.382 \log t - 2.475$	$v = 3.804 \log t - 2.533$
Feb. 3	$v = 2.456 \log t - 1.639$	$v = 2.680 \log t - 2.071$
Feb. 12	$v = 1.568 \log t - 0.785$	$v = 1.794 \log t - 1.220$
Feb. 19	$v = 1.789 \log t - 1.577$	$v = 1.626 \log t - 1.416$
Feb. 26	$v = 0.999 \log t - 0.573$	$v = 1.235 \log t - 0.905$

v -- Volume of Oxygen released in ml.

t -- Time of shaking in seconds.

TABLE XX(a) The Catalase Activity of Stored Cauliflower Following Treatment with Methyl Ester of Naphthaleneacetic Acid (Expressed as amount of O_2 released at various time intervals).

Date Sampled	Oxygen Released in ml.								
	10 sec.*	20 sec.	40 sec.	60 sec.	100 sec.	120 sec.	180 sec.	240 sec.	300 sec.
	"Head" Tissue								
Jan. 27	.60	.75	1.30	1.75	2.55	2.90	3.90	4.65	5.25
Feb. 3	.55	.75	1.25	1.70	2.50	2.85	3.80	4.50	5.10
Feb. 12	.55	.80	1.30	1.70	2.50	2.85	3.70	4.35	4.90
Feb. 19	.50	.70	1.2	1.65	2.40	2.65	3.65	4.30	4.80
Feb. 26	.50	.70	1.15	1.60	2.4	2.75	3.6	4.30	4.85
	Midrib Tissue								
Jan. 27	.60	.8	1.15	1.35	1.75	1.85	1.90	2.10	2.10
Feb. 3	.40	.6	.90	1.20	1.55	1.65	1.75	1.85	1.85
Feb. 19	.3	.5	.75	1.0	1.25	1.45	1.55	1.6	1.6
Feb. 12	.45	.65	.95	1.15	1.4	1.50	1.55	1.6	1.65
Feb. 26	.2	.3	.45	.7	1.0	1.1	1.25	1.35	1.4
	Leaf Blade Tissue								
Jan. 27	1.0	1.8	2.6	3.6	4.4	4.6	5.4	5.6	5.8
Feb. 3	.9	1.4	2.2	2.6	3.4	3.6	4.0	4.2	4.4
Feb. 12	.8	1.2	1.6	2.0	2.4	2.6	2.8	3.0	3.0
Feb. 19	.4	.7	1.0	1.4	2.0	2.3	2.6	2.8	2.8
Feb. 26	.4	.7	1.0	1.2	1.4	1.6	1.8	1.8	1.8

* Sec. -- Seconds.

TABLE XX(b) The Catalase Activity of Untreated Cauliflower During Storage (Expressed as amount of O_2 released at various time intervals).

Date Sampled	Oxygen Released in ml.								
	10 sec.*	20 sec.	40 sec.	60 sec.	100 sec.	120 sec.	180 sec.	240 sec.	300 sec.
	"Head" Tissue								
Jan. 27	.6	.8	1.2	1.7	2.4	2.8	3.8	4.5	5.2
Feb. 3	.6	.8	1.2	1.7	2.6	2.9	3.9	4.6	5.1
Feb. 12	.6	.9	1.3	1.7	2.5	3.0	3.85	4.6	5.1
Feb. 19	.4	.6	1.1	1.5	2.3	2.7	3.65	4.3	4.8
Feb. 26	.5	.7	1.2	1.6	2.55	2.8	3.75	4.3	4.7
	Midrib Tissue								
Jan. 27	.6	.8	1.1	1.4	1.9	1.9	2.1	2.2	2.2
Feb. 3	.4	.6	1.0	1.3	1.6	1.7	1.9	2.0	2.0
Feb. 12	.4	.6	.8	1.2	1.5	1.6	1.7	1.8	1.8
Feb. 19	.2	.4	.6	.9	1.2	1.3	1.5	1.6	1.6
Feb. 26	.2	.3	.5	.7	1.0	1.0	1.15	1.2	1.2
	Leaf Blade Tissue								
Jan. 27	1.4	2.0	3.6	4.2	5.2	5.6	6.2	6.6	6.6
Feb. 3	.8	1.2	2.0	2.6	3.4	3.6	4.1	4.4	4.5
Feb. 12	.6	1.1	1.6	1.8	2.4	2.6	2.9	3.1	3.2
Feb. 19	.4	.6	1.0	1.4	1.8	2.0	2.3	2.5	2.7
Feb. 26	.4	.6	1.0	1.3	1.5	1.8	1.9	2.0	2.2

* sec. -- Seconds.

TABLE XXI. The Effect of Leaf Order on the Catalase Activity of Untreated Cauliflower (Expressed as amount of O_2 released at various time intervals).

Lot No.	Leaf Order	Oxygen Released in ml.								
		10 sec.	20 sec.	40 sec.	60 sec.	100 sec.	120 sec.	180 sec.	240 sec.	300 sec.
		Leaf Blade Tissue*								
I	1-5	.9	1.4	2.1	2.7	3.4	4.0	4.9	5.5	6.1
	6-10	.8	1.3	1.9	2.4	3.1	3.6	4.4	4.8	5.3
	11-15	.6	1.0	1.6	2.1	2.5	2.8	3.2	3.5	3.7
	16-20	.6	1.0	1.5	1.8	2.3	2.5	3.0	3.2	3.5
	21-25	.6	1.1	1.6	2.0	2.2	2.6	2.7	2.8	2.9
II	1-5	.5	.8	1.4	1.7	2.2	2.6	3.3	3.8	4.2
	6-10	.5	.8	1.2	1.6	2.1	2.4	3.0	3.5	3.9
	11-15	.4	.7	1.1	1.4	1.7	2.0	2.4	2.7	3.0
	16-20	.5	.7	1.1	1.3	1.6	1.8	2.2	2.4	2.6
	21-25	.4	.6	1.0	1.2	1.4	1.6	1.9	2.1	2.2
		Midrib Tissue**								
I	1-5	.3	.4	.6	.8	1.1	1.2	1.4	1.4	1.5
	6-10	.3	.4	.7	.9	1.2	1.3	1.4	1.5	1.5
	11-15	.3	.5	.9	1.1	1.3	1.4	1.5	1.6	1.6
	16-20	.4	.6	1.0	1.2	1.4	1.5	1.6	1.7	1.7
	21-25	.4	.6	1.0	1.2	1.4	1.5	1.6	1.7	1.7
II	1-5	.3	.4	.7	.8	1.0	1.1	1.3	1.4	1.4
	6-10	.3	.5	.7	.8	1.0	1.1	1.2	1.3	1.4
	11-15	.3	.5	.7	.8	1.0	1.1	1.2	1.3	1.3
	16-20	.3	.4	.6	.7	.9	1.0	1.1	1.2	1.3
	21-25	.3	.4	.5	.6	.7	.8	1.0	1.1	1.2

* 15 gms. of plant material in 100 ml. water.
** 40 gms. of plant material in 100 ml. water.

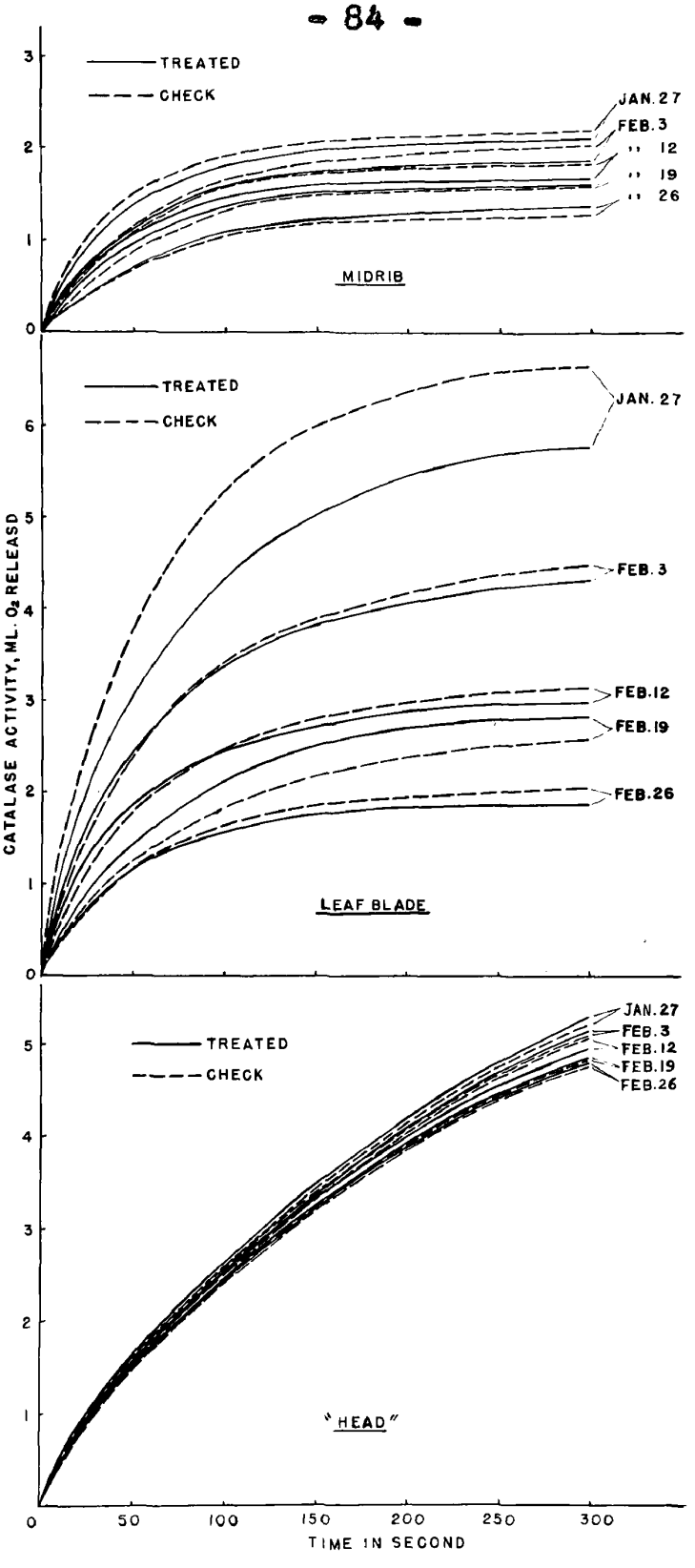

Figure 23. Effect of methyl ester of naphthaleneacetic acid on the catalase activity of stored cauliflower.

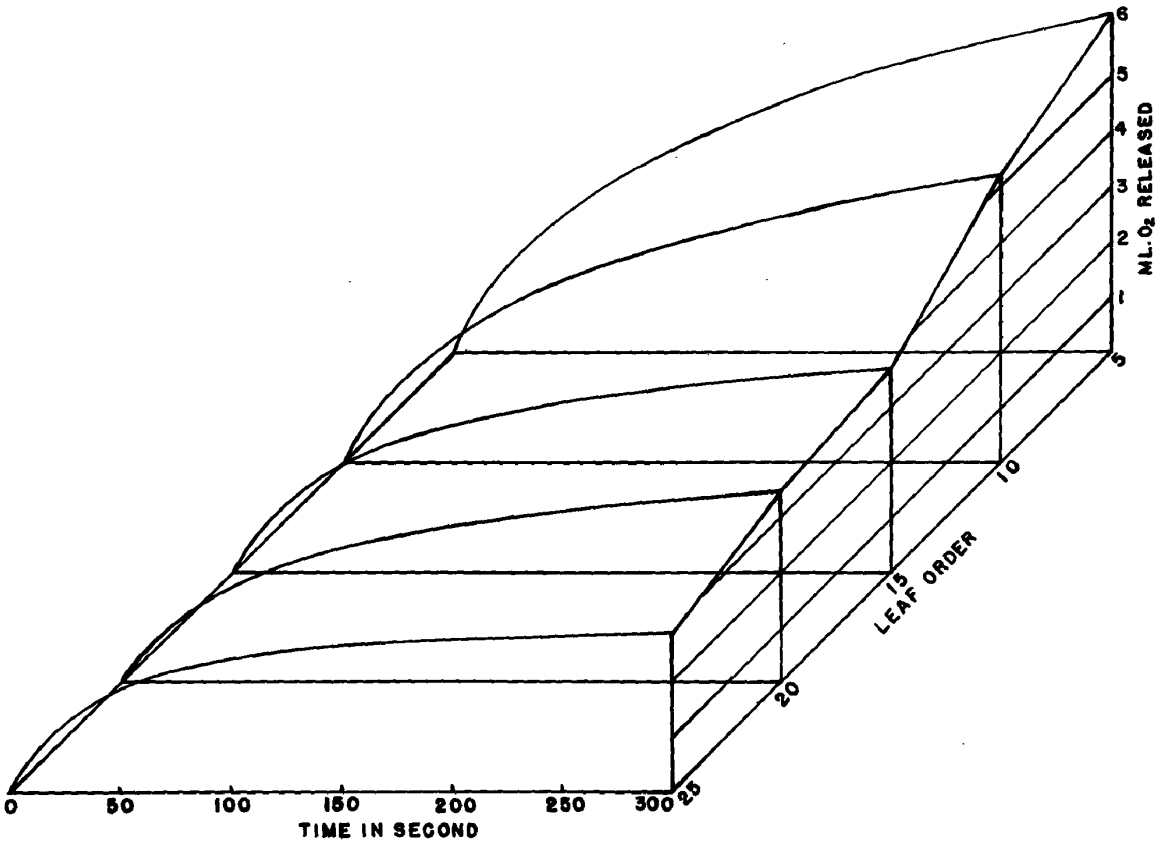

Figure 24. The relation between catalase activity and the leaf order of untreated cauliflower leaf blades. (Expressed in ml. of O_2 released per second).

DISCUSSION

The Role of Plant Hormone in Relation to Abscission Formation

Leaf fall has been generally recognized as being associated with a lack of sufficient growth hormone to inhibit abscission formation. Application of synthetic growth regulating substances augment or substitute for the dwindling supply of phytohormones that exist in the plant tissues. Since the phytohormone is a product of synthesis through physiological activity, any external condition or treatment that would alter physiological activity might influence the effectiveness of the applied growth substances. Maturity of the plant, the amount of the leaf blade attached to the midrib, temperature and humidity of the storage are possible external factors which be practically important.

The hormone content of different leaves is not the same. As found by Avery (1935) in Nicotiana leaves, the concentration of the hormone is roughly inversely proportional to the age of the leaf. In Morus alba, Okabe (1940a), using the Avena method to test the effectiveness of the growth substance, found that negative curvatures result from treatment of young leaves, and positive curvatures developed from treatment of mature

leaves. In the rosette leaves of Solidago, Goodwin (1937) reported that the total amount of auxin diffusing a leaf is very small in the early stage of development, but increases to a maximum, which is almost coincident with the most rapid growth period of the leaf, and then falls off with approaching maturity. Under natural conditions, therefore, older leaves always drop first, and the younger leaves later. This phenomenon is very significant from a practical point of view. In both cabbage and cauliflower, the leaves of the older mature heads, if other conditions are equal, drop earlier than the younger immature leaves. Apparently, the application of growth regulating substances is more effective for the older leaves in which the auxin content is lower than for the younger leaves in which the auxin content is higher.

Nearly all positional differences in effectiveness resulting from the application of growth substances, as reported by Hitchcock and Zimmerman (1941) in apples as well as the results of the present study, are caused by or related to the degree of maturity of the plant organ.

Since the growth hormone is believed to be synthesized in the leaf blade, and probably is associated with photosyntheses, deblading should hasten leaf dropping. This was true in the case of intact plants, but

was not true in the case of excised plants, such as cabbage and cauliflower in storage. The results of the trimming experiment indicate little effect of the leaf blade on leaf falling. This conclusion is probably due to the difference of metabolic activity between the growing plants and the stored crop. Under cold storage in the absence of light, the leaf blades are probably unable to synthesize the growth hormone so abscission could not be affected by the leaf blades.

To a certain extent, the length of the midrib is probably not a major factor in connection with the abscission formation. With respect to abscission, the role of the trimmed midrib (reduced about one half), was the same as that of the untrimmed one. But observations have also been made that if the midrib was cut too short (less than one inch in length), the leaf would fall much earlier, even in those treated with a growth regulating substance. This observation fonfirms the assumption of Gardner and Cooper (1943) that the action of the growth substances depends on or is conditioned by the presence of a necessary second substance or substances already present in the plant tissue.

The Transport of Growth Regulating Substance in Relation to Abscission Formation

As a result of the present study, the transport or movement of the growth regulating substances in the stored cauliflower is polar, that is, from the tip toward the base in the leaf as well as in stem. Not only can the growth regulating substances move downward vertically, but they also move horizontally in the stem. This phenomenon had been discussed by many investigators. Avery (1935) studied the movement of auxin in the Nicotiana leaf and found that it moves from the tip toward the base of the leaf, and from the lateral vein into the midrib. This polar auxin transport in plant tissues, according to Clark (1938), is not caused by the electrical polarity but by entirely different mechanisms, as shown by the different effects of light and gravity.

In Morus alba, Thea sinensis, Ginkgo biloba, and many other asiatic woody plants, Okabe (1940a) found that movement of growth-promoting substances in the petioles seems to be basipetally while that of the growth inhibiting substances has no polarity of movement. But according to the results obtained by Hitchcock and Zimmerman (1935) and Ferri (1945) the synthetic growth substances can be absorbed from the soil by plants and transported upward, as are mineral salts.

The apparent divergence of these results is probably due to differences in the physiological activity of intact and excised plants and of natural and synthetic growth substances. It is general known that natural growth hormones move or diffuse normally in a polar manner, whereas the synthetic growth substances may be transported upward in the growing plants. But this upward movement did not occur in the case of stored plants, such as the cauliflower used in this study.

Since the upward movement of the applied growth substances was affected by conditions influencing the rate of transpiration, Hitchcock and Zimmerman (1935) claimed that the movement occurred in the transpiration stream. If, as in the stored cauliflower and cabbage, the transpiration rate decreased, the movement of the applied growth substance would naturally be slowed down, so that no retarding effect on the abscission would be found when a growth substance, such as 2,4-dichlorophenoxyacetic acid, was applied only on the cut end of the stem. But it did not prove that the downward movement also occurred in the transpiration stream. The retarding effect of the growth substance which was applied only on the cut end of the midrib did not necessarily mean that the growth substance itself moved in a polar manner. Probably, the retardation

of the abscission formation is a secondary effect of the growth substance used, and was not caused by the direct contact with the abscission layer.

The polar movement and local effect of the growth regulating substance on the stored cabbage and cauliflower are probably the main reason for the "inverted relationship" between leaf order and the development of the abscission here discovered.

Under natural conditions, the formation of foliar abscission of untreated plants is mainly controlled by the maturity of the leaves. The older the leaves, the earlier the abscission will be formed; in other words, the degree of abscission is primarily governed by one factor, the gradient of maturity. The foliar abscission of the heads treated post harvest, however, is probably governed by two factors, the maturity of the leaves and the effectiveness of the growth regulating substance.

Based on this theoretical consideration, the relationship between the abscission formation and leaf order can be illustrated by the diagrams in Figures 25 and 26. In the case of cauliflower, in which there are no very young leaves at the time of harvest, the percentage of separation is directly proportional to the increase of the leaf order on the post harvest treated heads, and in-

versely proportional to the increase of the leaf order on the untreated heads.

If, for example, the outer leaves of a cauliflower, which had been treated with NA after harvest, show 40 per cent of separation. It can be expected that the innermost leaves might have completely abscissed. On the other hand, in the untreated cauliflower, the innermost leaves might have no abscission at all. The differences between the relationships occuring in treated and untreated cauliflower were not evident at either the early stage or the final stage of the storage life (see Figures 25 and 26).

In the case of cabbage, in which there are many young immature leaves at the time of harvest, the percentage of separation of the abscission on the untreated heads, as in cauliflower, is also inversely proportional to the increase of the leaf order. On the post-harvest treated cabbage, however, the intermediate leaves always have higher percentage of separation than the outer and inner leaves. In this work post-harvest treatment with the growth regulating substance only retard the abscission formation of the 20 outermost leaves. There was little difference between the treated and untreated cabbage in the percentage of separation of the inner young leaves.

From the diagram for cabbage, Figure 25, the maximum point of the separation curve at a given time is

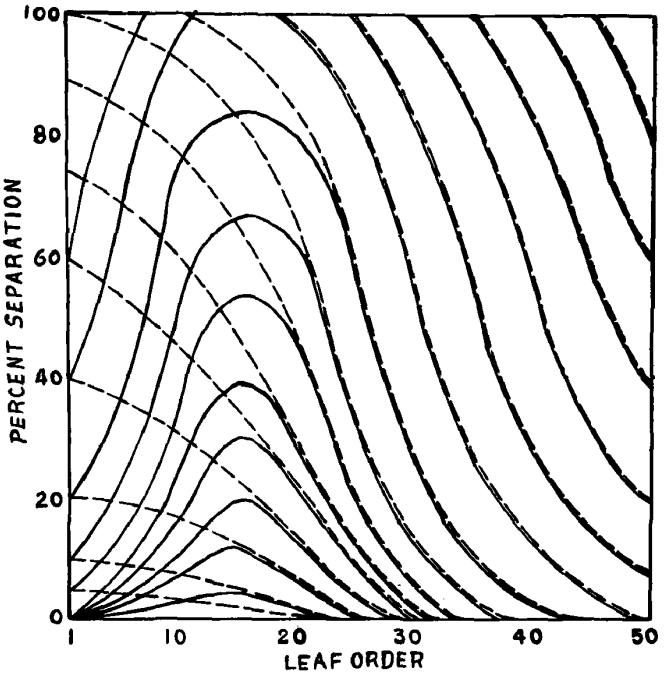

Figure 25. Diagram illustrating the theoretical relationship between the change of leaf order and the change of percentage of separation in cabbage, cf. figure 17. ——— treated, ---- check.

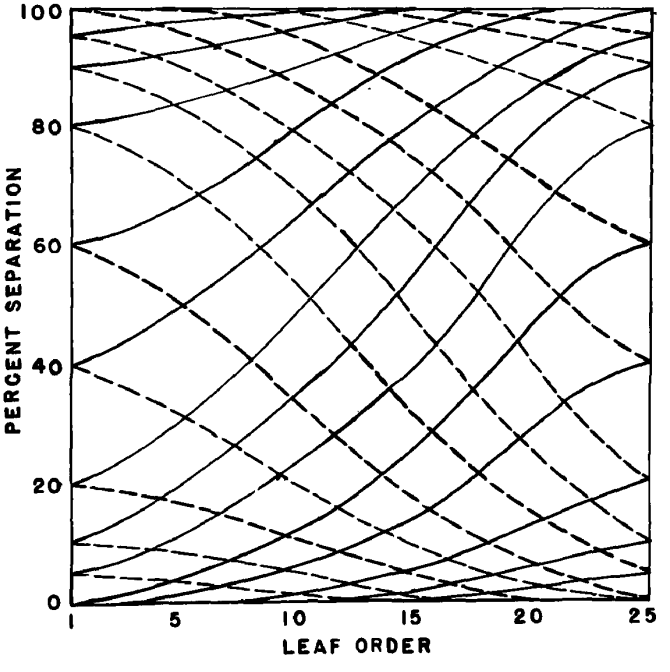

Figure 26. Diagram illustrating the theoretical relationship between the change of leaf order and the change of percentage of separation in cauliflowerm cf. figure 18. ——— treated, ---- check.

taken as the intersection of the theoretical curve of maturity at which the foliar abscission will naturally be formed, and the curve of the effectiveness of the growth regulating substance. As time advances, the curves move upward until all the leaves fall. It must be remembered, however, that the time required for a given percentage of separation of the outer leaves of the treated cauliflower or cabbage is much longer than that for the untreated heads.

Since these relationships of abscission formation and leaf order in either cabbage or cauliflower are not observed in pre-harvest treated plants, it is probably that the relationship is due to the slow and polar transport of the applied growth substances in the plant tissue.

Biochemical and Physiological Changes in Relation to Abscission Formation

Microchemically, the nature of the abscission zone is greatly changed in the course of development of abscission formation. However, the present study indicates that this change has little effect on the nutritive value of the edible product.

The variation of total sugar, reducing sugar, dry matter, and ascorbic acid between treated and untreated cauliflower, as found in this study, is considered mainly

to be the result of the abscission rather than the cause of it. The slightly higher sugar content of the untreated cauliflower "head" may be due to the decreased sugar consumption taking place in the leaves after abscission, but it is not true in the case of dry matter and ascorbic acid. Generally, the content of these substances varies more in the leaf, especially the leaf blade, than in the "head". It is thought that this variation is caused by the secondary effect of the abscission and is not the direct effect of the growth regulating substance.

As indicated before, the growth regulating substances may have a pronounced effect on the bio-chemical transformation of the growing plant, and may have comparatively little effect on the stored plant. Many workers have investigated this problem. Alexander (1938) found a movement of carbohydrate toward the apical swelling and an accumulation of starch below it after treatment with indole-3-acetic acid on bean plants. Mitchell, Kraus, and Whitehead (1940) showed that naphthaleneacetic acid sprayed upon the leaves of kidney bean plants hastened the hydrolysis of starch and dextrins in the leaf. Bausor (1942), in an experiment using indoleacetic or b-naphthaleneacetic acid in lanolin on tomato cuttings, reported that amylolysis follows treatment of tissues with a growth substance even in the presence of high carbohydrate supply.

Miller (1933), treating dormant potato tubers with sulphur compounds, found that the chemicals increased the sucrose content and respiration. As a result of this change, the maturity of the plant would naturally be hastened, as was found by Wittwer and Murneek (1946) who reported that snap beans sprayed with 2,4-D and several other substances in the flowering stage, matured earlier.

On the other hand, many instances have also been found in which growth regulating substances can retard the physiological activity. The use of methyl ester of naphthaleneacetic acid for inhibiting sprouting of potato tubers has been found by Denny (1942, 1945), Guthrie (1933, 1939) and many others. Recently stromme and Hamner (1948) also found that bean plants sprayed with solution of 2,4-D at a non-herbicidal concentration delayed maturity. The retardation of abscission formation is but one of these effects.

These apparently contradictory results are probably related to the potency of the growth regulating substances used and the metabolic activity of the plant organs treated. In the case of stored plants such as cabbage and cauliflower the chemical and physiological changes following treatment with growth regulating substances, is not as great as that observed in growing plants in the field.

Catalase activity was altered by the treatment in this work. Generally catalase activity is more closely

associated with the metabolism of the plant than the other chemical changes. The ability to decompose hydrogen peroxide, or the reaction of catalase activity, as pointed out by Heinike (1924), is a more sensitive measure of the metabolic status of the tissue than the ordinary chemical analyses. Ranjan and Mallik (1931) found that catalase activity is correlated with the monosaccharides, and is influenced more by hexose formation than by the actual amount of hexose present.

One of the main physiological effects of the growth substance is the mobilization of material toward the region of vigorous growth and a redistribution of storage material in the plant. After treatment of bean plants with indol-3-acetic acid, Alexander (1938) found a movement of carbohydrates toward the apical swelling. This mobilization seemed to be effected through the action of the growth substances on the enzymatic system. It was found by Miller (1933), that sulphur compounds may break the dormancy of potato tubers, and will result in an increase in catalase and peroxidase activity as well as respiration. Apparently, low concentration of the growth substance may retard the maturity, catalase activity, hydrolysis of starch and, to a certain extent, respiration of the treated plants; whereas, in higher concentration, the reverse may be true.

As a result of the present study, the catalase activity was inhibited by the action of the growth regulating substance, especially in the leaf blades of cauliflower. However, there was little effect on the "head" tissues, which were probably of little importance in connection with abscission, in comparison with the leaf organ that subsequently abscissed.

The relationship between the leaf order and abscission is probably related to the catalase activity of the leaves. The outer (older) leaves have higher catalase activity and thus they will drop earlier; the inner (younger) leaves have lower catalase activity, and thus they will drop later. The mechanism of the retarding of foliar abscission is probably due to the growth regulating substance which may inactivate certain enzymatic systems which are required for the formation of the abscission.

CONCLUSIONS

Leaf abscission formation in cabbage and cauliflower stored at temperatures from 34° to 70°F. was markedly retarded by treatment with α-naphthaleneacetic and 2,4-dichlorophemoxyacetic acid compounds. The methyl ester forms of these two chemicals induced some elongation of the cauliflower inflorescence during storage, while their sodium salts tended to reduce the chlorophyll content of the leaves. Low storage temperature at a high humidity also delayed abscission formation.

Results of experiments indicated that the stimulus of the growth regulating substances was transported vertically from the tip of the leaves or stems downward and also horizontally from one side to the other, but did not move upward.

Leaf blade tissue had little effect in delaying abscission of the stored cauliflower. Treatment with the methyl ester of α-naphthaleneacetic acid at a concentration of 100 p.p.m. can retard the leaf fall of both trimmed and untrimmed cauliflower with leaves either debladed or left intact.

Pre-harvest treatment of cauliflower or cabbage with the sodium salt of 2,4-dichlorophenoxyacetic acid at a concentration of 100 p.p.m. applied one or seven days prior to harvest more effectively and uniformly retarded foliar abscis-

sion during subsequent storage than any post-harvest treatment. However, the sodium salt of naphthaleneacetic acid, when applied by the same method and in the same concentration, had little effect.

Anatomically, leaf fall in both cabbage and cauliflower was shown to be due to a weakening of the leaf attachment caused by the differentiation of a meristematic layer, followed by the dissolution of the middle lamellae in the separation layer and the mechanical rupture of the vascular bundle. Formation of a separation layer on the lower side of the petiole usually did not follow the differentiation of a meristematic layer. Differences in foliar abscission between cabbage and cauliflower were in the cell shape in the meristematic layer and the position of the separation layer. Delay in the development of abscission formation through treatment with growth regulating substances may be due to either the impediment of differentiation of the meristematic layer or the retardation of cell wall weakening of the separation layer.

With untreated cabbage and cauliflower it was found that the outer leaves always drop before the inner leaves. In the case of heads treated following harvest, the inner leaves in cauliflower or the intermediate leaves in cabbage dropped first; however, in storage following pre-harvest treatment, little difference was found in the time of abscission between the older and the younger leaves.

Sugars, dry matter, and ascorbic acid were generally found to vary more in the leaf blade than in the "head" tissue of cauliflower. The differences in the content of sugars, dry matter and ascorbic acid observed between treated and untreated cauliflower tissues are considered to be the result or secondary effect of foliar abscission and are not the cause.

Not only did the catalase activity of cauliflower decrease with increase in the length of the storage, but also it decreased from outer (older) leaves to inner (younger) leaves. Also treatment with the methyl ester of naphthaleneacetic acid decreased the intensity of catalase activity, especially in the leaf blade. Apparently the partial inactivation of catalase activity by treatment is a possible cause for the delay in abscission formation.

LITERATURE CITED

Alexander, T.R. 1938. Carbohydrates of bean plants after treatment with indol-3-acetic acid. Pl. Physiol. 13: 845-858.

Association of Official Agricultural Chemists. Official and tentative methods of analysis. 1940. Washington, D.C.

Avery, G.S.,Jr. 1935. Differential distribution of a phytohormone in the developing leaf of Nicotiana, and its relation to polarized growth. Bull. Torrey Bot. Club. 62: 313-330.

Batjer, L.P. 1942. Temperature in relation to effectiveness of pre-harvest drop spray on apples. Proc. Amer. Soc. Hort. Sci. 40: 45-48.

_____, and Moon, H.H. 1945. Effects of naphthaleneacetic acid sprays on maturity of apples. Proc. Amer. Soc. Hort. Sci. 46: 113-117.

_____, and Marth, P.C. 1941. Further studies with sprays in controlling pre-harvest drop of apples, Proc. Amer. Soc. Hort. Sci. 38: 111-116.

_____, and Thompson, A.H. 1946. Effects of 2,4-dichlorophenoxyacetic acid sprays in controlling the pre-harvest drop of several apple varieties. Proc. Amer. Soc. Hort. Sci. 47: 35-38.

Bausor, S.C. 1942. Effects of growth substances on reserve starch. Bot. Gaz. 104: 115-121.

Beal, J.M., and Whiting, A.G. 1945. Effect of indoleacetic acid in inhibiting stem abscission in Mirabilis jalapa. Bot. Gaz. 106: 420-431.

Carolus, R.L., Lee, S.H., and Vandemark, J.S. 1947. Effect of the methyl ester of d-naphthaleneacetic acid on the storage life of cauliflower. Proc. Amer. Soc. Hort. Sci. 49: 367-369.

Clark, W.G. 1938. Electrical polarity and auxin transport. Pl. Physiol. 13: 529-552.

Davey, A.E. 1942. Experiments with sprays in the control of pre-harvest drop of Bartlett pears in California. Proc. Amer. Soc. Hort. Sci. 40: 49-53.

Denny, F.E. 1942. The use of methyl ester of d-naphthaleneacetic acid for inhibiting sprouting of potato tubers, and an estimate of the amount of chemical retained by tubers. Boyce Thompson Inst. Contrib. 12: 387-403.

_____, 1945. Further tests of the use of the methyl ester of d-naphthaleneacetic acid for inhibiting the sprouting of potato tubers. Ibid. 14: 15-20.

_____, and Guthrie, J.D. 1942. Effect of the vapor of the methyl ester of d-naphthaleneacetic acid on the sprouting and the sugar content of potato tubers. Ibid. 12: 253-268.

Dickson, G.H. 1939. Some factors affecting the dropping of McIntosh apples. Sci. Agr. 19: 712-721.

Ennis, W.B.,Jr., and Boyd, F.T. 1946. The response of kidney bean and soybean plants to aqueous spray applications of 2,4-dichlorophenoxyacetic acid with and without carbowax. Bot. Gaz. 107: 552-559.

Ferri, M.G. 1945. Preliminary observation on the translocation of synthetic growth substances. Boyce Thompson Inst. Contrib. 14: 51-68.

Feher, D. 1925. Untersuchungen über den abfall die Fruchte einiger Holzpflanzen. Ber. Deut. Bot. Ges. 43: 52-61.

Gustafson, F.G. 1946. Influence of external and internal factors on growth hormone in green plants. Pl. Physiol. 21: 49-62.

Gardner, F.E., Marth, P.C., and Batjer, L.P. 1939. Spray with plant growth substances for control of the pre-harvest drop of apples. Proc. Amer. Soc. Hort. Sci. 37: 415-428.

Gerhardt, F., and Allmendinger, D.F. 1946. The influence of d-naphthaleneacetic acid spray on the maturity and storage physiology of apples, pears, and sweet cherry. J. Agr. Res. 13: 189-206.

Goodwin, F.G. 1937. The role of auxin in leaf development in Solidago species. Amer. J. Bot. 24: 43-51.

Guthrie, J.D. 1939. Inhibition of the growth of buds of potato tubers with the vapor of the methyl ester of naphthaleneacetic acid. Contrib. Boyce Thompson Inst. 5: 83-94.

Hamner, C.L., Gartner, J.B., and O'Rourke, F.L. 1948. A non-toxic plastic coating to improve the keeping quality of cut foliage. Mich. Agr. Exp. Sta. Quart. Bull. 30: 268-271.

Hamner, K.C., and Kraus, E.J. 1937. Histological reactions of bean plants to growth promoting substances. Bot. Gaz. 98: 735-807.

Haller, M.H., and Rose, D.H. 1932. Apparatus for determination of CO_2 and O_2 of respiration. Science. 75: 439-440.

Hesse, C.O., and Davey, A.E. 1942. Experiments with sprays in the control of fruit drop of apricot and peach. Proc. Amer. Hort. Sci. 40: 55-62.

Heinicke, A.J., Reuther, W., and Cain, J.C. 1942. Influence of boron application on pre-harvest drop of McIntosh apples. Proc. Soc. Hort. Sci. 40: 31-34.

Heinicke, A.J. 1924. Catalase activity in dormant apple twigs: its relation to the condition of the tissue, respiration and other factors. N.Y. (Cornell) Agr. Exp. Sta. Mem. 74.

Hitchcock, A.E., and Zimmerman, P.W. 1935. Absorption and movement of synthetic growth substances from soil as indicated by the responses of aerial parts. Contrib. Boyce Thompson Inst. 7: 447-476.

_____, and _____. 1941. The use of naphthaleneacetic acid and its derivatives for preventing fruit drop of apple. Proc. Amer. Soc. Hort. Sci. 38: 104-110.

Hodgson, R.W. 1918. An account of the modes of foliar abscission in Citrus. Univ. Calif. Pub. (Bot.) 6: 417-428.

Hoffman, M.B. 1940. The pre-harvest drop of mature McIntosh apples as influenced by application of nitrogen carrying fertilizers. Proc. Amer. Soc. Hort. Sci. 37: 438-442.

Kendall, J.N. 1918. Abscission of flowers and fruits in the Solanaceae with special reference to Nicotiana. Univ. Calif. Pub. (Bot.) 5: 347-428.

Knott, J.E. 1926. Catalase in relation to growth and to other changes in plant tissue. N.Y. (Cornell) Agr. Exp. Sta. Mem. 106.

LaRue, C.D. 1936. The effect of auxin on abscission of petioles. Proc. Nat. Avad. Sci. 22: 254-259.

Lloyd, F.E. 1916. Abscission in Mirabilis jalapa. Bot. Gaz. 61: 213-230.

Livingston, G.A., and Addicott, F.T. 1947. Mechanical factors in foliar abscission of citrus. Amer. J. Bot. 34: 586. (Abstract).

Lucas, E.H. 1944. Determining ascorbic acid in large numbers of plant samples. Ind. and Eng. Chem. 16: 649-652.

MacDaniels, L.H. 1936. Some anatomical aspects of apple flower and fruit abscission. Proc. Amer. Soc. Hort. Sci. 34: 122-129.

McCown, M. 1939. Abscission of flowers and fruits of the apple. Proc. Amer. Soc. Hort. Sci. 36: 320. (Abstract).

_____. 1943. Anatomical and chemical aspects of abscission of fruits of the apples. Bot. Gaz. 105: 212-220.

Miller, L.P. 1933. Effect of Sulphur compounds in breaking the dormancy of potato tubers and in inducing changes in the enzyme activities of the treated tubers. Contrib. Boyce Thompson Inst. 5: 29-81.

Mitchell, J.W., Kraus, E.J., and Whitehead, M.R. 1940. Starch hydrolysis in bean leaves following spraying with alpha naphthalene acetic acid emulsion. Bot. Gaz. 102: 97-104.

Myers, R.M. 1940. Effect of growth substances on the abscission layer in leaves of Coleus. Bot. Gaz. 102: 323-338.

Okabe, Y. 1940a. Growth-promoting and growth-inhibiting substances in the petiole. Bot. Mag. (Tokyo) 54: 357-365. (in Japanese with English summary).

_____, 1940b. On the distribution of growth promoting and growth inhibiting substances in Morus alba L., and Cassia occidentalis L. Bot. Mag. (Tokyo) 40: 453-461. (in Japanese with English summary).

Penfound, W.T., and Mihyard, V. 1947. Relation on light intensity to effect of 2,4-dichlorophenoxyacetic acid on water hyacinth and kidney bean plants. Bot. Gaz. 109: 231-234.

Ranjan, S., and Mallik, A.K. 1931. A study of the catalase reaction, with special references to respiration. New Phytol. 30: 355-381.

Rice, E.L. 1948. Absorption and translocation of ammonium 2,4-dichlorophenoxyacetate by bean plants. Bot. Gaz. 109: 301-314.

Sampson, H.C. 1918. Chemical changes accompanying abscission in Coleus Blumei. Bot. Gaz. 66: 32-53.

Scott, F.M., Schroeder, M., and Turrell, F.M. 1947. Leaf abscission in the valencia orange and suberisation of the internal surface of the valencia and other leaves. Amer. J. Bot. 34: 589. (Abstract).

Smith, F.G. 1948. The effect of 2,4-dichlorophenoxyacetic acid on the respiratory metabolism of bean stem tissue. Plant Physiol. 23: 70-83.

Stewart, W.S., and Klotz, L.F. 1947. Some effects of 2,4-dichlorophenoxyacetic acid on fruit drop and morphology of oranges. Bot. Gaz. 109: 150-162.

Stromme, E.R., and Hamner, C.L. 1948. Delayed maturity of bean plants sprayed with solution of 2,4-dichlorophenoxyacetic acid of non-herbicidal concentration. Science 10:: 2772: 170-171.

Sweeney, B.M., and Thimann, K.V. 1942. The effects of auxin on protoplasmic streaming. Jour. Gen. Physiol. 25: 841-854.

Thimann, K.V. 1934. Studies on the growth hormone of plants VI. The distribution of the growth substance in plant tissues. Jour. Gen. Physiol. 18: 23-24.

Thompson, B.F. 1945. Tissue responses to physiologically active substances. Bot. Rev. 11: 593-610.

Tukey, H.B., and Hamner, C.L. 1945. Retardation of pre-harvest drop of apples through aerosol application of growth regulating substance. Proc. Amer. Soc. Hort. Sci. 46: 102-108.

Vyvyan, M.C. 1946. Experiments with growth substance sprays for reduction of pre-harvest drop of fruit. Jour. Pomol. Hort. Sci. 22: 11-37.

Weaver, R.J., and DeRose, H.R. 1946. Abscission and translocation of 2,4-dichlorophenoxyacetic acid. Bot. Gaz. 107: 509-521.

Wittwer, S.H., and Murneek, A.E. 1946. Further investigations on the value of "hormone" sprays and dusts for green bush snap beans. Proc. Amer. Soc. Hort. Sci. 47: 285-293.

Zimmerman, P.W., and Hitchcock, A.E. 1937. Comparative effectiveness of acids, esters and salts as growth substances and methods of evaluating them. Contrib. Boyce Thompson Inst. 8: 337-350.

_____, _____, and Wilcoxon, F. 1939. Responses of plants to growth substances applied as solution and as vapors. Ibid. 10: 363-376.