# T H E S I S

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# INVESTIGATIONS ON THE PHYSIOLOGICAL ASPECTS OF LETTUCE TIP BURN

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Submitted by

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for the Degree of Master of Science

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Fort Collins, Colorado

May 1, 1925

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# T A B L E  O F  C O N T E N T S

# I N T R O D U C T I O N

Head lettuce has recently become one of the important crops in Colorado. The commercial production of head lettuce is a new industry in the state, having had its beginning in 1918. The acreage devoted to the production of head lettuce had increased from a small plot of a few acres in 1918 to approximately 7000 acres in 1923. Head lettuce being a cool season crop requires a cool climate for proper development. The cool climate of the higher altitudes of Colorado is well adapted to the production of lettuce of high quality.

The development of the head lettuce industry in the mountain districts has greatly increased the total production. Along with this increase in production has come keener competition. The market requires a quality product, which means that only lettuce of good quality will yield a profit to the grower. Any factor which tends to lower the quality lessens the returns to the grower. The production of head lettuce is a highly specialized industry and the grower is confronted with many difficulties. Few truck crops are more difficult to grow and market successfully and profitably than head lettuce. The crop is very exacting in its cultural requirements and is easily affected by unfavorable growing conditions. Sudden changes of temperature and moisture relations are almost certain to be disasterous.

The future development of the head lettuce industry in Colorado is dependent upon the production of a product of which a large percentage will be marketable. At the present time lettuce growers in Colorado are often able to market only 50% of the crop. This low percentage of marketable lettuce is largely due to two limiting factors, shooting to seed, and disease. These two factors are making it difficult to produce lettuce at a profit in competition with other districts. A few days of extremely warm weather may destroy the entire crop by causing it to send up a seed stalk.

The disease, "tip burn," is probably the most important limiting factor in producing head lettuce profitably in Colorado. It is with this factor that we are to deal in this paper. It is difficult to estimate the loss resulting from tip burn. The disease is found wherever lettuce is grown. The loss is not limited to the heads which are never cut on account of tip burn injury, but much of the loss by rot in transit is very likely due to the secondary infection of the tip-burned tissue by decay organisms. If this disease could be controlled, the profits in growing head lettuce in Colorado would be greatly increased.

# HISTORY OF LETTUCE

Lettuce was probably native to Europe and Asia. It has been cultivated as a salad crop since ancient times. The name, Lactuca, was given to this plant by the Latins on account of the large amount of milky juice in the tissues of the lettuce plant. The French, for the same reason, called it Lactue. The English name Lettuce is probably a corruption of either the Latin or French word. It is in all probability a corruption of the Latin, for a number of early authors spell it Lectuce.

That lettuce in early times was held in high esteem as a pot herb and salad plant, is indicated by an anecdote related to Herodotus that Lettuce was served at the Royal tables of the Persian Kings at least 550 years before the Christian era.

Pliny tells us that the Romans knew but one kind of lettuce, which was a black variety that yielded a large quantity of milky juice which caused sleepiness.

The medicinal properties of lettuce as a food plant were noted by Hippocrates 430 B. C. It was mentioned by Aristotle 356 B. C. The species was described by Theophratus 322 B. C. and by Alioscorides 60 A. D. It was mentioned by Galen 164 A. D. In his discussion, Galen gives the impression that lettuce was in very general use and very popular among the Romans.

Columella notes the medicinal properties of the plant:

"And now let lettuce with its helpful sleep
Make haste, which of a tedious long disease
The painful loathings cure."

In 42 A. D. Columella describrs the Cascilian, Cappadeocian, Cyprian, and Tartesan.

Pliny, 79 A. D., enumerates the Alba, Caecelian, Cappadocian, Crispa, Graeca, Laconicon, Nigra, Purpurea, and Rubens. He reports that Antonius Musa, a physician, cured the Emperor Augustus Caesar of a dangerous disease by means of lettuce. Other authors noted that Augustus was cured of the violence of his disease by use of this plant, which circumstances seem to have brought the lettuce into esteem in Rome. As Pliny says, after that time there was no doubt about eating them and men began to devise means of growing them at all seasons of the year, and even preserving them, for they were used in pottage as well as salads.

We also learn from Pliny that the Greek lettuce was a variety that grew both high and large, and that the Roman, in his day, cultivated the purple lettuce with a large root that was called Cascilina. They had likewise, the Egyptian, Cilican, and Cappadocian besides the Astylis or Chaste, which he says was often called Eunuchion, because it was thought less favorable to Venus than other plants. This naturalist adds that they were all considered cooling, therefore eaten principally in summer.

Great pains were used to make them head. They were earthed up with sea sand to blanch them and give them heart. The white lettuce was noticed in that mild climate to be least able to endure cold.

The wild lettuce, as well as the cultivated, was used medicinally by the Romans. Palladium 210 A. D. mentions varieties and the process of blanching. Palladius, who was a Greek physician, notes the culture of lettuce in his treatise of fevers.

Lettuce has been reported in China as early as the 5th century. About 1340 Chaucer in England mentioned it in his prologue, "Well loved garlic, onions, and lettuce." Lettuce was mentioned by Turner in 1538. It is mentioned by Peter Martyr in 1494 as cultivated on Isabela Island. In 1565, Bensoni speaks of lettuce being found in Hayti. Neiuhoff, 1647, saw it cultivated in Brazil. In 1806, McMahon enumerates 16 sorts for the American garden. The Thorburn Seed Co., offered 13 kinds in their catalogue in 1828, and 23 kinds in 1881.

Goff, in 1885 describes 87 varieties with 585 names of synonyms. Vilmorin in 1883 describes 113 distinct kinds. The number of varieties mentioned at various times by writers are as follows:

France 1612, six
1690, twenty-one

1828, forty

1883, one hundred and thirteen

Holland 1720, forty-seven

England 1597, six

1629, nine

1726, nine

1763, fifteen

1765, eighteen

1807, fourteen

America 1806, sixteen

1885, eighty-seven

W. W. Tracy in 1904 describes as distinct varieties for America, 107 out of 404 listed by seedmen, the remainder being synonyms.

## B O T A N Y  A N D  C L A S S I F I C A T I O N

Botanists in general agree that the common garden lettuce Lactuca sativa originated from the wild species Lactuca Scariola. Wild Lettuce is native of Europe, Canary Islands, Madeira, Algeria, Abyssinia, and Eastern Asis. It has been introduced into the United States and is now a troublesome weed in many places. Garden lettuce is a tall annual herb. During the early part of the season, the plant consists of a cluster of leaves thrown up from a short stem. The shape, character, and color of the leaves varies with the different varieties. As the plant matures, the stem elongates and lateral growths develop, producing the seed stalk. The leaves are alternate, denticulate, or pinnatifid, sessile or auriculate, clasping sometimes spinulose-marginal, the lowest ones large the upper much smaller. The infloresence is a panicle. The flowers are yellowish or yellowish-white, the involucre cylindrical, the bracts of which are imbricated in several series, the outer shorter. The receptacle is flat and naked. The corrola rays are truncate and five toothed at the end. The anthers are sagittate at the base. The style branches are slender. The achenes are oval, oblong, or linear, flat three to five ribbed on each face, narrowed above or contracted into a narrow beak which bears a large number of soft capillary white or brown pappus bristles. The achenes vary in color, whitish, blackish, yellowish or brown.

There are three distinct types of lettuce grown in the United States, namely, head, cutting or leaf, and cos. There is a fourth type, called asparagus lettuce, little known in this country. It resembles the cos type. This does not form a compact head, but is grown for its thick stem.

These four types are recognized as botanical varieties or subspecies and are known under the following names: head lettuce, var. capitata; cutting or leaf lettuce, var. intybacea; cos or romaine, var. romana and asparagus lettuce, var. angustana.

Classification (Lactuca sativa)

Botanical

Basal leaves narrow distinctly lanceolate
L. SATIVA var. ANGUSTANA (Asparagus lettuce)
Basal leáves broad, spatulate, oval or roundish, always rounded at the tip.
Leaves deeply cut on edges (L. SATIVA var. INTYBACEA) Leaf lettuce.

Leaves entire but slightly toothed

Leaves forming compact round or flat head; leaves never decidedly stiff or flat (L. SATIVA var. CAPITATA) head of cabbage lettuce.

Leaves forming a conical or cylindrical shaped head; leaves straight and stiff (L. SATIVA var. ROMANA) cos lettuce.

Varietal

Tracy (86) has worked out a classification of lettuce based on the varietal characteristics. His classification is as follows:

Class 1.
Butter Varieties

Sub-class 1.
Cabbage Heading Varieties.
Color Division 1.
Plants wholly green.
Color Division 2.
Plants tinged brownish, larger portion green.
Color Division 3.
Plants brownish, small portion only greenish.

Sub-class 2.
Bunching varieties.
Color Division 1.
Plants wholly green
Color Division 2.
Plants brownish.

Class 2.
Crisp Varieties.

Sub-class 1.
Cabbage heading varieties.
Color Division 1.
Plants wholly green.
Color Division 2.
Plants tinged brown, larger portion greenish.
Color Division 3.
Plants brownish, small portion only greenish.

Sub-class 2.
Bunching Varieties.
Color Division 1.
Plants wholly green
Color Division 2.
Plants brownish, small portion only greenish.

Class 3.
Cos Varieties.

Sub-class 1.
Spatulate leaved varieties.
Heading Division 1.
Self-closing.
Color Division 1.
Plants wholly green
Color Division 2.
Plants brownish.
Heading Division 2.
Loose-closing
Sub-class 2.
Lanceolate-leaved varieties.
Sub-class 3.
Lobed-leaved varieties.

## THE PRESENT STATUS OF LETTUCE AS A TRUCK CROP

Lettuce is the most popular of the salad crops. In acreage lettuce is the leading salad crop and is exceeded in value only by celery. It is produced wherever vegetables are grown, in both city and farm gardens. The acreage and value of lettuce has steadily increased during the last ten years. Statistics compiled by the United States Department of Agriculture show that the acreage and value increased from 5,489 acres valued at $1,595,085 in 1909 to an acreage of 21,544 valued at $8,535,092 in 1919. There has been a very marked increase in the acreage devoted to lettuce production since 1918. A large part of this increase in lettuce production in the last few years has been due to the development of the head lettuce industry throughout the cooler mountain districts of Colorado, Washington, Idaho, Arizona, and California. The cool moist climate of the higher altitudes of these states is well adapted to the production of lettuce of high quality. The production of lettuce, being a new industry in the mountain districts, has presented the grower with many difficulties and uncertainties which have prevented a more rapid development. As the industry becomes more stable the mountain states will undoubtedly maintain their rank among the leading states in the production of lettuce.

Lettuce now ranks eleventh among the important truck crops grown in the United States according to figures compiled by the United States Department of Agriculture. Table I shows the relative rank of the 15 most important truck crops based on acreage production.

Table I.

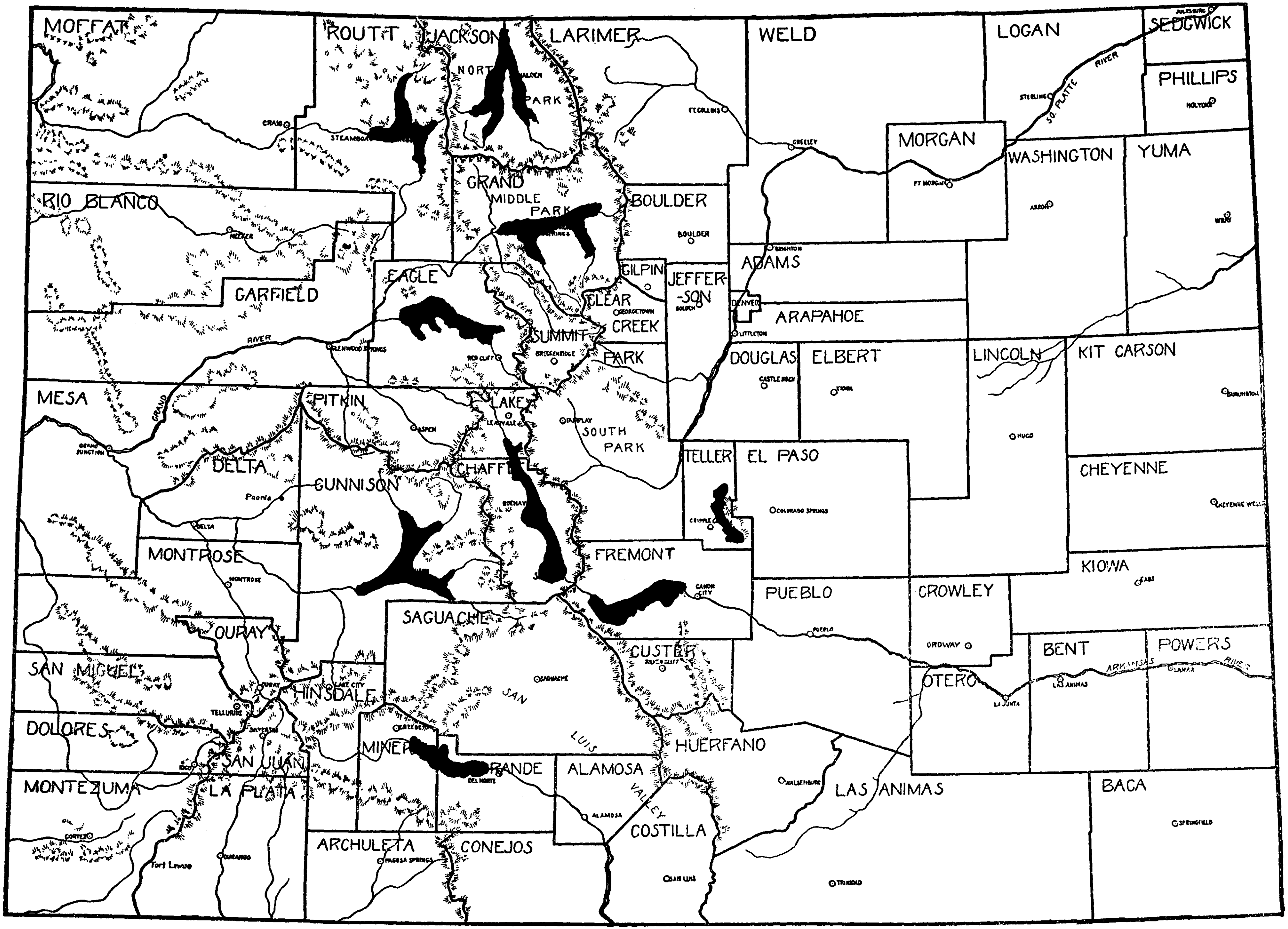
| Crop | Acreage 1923 | Average 1919-1923 | Rank 1923 | Rank 1919-1923 |
|---|---|---|---|---|
| Potatoes | 3,816,000 | 3,852,600 | 1 | 1 |
| Sweet Potatoes | 993,000 | 1,021,800 | 2 | 2 |
| Tomatoes | 405,990 | 323,960 | 3 | 3 |
| Sweet Corn | 250,160 | 219,400 | 4 | 4 |
| Peas | 270,590 | 161,690 | 5 | 5 |
| Watermelons | 155,730 | 158,944 | 6 | 6 |
| Cucumbers | 100,980 | 78,876 | 7 | 9 |
| Cabbage | 89,200 | 108,528 | 8 | 7 |
| Cantaloupes | 82,040 | 82,080 | 9 | 8 |
| Onions | 61,100 | 59,784 | 10 | 10 |
| Lettuce | 56,630 | 36,478 | 11 | 12 |
| Beans | 55,390 | 42,580 | 12 | 11 |
| Asparagus | 43,520 | 34,090 | 13 | 13 |
| Celery | 18,910 | 16,256 | 14 | 14 |
| Cauliflower | 10,520 | 9,104 | 15 | 15 |

## HEAD LETTUCE IN COLORADO

Prior to 1918 head lettuce was not grown on a commercial scale in Colorado. In the spring of 1918 a few acres were planted to head lettuce near Buena Vista. The crop proved so successful that quite a large acreage was planted in this district the following season.

Reports of the large returns per acre from the lettuce crop induced many farmers in the high altitude districts of the state to plant a part of their acreage to lettuce. The reports of profits were exaggerated and the industry over advertised. As a result many attempted to grow lettuce who did not have sufficient knowledge of the requirements necessary for the successful growing of truck crops. Many have attempted to grow lettuce on land not well suited to the crop and in localities not adapted to its production. These conditions resulted in many failures and much discouragement.

Lettuce growers are beginning to learn that head lettuce production is a highly specialized industry and that the difficulties confronting the growers are many. The large increase in acreage throughout the state and the Rocky Mountain Section in general has greatly increased the total production of head lettuce and made competition for market much keener. Lettuce growers now realize that the growing of head lettuce is not a get rich quick business and that only by hard work and careful study of local conditions and the requirements of the crop can it be produced at a profit.




CENTERS OF HEAD LETTUCE PRODUCTION

Some of the things necessary to make head lettuce production profitable and to put the industry on a stable basis are: A more careful study of the crop and its requirements by the grower; a better grade of seed; a more satisfactory control of diseases; and more economical packing and marketing.

The requirements of the crop under Colorado conditions are being slowly worked out. Work is under way to develop a strain on seed which will be more productive of marketable heads than the seed that is common on the market now. When a more satisfactory grading, packing and marketing system has been established the production of head lettuce is certain to maintain its rank as one of the most important truck crops grown in Colorado.

From table II, it will be seen that Colorado has risen since 1918 from a state producing practically no lettuce to the rank of fourth place among the lettuce producing states.

Table II - CARLOT SHIPMENTS OF LETTUCE, 1919-1923

| STATE | 1919 | 1920 | 1921 | 1922 | 1923 |
|---|---|---|---|---|---|
| California | 2,731 | 6,350 | 9,746 | 9,744 | 15,113 |
| New York | 1,761 | 2,138 | 3,167 | 3,167 | 3,817 |
| Florida | 2,134 | 3,120 | 2,286 | 3,323 | 3,146 |
| Colorado | 7 | 125 | 244 | 812 | 1,436 |
| Idaho | 1 | 26 | 182 | 889 | 1,241 |
| Arizona | 41 | 165 | 166 | 678 | 1,108 |
| Washington | 19 | 345 | 632 | 812 | 1,081 |
| North Carolina | 319 | 265 | 448 | 622 | 781 |
| South Carolina | 395 | 356 | 583 | 987 | 577 |
| New Jersey | 245 | 515 | 478 | 571 | 456 |
| Michigan | 63 | 110 | 97 | 81 | 208 |
| Oregon | 0 | 5 | 25 | 129 | 168 |
| Texas | 90 | 176 | 114 | 113 | 102 |
| Virginia | 31 | 26 | 135 | 106 | 70 |
| Others | 181 | 96 | 119 | 206 | 244 |

Since its advent as a truck crop in 1918 the increase in production of head lettuce has been rapid. As table III shows, it now ranks third in acreage as a truck crop in Colorado. The figures used in this table were taken from the Colorado Year Book for 1934.

Table III - TRUCK CROP ACREAGES IN COLORADO - 1923

| Crop | Acreage | Rank |
|---|---|---|
| Potatoes | 90,542 | 1 |
| Cantaloupes | 7,147 | 2 |
| Lettuce | 6,163 | 3 |
| Cabbage | 5,617 | 4 |
| Peas | 2,599 | 5 |
| Onions | 2,448 | 6 |
| Tomatoes | 2,272 | 7 |
| Beans (snap) | 1,350 | 8 |
| Cucumbers (pickles) | 1,206 | 9 |
| Watermelons | 1,189 | 10 |

# T I P   B U R N

Tip burn is one of the most serious diseases affecting lettuce. This disease causes more loss to the lettuce growers of Colorado than all other lettuce diseases combined. Tip burn is very wide-spread, being found wherever lettuce is grown. It attacks lettuce under both greenhouse and field conditions. It is usually very severe in the greenhouse. Many greenhouse vegetable growers have given up the production of head lettuce under glass largely for the reason that attacks of tip burn have made its production unprofitable.

Some varieties of lettuce are much more resistant to attacks of tip burn than others. Plants of the same variety vary greatly in their susceptibility to the disease. Cos lettuce is practically immune to the disease under field conditions in Colorado, although the disease did develop on plants under study in the greenhouse. The leaf varieties are very resistant to tip burn but may be affected under unfavorable greenhouse conditions.

Tip-burned heads have practically no value which makes the loss from this disease very great. The United States Grades for head lettuce limits the amount of tip burn injury to not more than 5% in grades Nos. 1 and 2. Tip-burned heads may be packed in grade No. 3, but third grade lettuce has a market value so low that is is not ordinarily profitable to market it.

It is difficult to estimate the loss due to tip burn. The loss is not limited to the heads which, due to visible injury are not packed for market, but a large part of the loss in transit is very likely due to infection of tip-burned tissue by decay organisms. The injury from tip burn is often confined to the inner part of the head and is often unnoticed in packing. Heads thus injured often find their way into the packed crates and cause considerable loss through decay in transit.

The first appearance of the disease is characterized by a breaking down of the tissue of the leaf at or near the margin. The affected area becomes somewhat transparent; loses its green color; and finally becomes brown or blackish. Microscopic examination of the diseased tissue shows that the cells have collapsed; the cell contents have disappeared and only the cell wall remains. At the time of the attack the plants appear to be in a normal healthy condition.

Under field conditions the disease does not generally appear until the plants near maturity and heads have begun to form. In the greenhouse, plants were attacked while very young before they showed any sign of forming heads. The tender inner leaves are always the first to show injury from tip burn. The older, outer leaves are seldom ever injured by this disease.

## R E V I E W   O F   L I T E R A T U R E

The literature on tip burn of lettuce is not extensive. Mention of the disease is found in a number of texts and experiment station bulletins, but very little of a scientific nature has been published. The loss to vegetable growers resulting from tip burn justifies a more extensive study of its cause and control.

The first reference to this disease was made in 1891 by L. R. Jones, then of the Vermont Experiment Station. In the Annual Report of the Vermont Experiment Station of 1891 Jones (38) mentions a disease of lettuce, which in a later report he describes and illustrates in such a way as to lead us to believe that it was tip burn. In his first report he says, "Much trouble and serious loss was experienced during the entire winter with the lettuce rot both in our own house and others in the city. This trouble appeared only in the head lettuce varieties, the Grand Rapids and other crinkly leaved varieties not being attacked. There was rarely any sign of the disease until just as the plants began to head. The tips of the larger outermost leaves would die and often rot away."

The next year Jones (39) describes and illustrates this disease, stating that is is easily confused with the rot caused by Botrytis vulgaris. He says, "Even more destructive, however, was another rot which may be easily confused with Botrytis trouble, but which is an entirely different thing.

This trouble has been worse in the Experiment Station greenhouse on the head varieties, but has attacked all more or less seriously. As indicated in the drawing the disease appears first at the tips, or the edges of the inner or heart leaves. In the tender moist leaves at the heart, the trouble first shows itself as a watery decay at or just under the margin near the tip. This marginal portion often becoming limp. In leaves more exposed, the tissues blacken soon and become crisp as they die. The decay then passes backward, especially along the veins. This progress is generally rather slow and as the leaves are growing very fast at this stage, they become curled. Often a growth of the Botrytis appears upon these diseased leaves, hastening their decay, but this is a secondary attack and not the primary cause of the trouble." His illustration of the diseased plant indicated that the disease referred to is tip burn.

Rawson (39) says, "I have seen lots of this trouble about Boston. The lettuce is all right until a certain stage of its growth, then it begins to rot in the heart. Some varieties are much more susceptible than others. With proper handling I can keep it out. The trouble is not caused by the sun, nor is it due to high temperatures. I consider the whole trouble of this rot at the heart to be caused by the dry air in the house at the time it occurs and I am able to run my house so there is no trouble."

Galloway (24) mentions a disease of rop burn and makes the following statement regarding it. "Top burh is the direct result of the collapse and death of the cells composing the edge of the leaves, it is most likely to occur just as the plants begin to head and may be induced by a number of causes. The trouble is most likely to occur on a bright day following several days of cloudy wet weather. During cloudy weather in winter the air in the greenhouse is practically saturated and in consequence there is comparatively little transpiration on the part of the leaves. The cells, therefore, become extensively turgid and are probably weakened by the presence of organic acids. When the sun suddenly appears as it often does after a cloudy spell in winter there is an immediate rapid raise in the temperature and a diminuation in the amount of moisture in the air in the greenhouse. Under these conditions the plant rapidly gives off water and if the loss is greater than the roots can supply the tissues first wilt and then collapse and die."

Stone and Smith (76) report on the disease as follows: "A disease occurring on greenhouse lettuce, and characterized as 'top burn' came under our observation the past winter. The disease can readily be distinguished by the withering and subsequent turning back of the tip and margin of the outer leaves, the blackened area sometimes extending inward an inch or more from the margin. This feature greatly disfigures the plant and consequently affects its market value, but the real damage to the lettuce plant is never sufficient to destroy it. Microscopic examination of the blackened areas frequently show bacteria in the cells, but more often the damping fungus (Botrylis) is present, and can be readily observed with the naked eye. In this instance, however, neither of these forms of organisms has anything to do with the cause of the disease. They are simply accompanying, which are always ready to seize upon any abnormal condition in the plant which is especially favorable to them.

The disease is a physiological one and has its origin in the unfavorable surroundings of the plant, especially those connected with the transpiration and sunlight."

Kinney (41) states that, "The top burn is a physical injury which seems to be due to excessive heat. The edges of the inner leaves wilt first and if this continues long the cells collapse and die. This is most likely to occur during a period of high temperature or where the plants are too near the steam pipes. Too porous a soil may cause it. Leaf burn does not usually occur until the head begins to form and it is not necessarily followed by decay of the tissues below the injured cells."

Ramsey (68) says, "A sort of tip burn was the first indication of any trouble, and examination was immediately made for fungi but no trace of any micelium was found nor was there any indication of bacteria or pathogenic disease. The tip burn which as far as could be determined was caused by no disease either bacterical or fungal in nature and must have been due to some physiological cause or functional disorder."

Fulton (23) makes the following statement regarding the disease, "Tip burn is due to the breaking down and dying of tissue that was not well formed or that could not be maintained under changed conditions of growth. Too rapid growth resulting from high temperatures at nights and on dull days is the frequent cause."

Selby (74) thinks that it is due to unfavorable moisture relations. He says, "Tip burn of lettuce leaves if often brought to notice. Usually it is associated with unsatisfactory watering in the greenhouse or with extreme changes of summer weather. The remedy consists in the methods of watering employed."

Lutman (45) states that lettuce both in the greenhouse and in the open air shows a burning or scalding of the tips of the leaves, which is undoubtedly the result of too much light."

Watts (92) in his "Vegetable Forcing" discusses the disease as follows: "Sometimes the margins of the leaves wilt and die. This is a disease, the result of a physiological disturbance called tip burn, that may occur on bright clear days when the temperature is above seventy degrees, following a season of cloudy weather. With good management in the regulation of soil and atmospheric conditions tip burn is not likely to occur."

Bailey (2) in his "Forcing Book" says: "Leaf burn or tip burn is the result of bad sanitary conditions, being especially favored by a soil which holds too much water. Also by insufficient care in ventilation and watering in dull weather."

Bailey (3) in his text "The Principles of Vegetable Gardening" states, "A blackening of the leaf margins frequently evident only on the inner leaves, is characteristic of tip burn. Apparently this disease is not due to a casual organism but to unfavorable environmental conditions. There is some indication that an excess of nitrates and excessive applications of fertilizers in midsummer may increase the development of tip burn."

Reed and Rodgers (69) say that under California conditions, "Severe losses are sometimes occasioned by tip-burn, which is characterized by the blackening of the edges of the inner leaves and which under favorable conditions will result in a partial or total decay of the interior of the head. It is however often impossible to detect any external symptons of this condition. The disease is of a non-parasitic nature and the damage is usually done after the plants have started to head. It is most prevalent when bright hot weather follows a period of cloudy or rainy weather.

Considerable variation of infestation is found in the different classes of soil. Lettuce growing in a soil of high water-holding capacity shows less injury than that grown in soil with an insufficient supply of moisture. In the Imperial Valley the disease seems to be closely related to alkali conditions."

In discussing the disease Taft (83) says: "The usual cause of the trouble is growing the lettuce at too high a temperature, especially if the changes are sudden and extreme. A deficiency of water in the soil, especially if the air is hot and dry, will also lead to its appearance. In short, it may be attributed to anything that will cause the water to be given off from the tips of the leaves faster than it can be supplied to them. If the soil is light the roots will penetrate deeply and water will be supplied much more readily than if heavy.

Erwin (19) believes that, "Tip-burn is caused by too rapid loss of water from the leaves and results in the collapse and death of the marginal cells of the leaf. Under conditions of extreme heat and dry atmosphere the foliage moisture is carried off faster than it can be replaced by the root system and tip-burn results. That this injury is thus brought on is evidenced by the fact that plants shaded by trees are less affected. In this case the average soil moisture supply is less than in the open but the foliage is protected from excessive transpiration by sun and wind."

Thompson (85) says: "Tip-burn is not due to any parasitic organism, but to unfavorable conditions. Blackening of the margins of the leaves is characteristic of the disease and this may be found on the inner leaves. Tip-burn is often very serious when lettuce matures in very hot weather and seldom of much importance during cool weather of fall. There is evidence that an excess of potash (and possibly nitrogen) may increase the development of tip-burn. No control measures have been found."

Valleau (89) thinks the burning of the margins of the leaves is due to the accumulation of salts in this area. He has found tip-burned plants to have a root-rot. As a result of the rot the selective absorption of the root is broken down and soil salts are taken up by the plant and deposited at the extremities of the leaves.

Regarding the disease, he says: "The lettuce plants which develop the tip-burn, are in every case that we have examined, and we have examined hundreds of plants,-- have a considerable amount of root-rot. The root-rot is not at first evident, but if the plants are carefully removed from the soil by slowly washing, numerous rotting roots may be seen on the badly tip-burned plants. As a matter of fact we have not yet found a single lettuce plant, either under greenhouse conditions or field conditions, that attained any size which did not have quite severe root-rot. It is known that the selective absorption of dead roots is broken down and as a consequence any of the soil salts may be taken up by the plant, transferred out to the extremities of the leaves, and there we expect that it is deposited, and results either in the poisoning of the leaf tip or an actual clogging of the veins which results in wilting, and finally in the death of the leaf margins.

The organism which causes the disease we believe to be carried in the seed, as there was an organism in practically every seed examined."

## THEORIES AS TO THE CAUSE OF TIP-BURN

The foregoing review of the literature pretaining to this disease shows that there is much variance of opinion as to the primary cause of the trouble. The several theories as to the cause of tip-burn which have been advanced by different investigators may be briefly stated as follows:

(1) Some have thought that the trouble is due to an organism. The author has been unable to transmit the disease by inoculation of healthy tissue with material from diseased plants. Organisms have been found in the diseased tissue after signs of tip-burn had been present for several days. The opinion expressed by those working in this field is that the organisms were secondary infections and not the primary cause of the trouble. The tissue broken down by tip-burn is readily attacked by fungi. After tip-burn injury has been present for several days a number of decay organisms may be found in the injured tissue. This is especially true under conditions of high humidity.

Binkley (7) in making pathological studies of the disease was unable to find what he thought to be a casual organism. Many organisms, among which were Bacteria, Botrytis and Sclerotinia, we found both in tip-burned tissue and in the roots of diseased plants. He expresses the opinion that none of these are the primary cause of the trouble.

He says, "The evidence at hand indicates that such organisms are not directly important as causal agents. The fact that organism evidence was negative and that similar organisms could be isolated from tip-burned and non tip-burned plants indicates that organisms do not apparently take a direct part in causing tip-burn."

(2) Some hold the view expressed by Lutman (45) that the trouble is caused by too much light.

(3) Poor physical condition of the soil is thought by some to be a factor in causing tip-burn.

(4) Fertilizers (potash and nitrogen) in excessive quantities have been mentioned as possibly increasing the amount of disease.

(5) The poisoning of the plant cells by accumulation of salts at the margins of the leaves, as a result of the breaking down of the selective absorption of the root hairs by root rot, is held by Valleau (89) as the cause of the trouble.

(6) The theory most generally expressed as to the cause of tip-burn is that the injury is due to improper moisture and temperature relations.

## EVIDENCES THAT TIP-BURN OF LETTUCE IS A PHYSIOLOGICAL DISTURBANCE RESULTING FROM UNFAVORABLE MOISTURE AND TEMPERATURE RELATIONS

Observations as to the nature of tip-burn and the environmental conditions under which it occurs, coupled with the inability of those working on the disease from a pathological standpoint to isolate a casual organism leads to the conclusion that the trouble is of a physiological rather than a pathological nature. It appears that the marginal cells of the tender lettuce leaves are caused to break down by unfavorable growing conditions. Observations of the conditions under which tip-burn is most prevalent both in the greenhouse and under field conditions show that the trouble is always most severe when the environment is favorable for rapid transpiration and when the temperature raises above the optimum for proper development of head lettuce.

Attacks of tip-burn very frequently occur in the greenhouse if bright warm weather follows a period of cloudy weather with little sunlight and low temperature. During a period of cloudy weather the tissue of the plant becomes very turgid and watery resukting from a continued absorption of water with a low transpiration rate. The soft watery tissue formed during such a period of high humidity and dull sunlight is apt to suffer injury from excessive loss of water and high temperature if there is a sudden raise of temperature accompanied by bright sunlight. A condition of high temperature and low humidity is frequently reported as resulting in an attack of tip-burn.

The conclusions as to the cause of tip-burn expressed in the literature on the disease have been drawn very largely from observations of the disease under greenhouse conditions. Observations made by the author in Colorado lettuce fields during the seasons of 1923 and 1924 have lead to some conclusions as to the possible cause of the disease and the field conditions which are favorable for its development.

## Field Observations

High temperature at heading time is very certain to result in tip-burn especially if the plants are very succulent and watery.

Bright warm days following a period of cloudy rainy weather are favorable for tip-burn development.

The disease seldom occurs when the weather is cool and cloudy at heading time.

The observations made show pretty clearly that excessive use of irrigation water produces plants which are easily injured by tip-burn.

Light soils containing much sand and gravel, requiring frequent irrigations to keep the plants supplied with sufficient water are inducive to tip-burn.

Plants grown on sub-irrigated land seem to be resistant to tip-burn. Such land is usually high in soluble salts which may raise the concentration of the soil solution to a point which prevents the rapid intake of water. The concentrating of the soil solution by salts present in the soil may account for the difference in the effect of sub-irrigation where there is always an abundance of water in the soil and excessive irrigation on the occurence of tip-burn.

Loam soils high in organic matter having a clay sub-soil are ideal for lettuce and the percentage of tip-burn seems to be less than on land having a porous sub-soil.

High fertility accompanied by excess water excites rapid growth and succulent tissue readily attacked by tip-burn.

Attacks of tip-burn are much more severe on south than on north slopes.

Another condition which seems to influence the extent of tip-burn injury, and which also points to temperature and water loss as the cause, is the position of the leaves of the head. It is well known that leaves which have a flat surface exposed to the sunlight transpire more rapidly and are more subject to sunlight injury than leaves which are in a position with the edge toward the direct rays of the sun. Some plants have the power of changing the position of the leaves to lessen the extent of moisture loss. It has often been observed in lettuce fields that round heads which have the top leaves in a position exposing a large area to the direct rays of the sun, tip-burn more readily, and the extent of the injury greater than conical shaped heads which have their outer leaves in a more upright position, hence having less leaf surface exposed to direct sunlight.

## POSSIBLE CAUSES OF THE INJURY KNOWN AS TIP BURN

If the disease is a physiological disorder resulting from unfavorable moisture and temperature conditions the injury may result from:

(1) Desiccation beyond the limit of physiological endurance.

(2) Breaking down of the tissue due to the plant attaining a temperature above the maximum it can withstand.

(3) Accumulation of poisonous metabolic products.

### Desiccation

The atmospheric conditions in Colorado are especially favorable for rapid loss of water from plant tissue and it seems justifiable to assume that these climatic agents may be factors in causing tip burn. While tip burn occurs wherever head lettuce is grown it seems to be more than usually severe in the dry rare atmosphere of the mountain districts.

The rareness of the air at our high altitude increases the intensity of the light. The rate of transpiration has been found to vary directly in proportion to the light intensity, hency in a rarified atmosphere with little cloudy weather it is probable that transpiration is greatly accelerated.

Temperature has direct influence upon transpiration just as temperature increases the rate of evaporation. The transpiration rate is much higher at high than at low temperature. Pfeffer (66) says that the rate of transpiration must be different during and just after a change of temperature from what it becomes subsequently, for the air acquires the new temperature more rapidly than the plant and the raise of temperature will cause the saturated air within the plant to expand and will temporarily accelerate the escape of water vapor. Moreover a change in the temperature of the air influences the rate of transpiration much more markedly than does the absorption of water.

Warm winds have a very desiccating effect on plant tissue, in that the water vapor is removed as fast as it is formed. Any movement of the branches of a plant favor transpiration.

Substances dissolved in the soil solution influence the rate of transpiration. Concentrated solutions retard the rate of transpiration. The alkalinity or acidity of the soil solution have opposite effect upon the passage of water from the plant. Pfeffer (66) states that Sachs and Burgerstein have shown that transpiration is decreased by the addition of small quantities of acids such as tartaric, oxalic, nitric, and carbonic to the soil, while the rate of transpiration is increased by alkalies such as potash, soda and ammonia, Colorado soils, like all arid lands, contain more or less soluble salts which undoubtedly influence the transpiration of plants and may be a factor in the presence of tip burn. Reed and Rogers (69) state that, "In the Imperial Valley the disease is closely related to alkali conditions."

## Temperature

It is characteristic of all vital processes that their maintainance is possible only within a comparatively narrow range and temperature changes therefore result in marked responses in the activity of the protoplasm. The range of the active life of higher plants lies between 0°C. and 50° C. The limits vary greatly with the different species. The optimum temperature for crop plants varies considerably. A vegetable plant like the tomato thrives at a much higher temperature and has a very much higher optimum temperature than cool season crops like peas and lettuce. On the other extreme temperature which would cause death to a tomato plant by freezing would not seriously effect a lettuce plant.

The higher plants are unlike the higher animal organisms which maintain a constant body temperature. The temperature of most plants follows very closely the temperature of their environment.

Pfeffer (66) states that, "Green and other colored leaves may absorb from 50% to 90% of the sun's rays falling upon them." A small part of this absorbed energy is used by the plant in the process of photosynthesis, but the larger part of it is converted into heat. If it were not for the cooling effect of transpiration the temperature of the plant would often raise above the physiological limit of endurance.

A raise of temperature above the maximum of a plant may be due to the direct rays of the sun if the atmospheric temperature be near the critical point, or due to the heat of respiration if the conditions are favorable for a rapid rate of respiration and the transpiration low.

The fact that tip burn is quite often found within heads which have no external evidence of the disease leads us to believe that the injury may be due to tissue disintegration resulting from temperature within the head becoming very high from the heat of respiration.

Askenasy (1) reports having observed a temperature of 52° C. when the thermometer bult was inserted between the rosetted leaves of Sempervivum alpinum when the shade temperature was 28.1° C. The inner leaves of Gentiana cruciata and Anbrietia deltoidia showed a temperature of 35° C.

It does not seem reasonable that the injury could be due to desiccation when the injured part is not exposed and the rate of water loss comparatively low. In a well developed lettuce head the outer leaves of the head are very tightly drawn over the top which undoubtedly greatly reduces the rate of transpiration from the under leaves. Since the plant is growing very rapidly at the time of heading, bright sunlight and high atmosperic temperature coupled with the heat of respiration may raise the temperature of the inner portion of the head to a point which results in injury to the protoplasm.

There will be shown later some relations which exist between the external conditions which cause water loss and high temperature and the extent of tip burn injury.

Internal Poisoning

Balls (95) in studying temperature in relation to growth reports that inhibition of growth at high temperature is very probably due to accumulation of injurious metabolic products within the cells. It is his opinion that these injurious products are formed at low temperature also but are rapidly decomposed while at high temperature the poisonous substances are produced more rapidly and injury results.

The fact that tip burn generally occurs at high temperature it seems reasonable to think that the injury may result from the accumulation of some poisonous metabolic product.

## THE RELATION OF WATER CONTENT OF PLANTS TO THEIR RESISTANCE TO DESICCATION AND INJURY BY HIGH TEMPERATURE

The higher the ratio of water to dry matter in plant material the more tender the tissue and the more susceptible it is to injury by external forces.

All of the environmental agents which influence transpiration have a more marked effect on tender plant tissue having a high moisture percentage than on hardened plants of high dry matter content. The percentage of dry matter in zerophytic plants is generally much higher than in plants growing under moist conditions.

Plants growing under conditions which produce plant tissue of high water content are much more easily injured by raise or fall of temperature or sudden change in water supply or light intensity than the same species when grown under conditions which produce plant tissue of high dry matter content.

Sinnott (94) in his Botany Principles and Problems says, "In general with any particular species the ability to withstand high or low temperature is correlated with the amount of water in the cells, particularly in the protoplasm itself. Where water is abundant the resistance is low; where it is scarce resistance is high."

Coulter, Barnes & Cooles (14) states that air dry seed withstand the lowest temperature yet tried, that of liquid hydrogen (250° C.) and germinated freely when planted. The same seed soaked until swollen were killed at much lower temperature.

# FACTORS WHICH INFLUENCE THE WATER HOLDING CAPACITY OF THE PLANT CELL

The explanation of the variations in the amount of water absorbed and retained by plants are generally ascribed by investigators to the difference in the osmotic pressure of the cell sap and the variability of the plant colloids in their affinity for water. In other words the power of the plant cell to hold onto its water content is primarily due to the concentration of the sap and the force of imbibition.

## Sap Concentration

It is a matter of common observation that plants grown under shade, or in a moist warm greenhouse under conditions which make the plant tissue very tender and watery with a sap of low concentration, loose their moisture and wilt down much more rapidly when removed from their protected surroundings, than plants grown under conditions which produce woody tissue and cell sap of high concentration. The freezing of plant tissue, which results in the withdrawal of water from the plant cell, has been found to injure plants of low sap concentration more readily than plants having a cell sap of greater density.

Chandler (11) in working with sap concentration in relation to resistance to desiccation by low temperature found that the lowering of the cell sap concentration by shading, lowered the power of resistance to desiccation, while an increase in the sap concentration resulted in greater resistance to water loss at low temperature. Rosa (71) in investigating the hardening process in vegetable plants found that any condition which increased the osmotic concentration of the cell sap resulted in an increase in the water retaining power of the plant cell.

Ohlweiler (63) in his investigation on The Relation Between The Density of Cell Sap And The Freezing Point Of Leaves, found, "That extreme differences of sap density in general are accompanied by a corresponding different in their resistance to freezing and that in plants of the same genus or variety of the same species differences in the sap density correspond to differences in their resistance to freezing."

## Imbibition

Imbibition or the power of any substance to absorb and hold a large amount of moisture is probably the most important factor influencing the water retaining power of the plant cell. Some organic substances like gelatin and agar have the property of imbibing and holding large quantities of water. A certain amount of these gelatinous substances are found in the tissues of practically all plants. It is believed that the imbibitional properties of these gelatinous plant materials are the important factor in the water holding power of plant cells. Water holding capacity of some plant cells is enormous. Pfeffer (66) reports that Nageli found the gelatinous envelopes of Nostocaceae to contain 200 parts water to 1 part solid material. Pfeffer states that, "The power of swelling, as well as the expansive force which the swelling may exert, rapidly diminishes as the amount of the imbibed water increases." In other words the fofce of imbibition of the plant cell increases with the decrease of the water content.

Wiegand (93) comes to the conclusion that, as the loss of water from the plant cell progresses, the osmotic concentration of the cell sap increased, and the force of imbibtion is magnified so that the water remaining in the cell is given up less readily than when the cell is very turgid.

## CONDITIONS WHICH INCREASE THE CELL SAP CONCENTRATION AND THE FORCE OF INBIBITION

If the conclusions of Pfeffer, Weigand, Chandler, Oheweiler and Rosa, that the water holding capacity of the plant cell is due to the density of the cell sap and the force of imbibition, then it is apparent that any condition which increases the sap density and the imbibitional forces will result in greater water retaining power. Sap concentration and the force of imbibition are so closely correlated and affected by similar conditions, that it is difficult to determine the role each may play in the water retaining power of the cell. Temperature, water supply, light and soil solution influence the forces which regulate the water holding capacity.

Rosa (71) states that the sap density or osmotic concentration can be increased by:

(1) Decreasing the water content.

(2) Increasing the amount of osmotically active sap solutes.

(3) Decreasing the amount of free water or conversely, by increasing the amount of unfree water held by colloidal absorption.

The imbibitional power of the plant cell he states can be increased by:

(1) Decreasing the total water content.

(2) Increasing the amount of hydrophylous colloids in the protoplasm.

(3) Increasing the water retaining power of such colloids by slight increase in acidity.

## THE EFFECT OF WATER ON THE CARBOHYDRATE CONTENT OF THE PLANT CELL

Spoehr (77) in working with Cacti found that the carbohydrate content of the cactus varied greatly with changes in the water supply. He found that a shortage of water supply reduced the water content of the cell which resulted in a decrease in the amount of monosaccharide and an increase in the total polysaccharide present in the cell. He found a marked increase in the pentosan content of the cell under conditions of low water supply.

McDougal and Spoehr (55) found by a long series of sugar analysis of the cacti made in all stages of development of the plant, at all seasons and of material subjected to various experimental conditions, that, prominent among the various transformations brought about in plants subjected to zerophytic conditions to be a change of polysaccharides into pentosans or mucilages; a conversion of carbohydrates of little hydration capacity into others which have a large coefficient of imbibition.

The conversion of polysaccharides into pentosans which take up and hold large proportions of water is only one of the possible results of zerophytic conditions. Under some conditions a low water content causes the formation of anhydrides of which cellulose is an example. This conditions causes the plant structure to become rigid.

McDougal and Spoehr came to the conclusion that succulence results from the conversion of polysaccharides into pentosans or mucilages and that zerophytism results from the conversion of polysaccharides into anhydrides, both conditions being induced by a depleted or lessened water supply. McDougal, Richards, and Spoehr (56) found in their investigations on succulence in plants that reduced cell water content converted some of the hexose polysaccharide of low imbitional capacity into pentosans of high hydration capacity. They have the following to say on succulence in plants, "The water relation of active tissues show the behavior of a biocolloid consisting largely of pentosans of which agar or plant mucilages would be an example, a small portion of protein or protein derivatives and some salts and free acids. It is to these features therefore that one would naturally turn for the factors which might increase the water holding capacity of cell or organ and in so doing the penotsans would claim attention first. Any action or condition which brings about a noticable increase in the proportion in the cell would have most important consequences. Such increase does result fмom a depletion of the water of the cell; for the polysaccharides under such conditions are reduced to pentosans and the reduction of the water content of the cell results in the conversion of the polysaccharides, which do not show marked imbibitions to pentosans, which take the form of an elastic gel with an enormous capacity for expansion, particularly when mixed with nitrogenous material.

Briefly stated, whenever the water content of a cell becomes low some of the hexose polysaccharides which have a low imbibitional capacity are converted into pentosans, which have a high hydration capacity, the action having the force of a regulatory adjustment and as the change is irreversible the pentosans are accompanied by a permanent succulence, with all the implied alternations in the metabolism including a very striking change in the type or respiration or of transformations in the carbohydrates. Whatever casual value is attributed to the action of soil salts or of arid conditions will rest upon their part in the conversion of polysaccharides to pentosans."

## STATEMENT OF THE PROBLEM

Previous investigations of the cause of tip burn of lettuce indicate that the trouble is not the result of a causal organism. The evidence at hand indicates that the disease is closely related to environmental conditions; that improper temperature and moisture relations play an important part in the extent of tip burn; and that conditions which tend to increase the loss of water from the leaves is likely to be followed by an attack of tip burn.

Some varieties of lettuce are much less susceptible to tip burn injury than others. Plants of the same variety show marked difference in their resistance to the disease. The question arises as to why some plants are more resistant to this trouble than others?

Since tip burn is so closely correlated with moisture and temperature relations the work of McDougal, Richards, Spoehr and Rosa on the ability of plants to withstand desiccation suggests that the apparent resistance of certain plants to tip burn may be due to their higher water retaining capacity.

The investigations of these workers show that there is a close relationship between the carbohydrate content of plants and their water retaining capacity. Their investigations show that plants which are resistant to dessication are higher in pentosan content than tender plants, which give up their moisture content readily.

This investigation was originally begun with the object of determining whether any correlation exists between pentosan content and tip burn, but has been extended to include other factors which may play a part in this disease, such as sugars, dry matter, acidity and environmental conditions.

# CARBOHYDRATE ANALYSIS OF LETTUCE PLANTS

The work of McDougal, Richards, Spoehr, and Rosa on the relation of mucilaginous substances and especially the pentosans to the water holding capacity of plant cells suggested the theory that the pentosan content of lettuce plants might have a relation to tip burn. That desert plants, which are subjected to very desiccating climatic conditions, are high in pentosan and that vegetable plants high in pentosan are more resistant to water loss at low temperature than plants low in pantosan hinted that the apparent resistance of some lettuce plants to the disease of tip burn might be due to their higher pentosan content.

As will be shown later, under the discussion of The Relation of the Water Content of Lettuce Plants to their Resistance to Tip Burn, plants grown with a limited water supply were injured much less by the disease than plants which received normal or excessive applications of water. According to the results obtained by the previously mentioned investigators the limiting of the water supply of plants magnifies their water retaining capacity by increasing the pentosan content. It was thought that carbohydrate analysis of tip burned and healthy lettuce plants might reveal some relationship between the pentosan content, the water holding capacity, and the extent of tip burn injury. To test out this supposition numerous lettuce plants both diseased and healthy were analyzed for the various carbohydrates as described later.

# MATERIALS AND METHODS

## Determination of Dry Weight

Since plants of tender watery tissue appear to be much more susceptable to tip burn injury than plants containing less water, it was decided to make dry weight determinations on a large number of plants both tip burned and healthy. About 15 grams of finely cut green material was placed in a tared dish and weighed. The dish containing material was then placed in a vacuum oven and dried for 12 hours at 70° C. After cooling in a desiccator the dish and dry material was weighed and the dry weight of the material determined.

## Preparation of Dry Material

The lettuce plants used for sugar analysis were all grown in the greenhouse under the same conditions except for the amount of water applied during the growing period. All material was handled as nearly alike as possible. The plants used were all cut at approximately the same time of day--about 9 o'clock in the morning. The entire plant was removed by cutting close to the soil. The plants were taken immediately to the laboratory. Any dirt or injured outer leaves were removed and the remainder of the head dried for analysis. A microtone razor was used to cut the material into small pieces without crushing the tissue any more than was necessary. After cutting into small sections the material was placed on a dish and dried for twelve hours in a vacuum oven at 70° C. After thoroughly drying the material it was ground in a mortar and placed in labelled bottles until analyzed.

## Preparation of Material for Analysis

The simpler sugars were separated from the condensed polysaccharides by extracting one portion with distilled water and by hydrolyzing another portion with 2% hydrochloric acid. For the water extraction one gram of over-dried material was placed on a filter paper in a funnel and washed six times with distilled water at room temperature. The filtrate after being clarified with alumina cream was made up to 250 cc. and aliquot portions used for sugar determinations as described later. Reducing sugars present were monosaccharide hexoses and pentoses.

100 cc. of the solution was hydrolized with 5 cc. of concentrated hydrochloric acid for exactly ten minutes at 70° C. After cooling the solution was neutralized with sodium hydroxide and made up to 200 cc. volume. The solution was then tested for sugars. The sugars present represented the monosaccharide hexoses and pentoses and invert sugar.

100 cc. of the solution of the hydrolyzed solution was then fermented with a suspension of bakers yeast to remove all hexose sugar. A suspension of carefully washed yeast was added to the solution in a 250 cc. Erlenmeyer flask and stoppered with cotton. The flask containing the solution was placed in a temperature of 30° C. to 35° C. for twenty-four hours. After fermentation the solution was filtered, clarified with alumina cream and boiled for ten minutes to remove the

alcohol formed in the fermentation. The solution was then made to volume and aliquot portions used for sugar determination. The sugars present were monosaccharide pentoses.

For estimation of the polysacchrides one gram of the powdered material was hydrolyzed for three hours with 60 cc. of 2% hydrochloric acid under a reflex condenser. The material was cooled, neutralized, clarified with alumina cream and filtered. The filtrate was made to volume and the sugars determined. This determination represented the total sugars.

A portion of the solution was transferred to a flask and a suspension of years added. After fermentation the solution was filtered, clarified, made to volume and the sugars determined. The sugars present represented the total pentoses and pentosans.

Calculation of Sugar Percentages From Analytical Data

The percentage of the various sugars were calculated from the following table used by Spoehr (77) in his determinations of sugar in Cacti.

In the Solution of the Acid Hydrolysis:

A. Total sugars of the hydrolized material, i.e., the monosaccharides as well as the hydrolyzed polysacchrides.

B. Fermented residue of A, or total pentose sugars, including monosacchride pentoses and pentosans.

In the Water Extraction:

C. Monosaccharide hexose and pentose sugars.

D. Hydrolyzed product of C or inverted disaccharides, original hexose, and pentose. There is no disaccharide pentoses present.

E. The fermented residue of D or monosaccharide pentose

Then the Copper Values of the Various Sugars Are:

A. Total sugars.

A-D. Total polysaccharides.

D-E. Disaccharides plus hexoses.

A-B. Total hexose sugars.

(A-B)-(D-E). Hexose polysaccharides.

C. Monosaccharides.

D-C. Disaccharides.

C-E. Hexoses.

B. Total pentoses.

E. Monosaccharide pentoses.

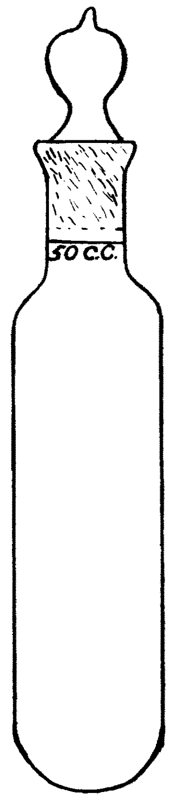
B-E. Pentosan.

## Analysis of the Sugar Solution

On account of limited time and apparatus it was decided to use the method of Peters (64) as described by Spoehr (77) for sugar analysis, which he found to be very accurate as well as rapid.

Special designed centrifuge tubes as illustrated were used. These tubes were made by Mr. Paul Anders of Urbana, Illinois.

The Soxlet modification of Fehling's solution was used. The copper sulphate solution was prepared by dissolving 69.28




grams of copper sulphate in a quantity of distilled water and made to 1 liter.

The alkaline tartrate solution was made by dissolving 346 grams of sodium potassium tartrate and 100 grams of sodium hydroxide in distilled water and the volume made up to 1 liter.

Copper Reduction

The copper sulphate Fehling's solution was carefully measured by use of a 50 cc. burette graduated to .1 cc. The tartrate solution was measured by a graduated pipette. These two solutions were run directly into the centrifuge tubes. The sugar solutions were run in from a graduated burette, the tubes stoppered and the contents mixed thoroughly by shaking. The tubes containing the Fehling's and sugar solutions were placed in a boiling water bath for exactly six minutes. After removal from the water bath the tubes were cooled as rapidly as possible by immersion in cold water. When cooled to about 15° C. the tubes were filled to the graduation mark wish recently boiled distilled water, stoppered, and the contents thoroughly mixed by shaking. The tubes were then placed in a centrifuge machine and rotated for five minutes at 2000 r.p.m.

## Copper Determination

The reduced copper was determined by the iodine method as outlined by Peters (65). Aliquot portions of 10 cc. of the supernatant solution in the tubes after centrifuging were transferred by means of a pipette into 125 cc. Erlenmeyer flasks. The tests were run in duplicate. The solution was acidified with 1 cc. of concentrated sulphuric acid. The temperature of the flasks was lowered to between 15° C. and 20° C. To each flask was added 5 cc. of a saturated solution of potassium iodide. The iodine liberated was titrated immediately with N/20 solution of sodium thiosulphate. A fresh preparation of starch solution, made by boiling 1 gram of soluble starch (Baker's) in 100 cc. of water, was used as an indicator being added near the end of the titration. From the amount of thiosulphate required for the titration the copper of sugar values were determined. For the pentosan percentage the glucose value was multiplied by .85. All other sugars are given the glucose value.

## Standardization of Solutions

Only C. P. chemicals were used throughout the work. The sugar value of the copper sulphate solution was determined by using a standard solution of dextrose. The copper value of the sodium thiosulphate solution was obtained by a blank determination. The entire procedure, heating, centrifuging, etc., was gone through without the addition of any sugar which also adjusted any self-reduction of the Fehling's solution.

## RESULTS AND DISCUSSION

The carbohydrate analysis of healthy and tip burned plants show some marked differences in the percentage of the different sugars. The total sugar content of the healthy plants was found to be considerably higher than the total sugar content of tip burned plants, using the average of six analyses in each case. The ratio of the total polysaccharide sugars to the total sugars was found to be higher in healthy than in diseased plants and the ratio of the total monosaccharide sugars to the total sugars was found to be highest in diseased plants.

The pentosan analysis gave reverse results from what was expected, if the water holding power of the cell is a factor in the resistance of some lettuce plants to the injury. The pentosan content of tip burned plants was found to be higher than the pentosan content of healthy plants. It was also very noticeable in making the analysis that the acid hydrolized material from diseased plants used in the total sugar determinations was more difficult to filter than the same material from healthy plants. This would seem to indicate that there was some colloidal material present in tip burned plants which is not present in healthy plants or at least not in as great a quantity. This would also indicate the ability of the plant material to absorb and hold water is greater in diseased plants than in healthy plants.

These analyses show that the pentosan content of lettuce plants is very low. They would seem to indicate that from a water holding standpoint the pentosans do not play an important role in the occurrence of tip burn. It is doubtful if the pentosans are present in lettuce plants in large enough quantities to have much influence on the water holding capacity of the tissues. Tables IV and V show the results obtained in the analyses.

The failure to find a significant correlation between the pentosan content and the disease led to further investigations regarding the effect of environmental conditions on the occurrence of the trouble.

Table IV — Carbohydrate Analysis of Healthy and Tip Burned Lettuce Plants

| No. | Condition of Plant | Percentage of the Dry Weight: Total Sugars | Percentage of the Dry Weight: Mono-saccharide Sugars | Percentage of the Dry Weight: Disaccharide Sugars | Percentage of the Dry Weight: Poly-saccharide Sugars | Percentage of the Green Weight: Total Sugars | Percentage of the Green Weight: Mono-saccharide Sugars | Percentage of the Green Weight: Disaccharide Sugars | Percentage of the Green Weight: Poly-saccharide Sugars | % of Dry Matter |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | Healthy | 13.400 | 6.716 | 3.626 | 3.068 | 1.015 | .509 | .274 | .232 | 7.58 |
| 16 | " | 15.823 | 7.491 | 5.425 | 2.907 | 1.350 | .630 | .462 | .248 | 8.53 |
| 18 | " | 19.116 | 6.858 | 8.124 | 4.134 | 1.556 | .559 | .662 | .336 | 8.15 |
| 20 | " | 23.672 | 12.400 | 5.600 | 7.672 | 2.151 | 1.039 | .469 | .643 | 8.38 |
| 22 | " | 23.875 | 11.045 | 7.135 | 5.695 | 1.836 | .865 | .548 | .438 | 7.68 |
| 31 | " | 22.474 | 11.120 | 6.755 | 4.600 | 1.689 | .836 | .508 | .346 | 7.50 |
| | AVERAGE | 20.060 | 9.271 | 6.111 | 4.679 | 1.599 | .739 | .4[illegible]7 | .374 | 7.97 |
| | | | | | | | | | | |
| 1 | Tip Burned | 18.390 | 8.331 | 7.169 | 2.890 | .943 | .427 | .367 | .147 | 5.11 |
| 4 | " " | 10.591 | 4.650 | 3.610 | 2.331 | .500 | .214 | .170 | .110 | 4.72 |
| 17 | " " | 15.500 | 9.300 | 3.100 | 3.100 | .852 | .511 | .170 | .115 | 5.50 |
| 21 | " " | 20.344 | 13.691 | 4.275 | 2.378 | .912 | .610 | .193 | .107 | 4.53 |
| 26 | " " | 10.591 | 4.650 | 3.610 | 2.331 | .500 | .214 | .170 | .110 | 4.72 |
| 32 | " " | 10.981 | 4.130 | 4.370 | 2.481 | .612 | .230 | .243 | .138 | 5.58 |
| | AVERAGE | 14.222 | 7.269 | 4.292 | 2.49[illegible] | .731 | .376 | .212 | .120 | 5.22 |

Table V. - Pentosan Content of Lettuce Plants

| No. | Condition of Plant | Date Sampled | Percentage of Dry Weight | Percentage of Green Weight | Percentage of Dry Matter |
|---|---|---|---|---|---|
| 19 | Healthy | 3/17/25 | 2.250 | .164 | 7.30 |
| 22 | " | 3/9/25 | 1.921 | .147 | 7.68 |
| 20 | " | 3/10/25 | .824 | .069 | 8.38 |
| 16 | " | 3/11/25 | .878 | .075 | 8.53 |
| 6 | " | 3/12/25 | .742 | .041 | 7.58 |
| 18 | " | 3/13/25 | .603 | .048 | 8.15 |
| 15 | " | 3/14/25 | 1.700 | .110 | 6.50 |
| 10 | " | 3/14/25 | .881 | .088 | 10.00 |
| 13 | " | 3/14/25 | .768 | .089 | 11.62 |
| 5 | " | 3/17/25 | .439 | .032 | 7.35 |
| 35 | " | 3/17/25 | 1.097 | .082 | 7.50 |
| 36 | " | 3/31/25 | .714 | .041 | 5.77 |
| Average for Healthy Plants | | | 1.066 | .082 | 8.03 |
| 26 | Tip Burned | 3/10/25 | 2.250 | .106 | 4.72 |
| 21 | " " | 3/13/25 | .933 | .042 | 4.53 |
| 1 | " " | 3/14/25 | 2.599 | .132 | 5.11 |
| 38 | " " | 3/17/25 | 1.100 | .052 | 4.73 |
| 37 | " " | 3/14/25 | 1.097 | .053 | 4.80 |
| 39 | " " | 3/25/25 | 1.594 | .097 | 6.05 |
| 40 | " " | 3/25/25 | 1.921 | .094 | 4.95 |
| Average for Tip Burned Plants | | | 1.639 | .086 | 4.98 |

# THE RELATION OF THE WATER CONTENT OF LETTUCE PLANTS TO THEIR SUSCEPTIBILITY TO TIP BURN

If the supposition that tip burn of lettuce is a physiological trouble resulting from the loss of water from the marginal cells of the leaves due to improper temperature and moisture relations is true, any change induced in the lettuce plant which results in greater water retaining power should increase the plant's resistance to tip burn.

According to the investigations of McDougal, Richards, Spoehr, Chandler and Rosa a gradual reduction of the water content of the cell induces chemical changes in the protoplasm which increases the water retaining power of the cell making it more resistant to desiccation.

If the reduction of the water content of the plant cell results in chemical changes which increases the water retaining capacity; and if tip burn is due to water loss it was thought that a minimum water supply might result in plants resistant to tip burn.

## Irrigation

To test out this supposition an experiment was conducted under greenhouse conditions in which plants were grown under various conditions of water supply. One hundred and forty-four lettuce plants of the variety New York were used in the experiment. The plants were started in flats November 20, and transplanted into the bed December 15. From December 15, to January 11, all plants were given normal irrigation.

Treatments

A. After January 11, 36 of the 144 plants were grown dry. These plants were given no water until March 15. At this time a light application of water was given to them and no more water was applied until March 25. After this date a minimum of water was used to the end of the experiment.

B. After January 11, the remaining 108 plants were grown with a little more than what was thought to be normal irrigation, until February 14.

C. From February 14, to the end of the experiment 20 of the 108 plants in (B) were grown under moisture conditions quite in excess of the normal requirements.

D. The remaining 88 of the 108 plants in (B) were grown without water after February 14, until the plants showed signs of wilting. From this time on the soil was kept just moist enough to prevent wilting.

Results

A. Of the 36 plants all were free of tip burn until after the irrigation on March 15. Two plants showed slight tip burn injury 8 days after the irrigation. By this time the plants were nearing maturity and heads were forming. Of the 36 plants 6 failed to produce heads. The other 31 plants produced heads varying considerably in size. No more cases of burning appeared among these plants than the two previously mentioned.

B. By February 14, all but 9 of the 108 plants in this group grown with slightly more than normal irrigation showed injury in varying degrees--some very badly injured and others very slightly. One end of the bed was somewhat shaded and the plants in this area were the most severely injured by the burn.

C. All of the plants grown continuously under conditions of excess moisture became badly affected by the disease. Every head in the lot was so badly injured as to be worthless. These plants were all very susceptible to attacks of fungus and most of them became badly infested with Botrytis.

D. The 9 plants in (B) which were free of injury on February 14, were included in this series. The remainder of the plants in the group showed some burning. The withholding of water after February 14, checked the development of the disease to some extent. 83 of the 88 plants developed fairly good heads.

The above data shows very clearly that a minimum of water greatly reduces the susceptibility of lettuce plants to tip burn, while an excess of water produces plants readily injured by the disease.

A similar experiment was conducted during the winter of 1924-25 with the same results. Healthy plants could be caused to tip burn within a few days by excessive irrigation if the temperature was above 70° C.

The following table gives the results of the 1923 experiment in tabulated form.

Table VI - Results of 1923 Experiment

| Group | No. Plants | Treatment | No. Tip-Burned Plants | No. heads formed |
|---|---|---|---|---|
| A | 36 | Dry | 2 | 31 |
| C | 20 | Very wet | 20 | 19 |
| D | 88 | Slightly less than normal | 81 | 83 |

## THE WATER CONTENT OF LETTUCE TISSUE

The fact that lettuce plants receiving excessive amount of water were most severely injured by tip burn suggested that dry weight determinations showing the amount of water present in the tissues of healthy and tip burned plants, might show more clearly the relation which existed between the water content of the tissue and the extent of tip burn.

For these determinations, lettuce plants of the variety Grand Rapids (a non-heading type) were used as it was possible to select plants of this variety which had grown under the same conditions, especially as regarded the amount of water received. In order to have growing conditions as near uniform as possible healthy and tip burned plants were selected in groups of two, one healthy and one tip burned which had grown side by side in the bed.

The following table shows the results of these determinations.

Table VII

| No. | Condition of Plant | Date | % Dry Matter |
|---|---|---|---|
| H | Healthy | 2/19/25 | 5.77 |
| G | Tip Burned | 2/19/25 | 4.95 |
| I | Healthy | 2/21/25 | 5.73 |
| J | Tip Burned | 2/21/25 | 5.16 |
| K | Healthy | 2/25/25 | 5.53 |
| L | Tip Burned | 2/25/25 | 4.67 |
| O | Healthy | 2/27/25 | 5.03 |
| P | Tip Burned | 2/27/25 | 4.51 |
| Ave. | Dry Wt. of Healthy Plants | | 5.51 |
| " | " " " Tip Burned " | | 4.82 |

In all cases the water content of tip burned plants was greater than in healthy plants. The relation of the water content of the plant and the extent of tip burn was more marked among plants subjected to different conditions of water supply. In no case out of some fifty dry weights determined was the percentage of dry matter greater in tip burned than in healthy plants which had been grown under the same conditions except for the amount of water supplied.

It was found that the dry weight of the outer leaves was somewhat higher in some plants than the dry weight of the inner leaves. This was especially true of the head variety New York after the heads began to form. In order to reduce the error which might enter the determinations of the dry weight of healthy and tip burned plants through variation in the water content of the inner and outer leaves, the entire head was cut into small sections and the mass mixed as thoroughly as possible without too much exposure to water loss through evaporation during the operation. The determinations were made in triplicate and the average of the three used as the percentage of dry matter for the plant.

## SAP ACIDITY AND TIP BURN

The discovery that the water content of tip burned plants was higher than in healthy plants even when grown under the same conditions presented the question as to why these plants took in more water from the soil than the healthy plants. It was concluded that some condition existed within the plant which caused it to absorb an excessive amount of water.

Daschnowski (15) found that different acids and alkalies increased the water absorbing and water retaining power of certain seeds. He says, "Seeds of Phaselos multiflorus swell more and retain greater quantities of water in a solution of any acid than in distilled water. The amount of water that seeds absorb and retain in an acid solution is not dependent on the concentration of the acid. A maximum is attained above which a further increase in the acid does not lead to a greater retention of water but to a diminishing one." He concludes that the water absorbing and water retaining power of the plant cell in acid solutions is due to chemical changes induced in the cell.

McDougal and Spoehr (53) found that the amino acids glycocoll, alanine, phenylalanine, asparagin and histidine accelerate hydration in biocolloids.

Upson and Calvin (88) show that wheat gluten has greater swelling capacity in weak acid solutions.

The results of these investigations suggest that the excess of water in plants attacked by tip burn may be due to the formation of some organic acid which increases the water absorbing power of the plant.

A few hydrogen ion concentration determinations were made using the colormetric method. A slightly higher acidity was found for the healthy plants. The average of four determinations was used for the pH of healthy plants and the average of three determinations for the pH of the tip-burned plants.

The following table shows the results of the hydrogen ion determinations.

Table VIII - pH of Healthy and Diseased Plants

| Number | Condition of Plant | pH | |
|---|---|---|---|
| K | Healthy | 5.60 | |
| 16 | " | 5.24 | |
| 30 | " | 5.60 | |
| 13 | " | 5.35 | |
| | AVERAGE | | 5.44 |
| L | Tip Burned | 5.51 | |
| U | " | 5.35 | |
| T | " | 5.95 | |
| | AVERAGE | | 5.60 |

The small number of determinations made are insufficient to give satisfactory data for drawing conclusions. The inconsistencies in these determinations seems to indicate that acidity is not an important factor. Of the three acid determinations made for diseased plants one is the lowest and one next to the highest of all the determinations made. If the pH of tip burned plants had been consistently higher than the pH of the healthy plants the data would be more conclusive.

## THE RELATION OF SOIL NUTRIENTS TO TIP BURN

Thompson (85) and Bailey (3) state that there is evidence that an excess of potash and possibly nitrogen may increase the amount of tip burn.

Data as to the influence of fertilizers on tip burn was kept on the fertilizer experimental plots at Buena Vista during the season of 1923. Table IX shows the results recorded.

Table IX - Results of Fertilizer Experiment 1923

| Plot | Treatment | Number Plants | Tip Burned Plants | % Tip Burned Plants |
|---|---|---|---|---|
| 4 | Check | 2117 | 407 | 19.2 |
| 5 | Phosphate, 450 lb. per A. | 2080 | 640 | 35.5 |
| 6 | Phosphate, 450 lb. per A. of each Sulphate of Ammonia | 1760 | 760 | 43.0 |
| 7 | Manure 2 T. per A. | 1589 | 223 | 14.0 |
| 8 | Sulphate of Ammonia 550 lb. per A. | 1820 | 203 | 11.0 |
| 9 | Swifts 3-10-7 550 lb. per A. | 1751 | 267 | 15.2 |
| 10 | Swifts 4-7-6 550 lb. per A. | 1862 | 276 | 14.8 |

Contrary to expectations the phosphate plots showed the greatest number of tip burned plants. It was expected that the percentage of tip burned plants might be greatest on the sulphate of ammonia plot since nitrogen had been noticed to increase the amount of tip burn.

From the table it will be seen that the percentage of tip burn was less than one third as great on the sulphate of ammonia plot as on the phosphate plots. The plots treated with well decomposed manure and complete fertilizers showed about the same extent of injury as was found on the sulphate of ammonia plot.

The fertilizer work was continued during the season 1924 on the College Farm at Avon and it was intended that the date on tip burn injury be continued. After the lettuce crop on the fertilizer plots was well along it was discovered that applications of manure on part of the plots during previous seasons had upset the fertilizer work and no data on tip burn was kept.

The fact that only one season's figures as to the influence of fertilizers on tip burn are available it is impossible to draw any satisfactory conclusions from the data even though the plots were large and a large number of plants were examined.

The general conclusions drawn from observations made during the two seasons of fertilizer work are that fertilizers affect the amount of tip burn to the extent that they excite rapid succulent growth. During both seasons it was noticed that wherever the fertilizer applied resulted in very rapid growth of the plants, the percentage of tip burn was greater than where the growth was not so rapid and the tissue not so watery.

## TRANSPIRATION AND TIP BURN

Critical observation of plants during the progress of the experiment gave evidence that the dying of the margins of the lettuce leaves was not due to loss of water from the leaves as was at first thought. A transpiration experiment conducted with ten plants selected in various parts of the bed indicated that the injury was not due to water loss. Ten healthy plants of the variety New York, just beginning to head, were placed under large bell jars to increase the humidity of the air. The bell jars used had an opening in the top which allowed circulation of air. The plants grew for about two weeks under the jars without very noticeable injury from being so treated. Six of the ten plants grown under the jars developed typical tip burn injury showing very clearly that loss of water from the cells is not the primary cause of tip burn. The air under the jars was very humid so the atmosphere could have no drying effect on the plant tissue.

The diseased areas on the plants grown under the jars was very characteristic of the tip burned tissue developing on plants under field conditions when a rain is followed by high temperature.

Throughout the experiments carried on in the greenhouse during the winters of 1923-1924 and 1924-1925 it was observed that tip burn occurred when the humidity of the house was high as well as when the air was comparatively dry. It appeared as though humid air was as inducive to tip burn as dry air.

In some cases it appeared as though a humid atmosphere was more favorable to tip burn than dry air.

## TEMPERATURE, RATE OF GROWTH AND TIP BURN OF LETTUCE

A study of the relation of growing conditions to the appearance of tip burn reveals the fact that the trouble is greatly influenced by temperature, The trouble never occurs if the temperature is kept sufficiently low. The question arises as to whether the trouble is the result of direct physical injury resulting from too high a temperature or whether the disease is indirectly influenced by heat. The fact that the disease often occurs at the inner part of the head where it is not exposed to direct sunlight makes it appear doubtful if sunlight is an important factor.

The observations of Askenasy (1) in which he found the temperature of certain species of plants to be much higher than the shade temperature suggested that the injury of tip burn might be due to the temperature in the inner part of the head becoming too high from the heat of respiration. A number of heads of lettuce which were beginning to tip burn were examined, and the temperature of the inner part of the head determined during the warmest part of the day. The temperature of the inner part of the heads was found to be practically the same as the surrounding air. Tip burn occurred in the greenhouse when the temperature was around 70° F.

The maximum temperature which lettuce will withstand under normal conditions is much higher than 70° F.

The action of temperature as a causal agent in the development of tip burn of lettuce is undoubtedly a direct one. The burning can hardly be a direct physical injury resultion from too high temperature when it occurs at a temperature so far below the maximum for lettuce.

Attacks of tip burn being most severe at heading time, in plants making a very rapid growth, and in plants high in water content indicate that too rapid growth is not desirable. High temperature, bright light, humid atmosphere, and high soil moisture content are all conducive to rapid growth and are the conditions most favorable for tip burn injury.

The evidence at hand indicates that the burning is not a direct physical injury by external conditions. It seems more reasonable to assume that high temperature coupled with other conditions favorable for the development of tip burn injury causes internal changes in the plant which result in death of the cells.

## CONCLUSIONS AND DISCUSSION

The results obtained from the foregoing investigations indicate:

1. That the pentosans are not an important factor in tip burn injury.

2. That tip burn of lettuce is a physiological trouble.

3. That the dying of the plant tissue is not due to loss of water from the cells.

4. That an over supply of water rather than a shortage of water is inducive to the development of the disease,

5. That the occurrence of the burning is very much influenced by temperature.

6. That the disease will not appear if the temperature is kept sufficiently low.

7. That the action of heat in causing the disease is indirect and that the injury is not due to the leaves attaining a temperature above the maximum for normal lettuce tissue.

8. That high fertility and other conditions favorable for rapid growth produce plants susceptible to the disease.

Numerous efforts to transmit the disease from affected to healthy plants by inoculation were all unsuccessful. Failure to transmit the disease in this way indicates that the trouble is physiological and not due to an organism or a virus.

The development of typical tip burn injury on plants grown in a very humid atmosphere proves that the dying of the tissue is not due to desiccation.

Observations made of plants grown under various conditions of moisture supply show that an over supply of water is more conducive to the development of the disease than a deficiency in water supply. Many of the references to the disease express the opinion that the injury is due to water being lost from the leaves faster than it can be supplied by the roots and that a shortage of water increases the severity of the disease. The data obtained from the investigations carried out as outlined in this paper show very conclusively that a depleted water supply increases the resistance of the plant and that an excess of water lowers its resistance.

There is no question but what temperature is an important factor as a causal agent. If the temperature is held sufficiently low, no disease will appear. The temperature at which the burning will show up depends on other conditions, as the succulence of the plant. Under field conditions, it is apparently impossible to prevent the trouble as it is not possible to regulate growing conditions. The trouble may be lessened to some extent by minimum irrigation. In the greenhouse, there is little doubt that the disease can be controlled by the maintenance of low temperature.

These investigations show that burning will develop on plants growing in partial shade as quickly as on plants in direct sunlight. Sunlight does not appear to be an important factor as a causal agent. That the dying of the tissue is not due to direct heat injury seems apparent from the fact that the injury is often found at the center of heads which have no outward indications of the trouble and that the temperature of the inner portion of the head is not higher than the surrounding air as far as our observations go.

The study of the influence of fertility on the development of this disease indicates that any soil nutrient which produces rapid growth lowers the resistance of the plant.

ACKNOWLEDGEMENTS

The author acknowledges his indebtedness to Doctor E. P. Sandsten for the suggestion of the problem and for interest shown throughout the investigation, to Professor R. A. McGinty under whom the work was carried out for encouragement and assistance and for reading of the manuscript, to Doctor W. G. Sackett for apparatus and for many helpful suggestions and criticisms, to the Experiment Station Chemists and the Department of Botany for apparatus, and to the College Librarians for aid in securing literature.

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