University of Alberta Library

0 1620 3448147 1

For Reference

NOT TO BE TAKEN FROM THIS ROOM

Library of the University of Alberta, Edmonton, Alberta

Tuttle, G.

The effect of low temperature on plants.
1920.

1920

2

C.2

Ex LIBRIS
UNIVERSITATIS
ALBERTAENSIS

Thesis
1920
#2

THE EFFECT OF LOW TEMPERATURE
ON
PLANTS.

By

Gwynethe M. Tuttle, B.A.

TABLE OF CONTENTS.

A.	<u>General Considerations.</u>	<u>Page</u>
	1. Variation in ability to withstand low temperatures	1
	2. Phenomenon of freezing	4
	3. Effect of extreme low temperatures on protoplasm	5
	4. Concentration of cell sap and freezing	7
	5. Coldest points on the earth inhabited by plant life	8
	6. Functions of plant dependent on temperature	10
	7. Low temperature and the structure of the plant	14
	8. Low temperature and plant food reserves	18
B.	<u>Report of Investigation - Induced Changes</u> <u>in Reserve Materials in Evergreen</u> <u>Herbaceous Leaves.</u>	
	1. Preliminary tests of reserves	23
	2. Conversion of oils and fats to starch	25
	3. Reconversion of starch into fats and oils	33
	4. Cause of conversion	36
	5. Summary of results	45
C.	<u>Conclusions</u>	47
D.	<u>Bibliography</u>	49

The relations between a plant and the temperature conditions of its environment, form a problem which can only be solved indirectly, by regarding the result of temperature upon the many life processes of the plant. These are the factors which determine its growth, and upon which depend its successful existence and propagation. The ability which plants possess to withstand low temperatures varies widely, not only with those of different species, but even amongst various individuals of the same species which have grown under different climatic conditions.

Some hydrophytes, i.e. plants living under moist conditions such as *Nitella syncarpa*, are frozen at a temperature of -4°C while *Sphaerella nivalis*, living in the snow of Arctic regions is often exposed to a temperature of -20°C for months without being destroyed. One of the most striking illustrations of the ability of plants to withstand extremely low temperatures, is given by Kjellman, reported by Schimper (1),

Digitized by the Internet Archive
in 2017 with funding from
University of Alberta Libraries

<https://archive.org/details/tuttle1920>

in a description of the Arctic flora as seen by him on the "Vega" expedition. In speaking of *Cochlearia fenestrata* he says:

"There are few places on earth where the winters are so severe as the spot on which the "Vega" expedition passed the winter. The cold was very persistent, and the temperature went below -46°C . The individual in question grew on the top of a fairly high sand hillock near Pittlekaj, exposed to the constant and cutting North or Northeast wind. It had commenced to bloom in the summer of 1878, but its blooming was far from being completed, when the winter came on and stopped further progress. Its system of flowers consequently included flower-buds in the various stages of development, recently opened flowers, blown flowers, and some more or less ripened fruits. Only an insignificant, shrivelled remnant of the basal rosette of leaves remained, but the upper ones were fresh and vigorous. In this condition the plant was overtaken by winter, and exposed to its utmost rigour. We might well have believed that it must have been killed, and especially that the tender

flowering parts which were attacked during the development would have been destroyed by the frost and so prevented from developing further. This however, was not the case. When the summer of 1879 began, the plant continued its growth from the point at which it had been arrested by the approach of winter, the flower-buds opened, and from the axils of the fresh upper leaves fresh inflorescences sprouted forth."

On the other hand, there are plants whose critical temperature lies above freezing point. Plants such as the tropical Acanthaceae, the variegated Coleus, tobacco plants and melons wither, dry up and die if exposed for any length of time to a temperature of 2°C , due to their roots being unable to absorb sufficient water at this temperature to balance that given off by the leaves during transpiration. such plants, after an exposure to this temperature, resemble closely plants that have been actually frozen, but, as will be seen later, the phenomenon here does not include the processes which take place when a plant actually freezes.

If, then, plants respond so differently to temperature, the question arises as to the cause of

or factors which regulate their ability to withstand low temperatures. But in order to deal with these factors, one must first determine clearly as to what is meant by freezing of plant tissues, and whether a frozen plant need thereafter be a dead one.

At one time it was thought that the freezing of a plant entailed the rupturing of the cell walls, but it has now been determined that such is not the case. It is a fact known to all, that plant tissues contain a very high percentage of water. A small amount of this water enters into chemical combination in the formation of the different plant products, but by far the largest percentage of it is present in the cell wall, the cell body, and in the cell sap of the vacuoles.

If frozen plant tissue be examined, ice crystals will be seen to be most abundant in the intercellular spaces. Now, under ordinary conditions these intercellular spaces are filled with air and contain a very small amount of water, if indeed, any at all. Hence, some force must have been exerted during the process of freezing, by means of which the water was expelled from the plant cells into the intercell-

ular spaces where it crystalised. According to Kerner (2), the phenomenon of freezing is best explained as follows: " The protoplasm becomes stimulated and roused by the lowering of the temperature to transport a portion of the water from the interior to the exterior of the cell, by means of contraction and pressure.....In the cooled leaves the benefit, of course, is to be sought for in the fact that the living portions of the cells are protected from destruction as long as possible by the formation of ice crystals in the intercellular spaces. If the water were forthwith frozen inside the cells, between the groups of molecules of the living cell body and its wall, by a few degrees of cold, fundamental displacements and disorganizations of the groups of molecules would be unavoidable. On the other hand, the ice crystals on the exterior of the cells do not produce such destruction."

It has been shown that this excretion of water need not connote the death of the cells. If the material be slowly thawed, the water can be again received back into the cells. If, on the other hand, the cold has been so severe as to change to ice the water held in the protoplasm, which is

essential to its vitality, then a disorganization of that substance takes place. It is then that the plant would be frozen dead.

It would seem then, that with the protoplasm of the individual plant, rested the determination of the critical degree of cold for that individual. The protoplasm of *Cochlearia*, mentioned above, was able to withstand -46°C , while that of *Nitella* was killed at -4°C . The definite composition of protoplasm has not, as yet, been exactly determined, but one is justified in contending that a variation in its composition in different plants is largely responsible for their varying response to temperature; just as the presence of different salts in solution causes a change in the freezing point of water.

Because of the important part which water plays in the process of freezing, it follows naturally that the water content of the plant at the time of freezing would be an important consideration. Young shoots, flowers and leaves are frozen at such a temperature as -1°C , while woody trunks and mature seeds are able to withstand extreme winter temperatures. This is generally attributed to the difference in water content in the two cases.

That this is true in the case of seeds, is shown by an investigation reported by Detmer (3):

"I have taken, on the one hand air-dry wheat grains, and on the other wheat grains which had been lying in water for seven hours, and exposed both for fifteen hours to a temperature of -10°C in small glasses. I found that the former, when placed on moist sand under normal conditions for germination, are still capable of germinating, while the latter do not germinate and perish."

There is still another factor which must be regarded as one which affects the temperature at which plants freeze, and that is the concentration of their cell sap. It is this concentration which determines the osmotic pressure, and because of the relationship existing between the freezing point of a solution and its osmotic pressure, the importance of the concentration of the sap within the cell is very great. The following data obtained by Dixon (4) are of interest in this connection:

<u>Plant from which cell sap was taken.</u>	<u>Depression of freezing point.</u>	<u>Osmotic pressure.</u>
Aloe plicatile	0.292	3.52
Anthurium andreanum	0.632	7.49
" cristallinum	0.727	8.73
Iris germanica	0.764	9.20
Catalpa bignoniodes	1.905	22.92
Fraxinus excelsior	2.097	25.22
Syringa vulgaris	2.119	25.50
Magnolia acuminata	1.858	22.34

From these figures it is seen that such plants as Aloe, Anthurium and Iris are not frozen at ordinary freezing point but at approximately one degree below, while Syringa and Fraxinus are able to withstand more than two degrees of frost as a result of a fairly high concentration of their cell sap. No doubt, figures could be obtained from other groups, the halophytes for instance, which would yield still more striking results.

It has been mentioned earlier that Cochlearia fenestrata is able to withstand even so low a temperature as -46°C , and so the question arises as to whether there is any temperature so low as to prohibit the existence of plant life. As yet none has been recorded. The coldest known places on the earth are those districts covered

by the Siberian forest. Details of the temperatures recorded at Verkhoyansk, lying within that region, are given in the following table:

Temperature at Verkhoyansk (Siberia)

67° 34'N. 135° 51'E. 107 meters above sea level.

	<u>Mean</u>	<u>Minimum</u>	<u>Maximum</u>
December	-48.4	-61.9	-28.7
January	-51.4	-64.1	-31.5
February	-46.2	-60.5	-24.3
March	-35.2	-55.7	-16.6
April	-15.8	-33.6	1.9
May	- 1.1	-17.2	11.9
June	9.4	- 0.7	22.4
July	15.6	5.0	29.8
August	9.3	0.4	30.1
September	0.4	-10.3	12.4
October	-18.1	-36.7	- 1.2
November	-39.7	-54.4	-14.6

So far as is known, then, at no place on the earth is the temperature so low that no plant can withstand it. The alleged complete absence of all terrestrial vegetation in the Antarctic polar zones is not due to an absolutely greater cold there - for the thermometer does not fall so low as in the North polar zones - but to the fact that the temperature remains so low, and almost constantly under the minimum of the essential functions.

The life of a plant is made up of very different actions, each of which is performed within its own range of temperature. Just as a wide range of temperature exists in the phenomenon of freezing, so a similar range occurs in connection with all the vital processes of the plant.

For the process of germination the degrees of temperature given for minima, as quoted by Schimper, are instructive: "*Sinapsis alba*, *Camelina dentata*, *Trifolium hybridum* and *Medicago sativa* germinated between 0°C and 1°C ! Of plants from hot countries *Solanum Melongena*, *Nicotiana Tabacum* and *Cucurbita Pepo* germinated between 11°C and 16°C , but *Cucumis sativa*, *C. melo* and *Theobroma cacao* only above 16°C . Uloth observed the germination of several grasses *Cruciferae* and *Papilionaceae*, at 0°C but the process was very protracted. Kerner placed glass tubes containing earth and seeds in a spring, the temperature of which remained constant at 2°C and he found that numerous alpine plants germinated even at this low temperature.

Growth after germination depends on conditions of temperature similar to these required for that process. Some alpine plants are able to grow at temperatures approaching freezing point. Flowers of *Soldanella alpina* have been found breaking through the snow and those of *Anemone vernalis* and *Crocus* in half melted snow. More remarkable than this however, is the growth of the seaweeds of Arctic seas in water whose maximum temperature seldom exceeds 0°C , and their production of sexual organs in the winter at -1°C .

As regards the processes of assimilation and respiration, fewer data are available, but it is a well established fact that these two functions do exhibit very dissimilar temperature curves. The lower zero point for assimilation is lower than that for any other function of the plant. Jummelle observed a distinct though weak assimilation in *Abies excelsa*, *Juniperus communis* and *Evernia prunestris* even at -40°C , while he could not observe any distinct respiration below -10°C in the same plants. The optima for these functions lie, of course, amongst the higher temperatures,

that of assimilation lying between 30°C and 56°C , while respiration increases in proportion to the temperature up to nearly the higher limit of plant life.

There are, however, some physiological processes for which not only the optima are low, but which can take place only in winter, late autumn, and early spring. These, obviously, concern plants of temperate and higher latitudes only. Certain of these will be dealt with at length in connection with the reserve food materials of the plants of the colder zones.

Low temperatures exert a favourable influence on the sexual organs and on the parts connected with them, in many plants of the temperate and frigid zones. A further quotation from Schimper (1) is of interest at this point:

"The cardinal degrees for the growth - and perhaps for the inception - of the primordia of flowers are often much lower than for the growth of the vegetable shoots, so that the former are favoured by a relatively lower temperature, and the latter by a higher temperature during development. It is well known

that Crocus, Hyacinthus and other perennial herbs do not send out flowers or inflorescences at a high temperature, but shoot out luxuriantly into leaf. Also in the forcing of fruit trees the temperature must be kept moderate before, and especially during the blossoming period. For the same reason many temperate plants seldom blossom in the tropics, for example most of our fruit trees,.....Kurz found in the mountains of Burmah that increased coolness due to increased altitude expedited the blossoming of temperate plants such as the Rhododendron and Gentiana but delayed that of tropical ones."

It has been observed that although there has been no lower limit of temperature recorded of plant life, yet, in many respects, low temperatures are fatal to the life functions of the individual when these temperatures are of considerable duration. It would be expected then, that plants would possess protections against extreme temperatures, but as yet our powers of observation have not enabled us to recognize any such protections.

Attempts have been made to establish some distinguishing points between individual plants belonging to species growing respectively in alpine and temperate climates. According to Lindberg, mosses growing in Spitzbergen have shorter stems, more richly branched; leaves which are more crowded, shorter and less pointed. Kjellman gives similar results for plants of higher orders. He mentions that *Picea* and some of the *Ericaceae* show the leaves broader and shorter. Warming (according to Schimper (1)) observed in *Juniper communis*, *Lycopodium annotinum* and *L. selago* a tendency to bear leaves which are straighter, adpressed, and do not stand off from the stem as is elsewhere the case. These modifications, however, have not been shown to depend only on temperature.

On the other hand Kjellman records that all contrivances which might be considered as protective against cold are wanting in the above-mentioned *Cochlearia*, which proved itself so resistant to extremely low temperatures. In central Europe the absence of any

protective means is evident in all the meadows and fields during a frost. There, delicate plants such as *Bellis perennis* and *stellaria media* are frozen hard and brittle. When the thaw comes these plants continue to grow undisturbed, despite the fact that during the cold periods they were entirely unprotected by any external contrivances.

Accordingly, as Schimper states, "We always come back to this, that the freezing of a plant to death, or not, depends upon whether or not the condition of the protoplasm is such that its molecular constitution becomes permanently disorganized in consequence of the cooling, and that the most effective protection must be sought for in the constitution of the protoplasm itself. Since we do not know the constitution of the protoplasm, it is idle to puzzle ourselves in surmises about it, this only being certain, that the resisting capacity of protoplasm differs much from plant to plant, as well as at different times in one and the same plant."

The process of assimilation, or the means by which a green plant manufactures organic compounds from inorganic substances through the agency of Chlorophyll in the presence of light and water, has been mentioned above as one whose optimum temperature lies rather high, although extreme cases have been determined where certain plants were able to assimilate at a very low temperature. Hence it follows that the greatest amount of organic food material possessed by the plant, is manufactured during the seasons of higher temperature and greater water supply, and that the plant is dependent upon reserve food for the remainder of the year, during the period of comparative quiescence of its functions.

The fact that plants in the temperate zones undergo definite periods of growth followed by a resting period has as its conclusive proof the presence of the so-called "annual rings" in the woody perennials. Here the wood is composed of a regular series of concentric bands, every pair of which represents one year's growth, with the large thin-

walled cells formed in the spring, and the smaller thick-walled cells of the autumn wood. The variation in the two kinds of wood is due to the smaller amount of reserve materials available to the plant for food in spring, while, as the season advances, temperature and the water supply increase, and with the resultant increase in assimilation, the character of the wood changes.

The phenomenon of leaf-fall of deciduous trees is a further demonstration of the fact that during the winter season the plant undergoes a rest period, so far as the process of assimilation is concerned, for without the chlorophyll present in the leaves, no fresh organic food can be manufactured. The assimilation mentioned above at the extremely low temperature, referred to evergreen plants only.

The exact products of assimilation and their sequence in formation have not as yet, been determined. Investigation at the present time is yielding startling results. Many distinguished plant physiologists are devoting themselves to research in this subject,

and their latest results tend to upset, materially, existing theories.

Whatever may be the earlier products, however, it has been conclusively demonstrated that the carbohydrates of most plants are transferred from one part to another in the form of soluble sugars, and stored in the insoluble form of starch. When required as food material the starch is changed into sugar again through the action of enzymes, and in the soluble form migrates to the part of the plant where it is required.

In the summer, during the period of greatest assimilation the majority of plants show their starch content to be very high. If these plants are examined several months later, at the beginning of winter, it will be noticed that a great many, especially those of the high temperate regions, have lost their starch altogether, and on the other hand, contain a large percentage of oils and fats, another form in which food reserves are frequently stored. Hence in many trees the starch is altered during the winter into

fats, which are again changed to starch in the spring. Both these processes, according to Jost (5), "have been seen to depend in a variable manner on temperature; low temperatures tend to produce the formation of fat, high temperatures starch."

Since all trees do not undergo the change of starch to fats, but retain the former as a winter food reserve, they are generally divided, especially evergreen trees, into two classes, (a) "Starch" trees, whose reserve is stored in the form of starch, and (b) "Fat" trees, in which the starch is converted into oil during the autumn. As this change is only found associated with a lowering of temperature, the idea is at once suggested that this may in some way protect the protoplasm against extreme temperatures. This suggestion is supported by Warming (6) in the following statement: "The presence of oils and fats in the latter ("fat" trees) may enable the plant to withstand lower temperatures in that the fatty oil in the form of an emulsion prevents the sub-cooling of the plant tissues

and increases the power of resistance to frost." The distribution of the two groups lends further support, in that "fat" trees are largely distributed throughout the colder regions, while the "starch" trees are found in localities of higher winter temperature.

Much attention has been given this phenomenon in trees but no information is available in connection with herbaceous plants. Since many evergreen herbaceous plants grow in the shade of the "fat" trees subjected to the same extremes of cold, it would seem possible that they should exhibit similar features. It was with a view to ascertaining a few facts in this connection that the following investigation was undertaken. My attention was called to the need for investigation in this direction by Dr. F.J.Lewis, to whom I am greatly indebted for helpful criticism and suggestions given throughout the progress of the work.

The only recent investigation bearing
(7)
on this subject is that of Kuchi Miyake of Tokyo. He confined his attention solely to the calculation of the winter starch content of a number of

trees and herbaceous plants from different parts of Japan. The conclusion is reached that the starch content of trees in winter is very small compared with that during the summer, but that this content varied with trees of different species and also with the region from which the material came - that from northern Japan, where lower winter temperatures occurred, showed less starch than that from the southern districts. Kuchi Miyake simply recorded the starch content as he found it. He did not try to account for its variation. It would have been interesting to know whether part of this decrease of starch was due to the formation of fats and oils at the beginning of winter, but no mention is made of investigation in this direction.

-investigation

The following[^] was undertaken not so much with a view to ascertaining the food reserve in a large number of plants as that of a single one, *Linnaea borealis*, var. *Americana* (Forbes) Rehdner, and to investigate the changes which these reserves undergo when subjected to different temperature conditions.

In view of the fact that temperature is vitally connected with the problem, a brief statement may be given of the meteorological conditions of the district. Early frosts are charactic of Western Canada, occurring usually from the 6th to the 10th of September - frequently followed by warmer periods. The precipitation during September and October is usually high but varies considerably with the different years. The winter seasons are quite variable but the extreme temperatures remain the same from year to year, ranging from about -40°F (-40°C) to an occasional 50°F (10°C). The humidity of the air is moderately low and high winds are rare. The depth of snow averages from one to two feet on the level.

This season, however, has been unusual. Cold periods of five to ten days, in which the thermometer ranged from 0°F (-17°C) to -40°F (-40°C), have alternated quite regularly with slightly longer periods of rising temperature, reaching on one occasion 47°F (8°C). As will be mentioned later, these fluctuations have been useful during the ptogress of the investi-

gation, especially in connection with reconversion.

I. Preliminary Tests of Reserves.

The work was commenced late in October, just about the time of leaf fall of deciduous trees. A number of leaves were examined then, for their starch and oil content. A solution of iodine and another chloral hydrate and iodine were used in the tests for starch, the former in the preliminary tests and the latter in the material during conversion as the chloral hydrate swelled the starch grains and also decolorised the sections thus making the reaction much clearer. For fats and oils, sections of the material were tested with a 1% solution of Osmic Acid and also with neutral red solution, both tests yielding similar results.

Table I. shows the starch and oil content of the majority of local plants which still retained their leaves.

It will be seen from the table that all the evergreens are practically destarchified, and on the other hand, contained a large amount

Table I.
Fat and Starch Content.

Date	Material	Starch	Oils and Fats
Oct. 23	<i>Picea Canadensis</i> Mill.	very little	large amount
" 23	<i>Pyrola rotundifolia</i> L.	none	" "
" 23	<i>Linnea borealis</i> L. var. Americana (Forbes) Redner	very little	" "
" 24	<i>Cornus Canadensis</i> L.	none	" "
" 26	<i>Hypnum</i> (sp)	good reaction	none
" 26	<i>Marchantia</i> (sp)	very little	large amount
" 26	<i>Mitella nuda</i> L.	none	" "
" 28	<i>Pinus Banksiana</i> (Lamb)	very little	" "

of oil.

II. Conversion of Oils and Fats to Starch.

A series of experiments was then started to induce the formation of starch in the destarchified plants. The following plants were brought indoors and subjected to higher temperatures - *Linnea*, *Pyrola* and *Picea*. The first two plants were potted, but in the case of *Picea*, shoots were kept in water. The experiments were kept in a dark room at a temperature of 68°F (20°C), and in each case a control was placed in the dark outside, at the lower temperature.

The material of *Linnea* showed starch appearing as early as the second day while *Pyrola* and *Picea* showed no increase, although allowed to remain at the higher temperature for five weeks. This points to the fact that different species vary in their response to the change in temperature.

As *Linnea* responded most readily to experiment, the following observations were all made from that plant. They are based on mater-

ial collected and potted at intervals of five or six days during November and December, 1916, and January, 1917. In each case the result was based on a test from six to twelve leaves.

Table II. shows that material which has been inside for two days shows a starch reaction in all parts of the leaf. At the end of four days the cells are quite crowded with starch but the amount increases until the seventh or eighth day. (Fig. 1), after which time the starch content seems to remain constant.

The starch in all cells is present in a very finely divided state and the individual grains exhibit Brownian movement to a marked degree.

It is interesting to note the large amount of starch in the epidermal cells of material undergoing conversion (Fig. II.0) as none is found there during the summer, when the starch is being formed as a product of photosynthesis - this apparently suggesting that the starch has arisen by conversion of oils and fats which are abundant in the epidermic during the winter (Fig. III). It has

Table II.

Starch in Material exposed to Higher Temperature.

Date	Condition when potted	Condition						
		1st day	2nd day	3rd day	4th day	1 week		
Nov. 8 (a)	Experiment	No starch	No test	No test	No test	No test	Good reaction	Good reaction
" " (b)	Control	" "	" "	" "	" "	" "	No starch	No starch
Nov. 14	Experiment	No starch	" "	Good reaction	" "	" "	Strong reaction	Strong reaction
" "	Control	" "	" "	No starch	" "	" "	No starch	No starch
" 16	Experiment	No starch	" "	No test	Good reaction	Good reaction	Strong reaction	Strong reaction
" "	Control	" "	" "	" "	No starch	No starch	No starch	No starch
" 23	Experiment	No starch	" "	" "	Good reaction	Good reaction	Strong reaction	Strong reaction
" "	Control	" "	" "	" "	No starch	No starch	No starch	No starch
Dec. 4	Experiment	No starch	No Starch	Slight Reaction	" "	" "	Strong reaction	Strong reaction
" "	Control	" "	" "	No Reac.	" "	" "	No starch	No starch
" 18	Experiment	No starch	No Starch	Slight Reaction	" "	" "	Strong reaction	Strong reaction
" "	Control	" "	" "	No Reac.	" "	" "	No starch	No starch
" 30	Experiment	No starch	NB test	Slight reaction	" "	" "	Strong reaction	Strong reaction
" "	Control	" "	" "	No Reac.	" "	" "	No reaction	No reaction

Fig. I. - Cross section of *Linnaea* leaf showing starch in material exposed to higher temperature for one week. Section treated with chloral hydrate and iodine.

-Fig II. Horizontal section of *Linnaea* leaf showing starch in epidermal cells. Material had been exposed to higher temperature for one week. Section treated with chloral hydrate and iodine.

- Fig III. - Horizontal section of *Linnaea* leaf showing oil in epidermal cells. Material fresh from outside, before exposure to higher temperature. Section treated with 1% Osmic acid.

been observed that the starch grains are grouped as though each group had arisen from a single oil globule.

No regular variation in the conversion has been determined. Entire plants have been tested and it has been shown that they do not possess "fat" leaves and "starch" leaves - all leaves tend to form starch when exposed to higher temperatures. Figure IV. shows the test of three plants from material which had been exposed to higher temperature for four days.

In this test, plants No. I and III. gave a strong reaction in all of the leaves but very little reaction was obtained in any leaves from Plant No. II. This may be due to the fact that the leaves were not so vigorous as those of Plants No. I. and III. Hence any variation seems to be due to some plants being more vigorous than others and also to the age of the leaves, as it has been observed that younger leaves carry on the conversion much more rapidly than do the older ones.

The increase in the starch content of these leaves is much more evident than the de-

Fig IV. - *Linnaea* material exposed to higher temperature for four days.
 Sections of which were treated with chloral hydrate and iodine.

Strong starch reaction in sections from	a ₁ -a ₂ -b ₁ -b ₂ -c ₁ -c ₂ -g ₁ -g ₂ -h ₁ -h ₂ -i ₁ -i ₂
Slight " " " " "	e ₁ -f ₂
no " " " " "	e ₂ -d ₁ -d ₂

crease in the oils and fats which one would suppose must be taking place, as that seems the only source from which the starch can be derived, for the factor of photosynthesis has been eliminated by placing the material in darkness. All the leaves showing a large amount of starch, gave a strong reaction with the test for oils and fats. In horizontal sections passing through the epidermis there seems to be quite as much oil as starch present. The palisade tissue shows no appreciable decrease. It is only in the cells of the mesophyll that an actual difference has been recognized. Here the decrease is quite evident as will be seen by comparing Figures V. and VI.

III. Reconversion of Starch into Fats and oils.

Having shown that starch appears in those leaves subjected to a higher temperature, it was then attempted to cause a reconversion of the starch to oils and fats by lowering the temperature. Material of *Linnea*, which had been kept at 68°F (20°C) for a week and which showed as a result a high starch content, was placed outside in

Fig. V. - Cross section of *Linnaea* leaf showing fats and oils before exposure to higher temperature - section treated with 10% osmic acid.

Fig VI.-- Cross section of *Linnaea* leaf showing decreased oil content, Material exposed to higher temperature for one week. Section treated with 10% Benic Acid.

darkness on two different dates, November 25th and December 13th. As will be seen from Tables III. and IV. the temperature conditions were very different during the two experiments.

From Tables III. and IV. it is seen that material containing a large amount of starch undergoes reconversion in which the starch is changed back into oils and fats if subjected to moderately low temperature. If however, the material, full of starch, and hence in a summer condition, be subjected to extremely low temperatures no reconversion takes place and the material is killed. The strong plasmolysis of the cell contents exhibited in this material is shown in Fig. VII.

IV. Cause of Conversion.

The conversion of fats to starch is quite impossible as an unaided chemical change, and hence the action of an enzyme is suggested. Tests were made for the presence of an enzyme, both in material fresh from outside, and in that which had been exposed to a higher temperature for some time.

Material containing Starch exposed to moderately low Temperature.
Table III.

Date	Av. Temperature	Experiment	Control
Nov. 25	25°F (-3°C)	Strong starch reaction	Strong starch reaction
Nov. 30	36°F (2°C)	Decreased starch content, four leaves out of six destarchified.	Strong starch reaction
Dec. 4	28°F (-2°C)	All material destarchified.	Strong starch reaction

Table IV.

Material containing Starch exposed to extremely low Temperature.

Date	Average Temperature	Experiment	Control
Dec. 13	40°F (-15°C)	Strong starch reaction	Strong starch reaction
" 20	-16°F (-26°C)	No decrease in starch. Cells strongly plasmolysed	" "
" 23	-19°F (-28°C)	No decrease in starch. Cells strongly plasmolysed.	" "

Fig. VIII. — Cross section of *Linum* leaf. Material outside for reconversion during extremely low temperature. Section treated with iodine solution. Starch grains visible in epidermis. Owing to the high starch content the plasmolysed cell contents were stained deep blue.

The test in this case, was an alcoholic solution of guiacum resin which, with the addition of hydrogen peroxide gives a blue reaction in the presence of any enzyme. In enzyme tests made December 7th and 18th on fresh material no reaction was obtained. Material, however, which had been exposed to higher temperature for several days gave a strong reaction when tested.

About a month later (Jan. 11-16) material in the same condition was tested which yielded rather different results. In the interval, however, the weather had modified and some very high temperatures had been recorded. All Linnea leaves, both from outside and inside then responded very strongly to the enzyme test. There was an inclination at first to doubt the test but when leaves of Begonia and Geranium from a greenhouse gave negative results it was concluded that the change in temperature must have affected the outside material - still the change had not been great enough to cause the formation of starch for the outside material gave no reaction to the iodine test. The effect of changing temperature was watched and it was found that

the enzyme response diminished after a fortnight of lower temperature, although at that time a slight reaction was given despite the fact that the material was gathered when the temperature was lower than $0^{\circ}\text{F}(17^{\circ}\text{C})$.

The important point, however, was that the material undergoing conversion gave a strong enzyme reaction. The next step was to determine whether or not this was due to the fat splitting enzyme lipase. The test applied was that used by G. J. Fowler (8) and Reynolds Green (9) in work with germinating Ricinus seeds. Here the solution which is to be tested for the presence of the enzyme, is allowed to act on an oil emulsion causing, if Lipase be present, the hydrolysis of the oil and the formation of a fatty acid.

An extract of the leaves was made by grinding them in a mortar with a solution containing 5% sodium chloride and 0.2% thymol as an antiseptic. This was allowed to stand for twenty-four hours and then filtered. The filtrate was treated as follows: An emulsion of olive oil

With a little gum arabic was made and to this was added a small amount of the extract, phenolphthaleine being used as an indicator. As the leaf extract and oil emulsion were both highly acid, they were carefully neutralized with a few drops of very dilute caustic potash. From two to five experiment tubes were made up in this manner. A control was made up in a similar manner, except that the extract was boiled for two or three minutes before it was added to the emulsion. Both experiments and control were placed in an oven at 95°F (35°C). Table V. gives the results of several tests for Lipase in extracts from material of different conditions. The test made on January 9th shows that fresh material from outside, without exposure to higher temperature, does not respond to the test for Lipase.

It has been recorded earlier in the paper that material fresh from outside responded to the general enzyme test. Experiment of January 9th in Table V. shows that the enzyme in fresh material is not Lipase - hence the presence of another enzyme is suggested. Owing to the browning of the leaf extract on standing, oxidases

Table V.

Action of Lipase

Date	Exposure to higher Temp.	Experiment	Boiled control	Indicator
Dec. 4	4 days	Acid reaction after 4 hrs.	No reaction	Litmus
" 8	4 days	Acid reaction after 4 hrs.	No reaction	Phenolphthaleine
Jan. 2	2 days	Acid reaction after 2 hrs.	No reaction	Phenolphthaleine
" 9	None (Material tested when brought in)	No reaction	No reaction	Phenolphthaleine
" 16	2 days	Acid reaction after 2 hrs.	*slightly acid	Phenolphthaleine
Feb. 8	2 days	Acid reaction after 4 hrs.	No reaction	Phenolphthaleine

*Due to insufficient boiling

were suggested.

It was determined (8) that guaiacum resin, without the hydrogen peroxide, was a test for oxidase alone and so this material was subjected to this test. Sections of the leaves, both fresh and converting, gave only slight reactions with the guaiacum resin, but the extract gave a good response. This is due to the fact that, on standing, a substance is formed in the extract which increases the oxidation (10).

In the tests for Lipase given above, it will be noticed that phenolphthaleine was used as an indicator instead of the litmus solution used by Reynolds Green in his experiments with extract from endosperms of ricinus. Litmus was used in early experiments in this work but it was found that the plant extract decolorised the litmus directly it was added. This fact was a further proof of the presence of oxidases, as they cause an immediate decolorisation of litmus solution. Hence Litmus was of no use as an indicator in the presence of oxidases, and so phenolphthaleine was substituted, with whose action they do not seem to interfere.

The results of this investigation may

be briefly summarised as follows:

1. Most evergreen plants are destarchified in northwestern Canada as early as October, and then contain a large amount of oil.
2. Exposure to a higher temperature in the case of *Linnea* induces the formation of starch in darkness.
3. Starch is present in the material after two days and increases in amount until about the eighth day.
4. The starch is in a very finely divided state and the individual grains exhibit Brownian movement.
5. All healthy leaves are capable of carrying on conversion. The plant does not possess "fat" leaves and "starch" leaves.
6. The starch disappears when again exposed to moderately low temperature for about eight days, but the leaves are killed if exposed to extremely low temperature when filled with starch.
7. A decrease in the oil content is evident

in leaves which have formed starch by conversion.

8. Enzymes are present in material undergoing conversion.
9. Lipase has been demonstrated in material undergoing conversion.
10. Oxidases are present in the leaf of *Linnea* even at quite low temperature.

The presence of Lipase and its action in the transformation of the oily reserves in certain seeds, at the time of germination, has been a fact familiar to plant physiologists for several years. The chemical changes taking place in the germination of *Ricinus*, the castor oil plant, were referred to above. But so far as definite information went, the presence of Lipase in plant tissues was confined to seeds. The similarity in the chemical changes probably led Sachs to formulate the following hypothesis, regarding the transformation of food reserves in processes other than that of germination, that in this, and other similar cases it may be a question of the gradual formation of ferments. To this Schimper adds "An experimental proof of this suggestion

has not yet been attempted." Although confined to one single species, the piece of investigation reported above seems to give evidence of the fact that Sachs's hypothesis was not an idle one, but one which would amply justify further research in order that the metabolism of plant reserves might be more fully understood.

From what is known then, of the action of low temperatures upon plant tissues, one is inclined to draw the following conclusions. In the first place the effects of temperature is not evidenced directly on the plant tissues, but only as it is seen in the performance of the life functions, and that so far as is known there is no spot on the earth from which plant life is absolutely excluded by reason of the thermal conditions.

Varied as are the temperatures in the different zones, just so varied are the abilities of different plants to withstand them. The inability or ability to withstand low temperature, so far as we are able to discern, does not depend on external difference of structure as is the case with the regulation of water supply,

but rather to internal variations. Primarily, it is the result of the variability in the structure of the protoplasm in plants of different species, or even those of the same species which have grown under different climatic conditions. Secondary to this, one places the importance of food reserves. In the investigation above, the fact that *Linnea borealis* was not able to withstand low temperatures, when its food reserves consisted largely of starch, yields evidence of efficient protection afforded by oily food reserves against the sub-cooling of the plant tissues, and especially of the protoplasm upon the vitality of which, the life of the plant depends.

B29737