FACTORS DETERMINING CHARACTER AND DISTRIBUTION OF FOOD RESERVE IN WOODY PLANTS

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(WITH TWO FIGURES)

Introduction

The investigations of RUSSOW, FISCHER, and others upon the character and seasonal changes of the food reserves in woody plants, and the considerable attention which this problem has more recently received, have made us familiar with many of the important facts which it involves; but as to the underlying causes which determine the type of reserve food occurring in any cell and which direct its changes in form and location we are still uncertain. Our present knowledge, derived in greater part from a study of twigs, branches, and small trunks and roots, may be summarized substantially as follows. The major part of the reserves stored up by trees and shrubs during the productive season is evidently composed of starch (fat is also demonstrable, and SABLON (5) has emphasized the importance of reserve cellulose as a center of storage). At about the beginning of winter there is a decided reduction in the amount of starch, leading to its disappearance in the phloem and cortex of practically all woody plants in our latitude. At the same time the amount of fat seems to increase greatly. In certain forms, called by FISCHER (2) "starch trees," there is no further change, the food reserves in the pith and wood persisting in the form of starch throughout the winter. In others, called by him "fat trees," the starch vanishes in these portions of the stem as well, and fat appears in abundance, constituting the only visible food reserve during the winter. In all woody plants, late winter or early spring sees a regeneration of starch throughout the tissues of the stem and an apparent diminution in the amount of fat. This regenerated starch is used up in the formation of the spring growth, and it is not until summer that a fresh supply begins to be deposited. It has been supposed that at the seasonal changes starch was converted directly into fat or fat directly into starch, but as microchemical methods have been employed almost entirely this cannot well be proven. The only work involving a quantitative analysis, that of NIKLEWSKI (3), seems to indicate that changes in the two types of reserve food occur independently of each other. It has been observed that the seasonal changes are most marked in twigs and small branches, less so in main stems, and least of all in roots, where fat is scarce and starch persists practically unaltered throughout the winter. The work of FABRICIUS (1) seems to indicate that in the large trunks of spruce conditions may be different from those in small trunks, branches, and twigs, and that starch there may have its maximum in winter and fat its maximum in summer.

That temperature is of importance in producing changes in the character of the food reserves is shown by the fact that starch regeneration may be induced in the winter by bringing twigs from out-of-doors into a warm place. That a subjection to cold during the summer will not cause the characteristic winter changes, however, and that these changes will nevertheless occur in the fall, even though the plants remain under a warm environment, indicate that factors other than temperature must be operative.

The present paper is an attempt to throw light on this general problem by a careful anatomical study of the storage regions of woody plants with a view to determining the exact distribution of starch and fat there and its change from season to season. It contains the results of nearly three years' observations on about 300 species of trees and shrubs belonging to over 100 genera and including all the common species of the northeastern United States, together with many exotic ones in the collections of the Arnold Arboretum. With the exception of a little received from the southern states, all the material studied was gathered in Massachusetts and Connecticut. Special attention was paid to conditions in twigs and young branches, where seasonal changes are most marked. Thin sections of freshly gathered material were cut on the microtome and treated with iodine and Sudan III to bring out the starch and the fat, respectively.

Observations

The results of previous workers as to seasonal changes were substantially confirmed. Although fat is evidently most abundant in the winter months, it is by no means absent during the summer, but at that time it is apt to be masked by the starch. Microchemical evidence as to the relative abundance of either starch or fat at different seasons is necessarily unreliable. It is certain, however, that much of the starch which disappears in the fall does not become converted into fat, but changes to glucose or some other non-visible substance, since in many starch trees large numbers of cells are emptied of starch without causing the appearance of fat. The twigs of some trees, notably species of *Catalpa*, are almost emptied of visible food reserves of all sorts during the winter. There are marked differences between species in their ability to produce fat, as indicated by its abundance in the phloem and cortex. This type of food substance seems to be practically absent in species of *Carya* and is very small in amount in *Fraxinus*, *Acer*. *Syringa*, and others. It is particularly abundant in such forms as *Liriodendron*, *Populus*, and *Pinus*. In general, fat is less abundant in the phloem and cortex of starch trees than of fat trees. There are certain exceptions to this rule, however; notably *Liriodendron*, a starch tree, but rich in cortical fat; and the soft birches, fat trees, but poor in cortical fat. Fat was universally found to be more abundant in the phloem than anywhere else in the plant.

The observations of others that seasonal changes are more marked in twigs than in larger branches and trunks was confirmed. This conservatism is apparently still greater in the roots, where starch was found to be practically unreduced in amount during the winter, a fact recorded by PRESTON and PHILLIPS (4). In the root, too, the amount of fat is very much less than in the stem.

FABRICIUS (1) and others have noted the fact that starch trees are predominantly hard-wooded species and fat trees soft-wooded ones. This rule was in general confirmed by the present study, but a number of exceptions were noticed which we shall later find to be significant. The hard pines, for example, are clearly fat trees; and *Liriodendron*, *Magnolia*, *Ailanthus*, and *Platanus*, all soft-wooded, are clearly starch trees.

Two other general relations between anatomy and the character of the food reserve were noted. Species with diffuse-pored woods are usually either fat trees or have an abundance of fat; those with ring porous wood are almost always starch trees. Narrow-rayed species may belong to either category, but broad-rayed types are prevailingly starch trees.

By no means all the species studied could be classed definitely as starch trees or fat trees. The oaks, ashes, and hickories belong clearly to the former category, and the pines and lindens to the latter; but very many species are intermediate in character, possessing both fat and starch in the wood of the stem. In many instances, also, storage material was noted which was neither starch nor fat but seemed somewhat intermediate in character between the two. The outlines of the original starch grains could sometimes roughly be made out, but the starch content of the cell was apparently coalescing into an irregular brownish mass. This was insoluble in ether and stained neither with iodine nor Sudan III. Its bulky, opaque character indicated that it was actually storage material and not merely the cytoplasm of the cell. It was evident chiefly during the winter, occurring frequently in the cortex as well as in the wood, in cells which had been filled with starch. SUROZ (6) called attention to the existence of such material, but apparently it has not been noted by others. If it is indeed a stage in the transition from starch to fat, its composition might perhaps throw light on the difficult problem of the chemistry of fat production in the cell.

Table I presents a rough outline of the character of the food reserve in the pith and wood of the stem (twigs and young branches) of the more common trees and shrubs during the midwinter months, dividing them into those where fat predominates, those which possess considerable amounts of both starch and fat, and those in which starch predominates. This classification should not be regarded as rigid, since a considerable variation has been noted in some of the species and genera, but it represents the average condition observed for each. The character of the reserve in phloem and cortex of course is not included in this table.

The most noteworthy facts brought out by these anatomical investigations, however, concern the exact distribution of the reserve foods in the tissues and their changes from season to season. In all

TABLE I

TYPE OF FOOD RESERVE IN PITH AND WOOD OF STEM (TWIGS AND YOUNG BRANCHES) OF VARIOUS WOODY PLANTS DURING MIDWINTER

PREDOMINANTLY FAT	BOTH STARCH AND FAT
Aesculus	Abies
Betula (some species)	Alnus
Catalpa	Betula (some species)
Cornus (some species)	Chamaecyparis
Dirca	Ginkgo
Juglans (some species)	Gordonia
Leitneria	Juglans (some species)
Picea	Populus (some species)
Pinus	Prunus
Populus (most species)	Rhus (most species)
Pseudotsuga	Robinia
Rhus (some species)	Salix
Taxus	Sambucus
Tilia	Viburnum (some species)
Tsuga	
Viburnum (some species)	

PREDOMINANTLY STARCH

Acer	Itea
Ailanthus	Jamesia
Berberis	Kalmia
Carpinus	Lindera
Carya	Liquidambar
Castanea	Liriodendron
Celtis	Lonicera
Cephalanthus	Magnolia
Cladrastis	Nyssa
Cornus (some species)	Philadelphus
Crataegus	Platanus
Deutzia	Quercus
Diervilla	Rhamnus
Diospyros	Ribes
Elaeagnus	Rosa
Evonymus	Sassafras
Fagus	Styrax
Fraxinus	Symphoricarpos
Gleditsia	Syringa
Hamamelis	Ulmus
Hydrangea	Vitis
Ilex	Xanthoxylum

species starch disappears in the fall almost completely from phloem and cortex, and even in the starch trees it is much reduced in the wood as well. The reduction in the wood takes place first and most extensively in the regions *immediately around the vessels.* In many species starch is poorly developed here even in summer. It is in these regions, too, that the "transitional" material is very apt to appear. Furthermore, even in typical starch trees the wood parenchyma cells or ray cells which directly adjoin a vessel frequently contain fat; and in species where both starch and fat occur in the wood the fat is conspicuously abundant near the vessels. In the medullary rays of such forms as most of the poplars and willows, for example, fat is found chiefly in those ray cells which touch a vessel and starch in those which adjoin nothing but fibers. This tendency for starch to be absent and fat to be present in the immediate vicinity of the vessels is obvious in all woody plants in the midwinter season, and suggests that the character of the food reserve may be related in some way to the water supply.

Another anatomical feature which is clearly associated with the kind of food stored in a cell is the character of the cell wall. Wherever this is strongly lignified, thick, and provided with few and small pits, starch tends to remain unchanged throughout the winter. When it is thin or provided with many and large pits, starch tends to disappear and fat to be abundant. Thus in the storage cells of phloem and cortex, the walls of which are quite unlignified, starch vanishes early and completely and fat is very common. In the heavily lignified, thick-walled pith cells which occur in so many species starch remains throughout the winter, and in such cells the reserve food is less modified than in almost any other part of the stem. In branch gaps, where such a pith meets the cortex, the line between the starch-containing and the fat-containing cells is absolutely sharp and coincides exactly with the line between the lignified and the unlignified tissue.

A study of the vertical and ray parenchyma of the wood, the chief seats of food storage in the xylem itself, is particularly instructive in this connection, and furnishes us with a definite anatomical distinction between starch trees and fat trees. Where these cells are thick-walled and have few and small pits, starch predominates; where the walls are thin and well pitted, fat predominates. In hard-wooded species (fig. 1), long noted as starch trees, the parenchyma shares certain of the characters of the other wood cells and has thick, well lignified, square-cornered, and small-pitted walls.

In soft-wooded species (fig. 2), on the other hand, well known as being prevailingly fat trees, the vertical and ray parenchyma is thinner-walled and less heavily lignified, and the cells of the rays tend consequently to be irregular in shape, with oblique or bulging end walls, an outline quite different from the prevailingly rigid and rectangular one of starch tree parenchyma. They are well provided with pits or are so thin as not to require pitting.

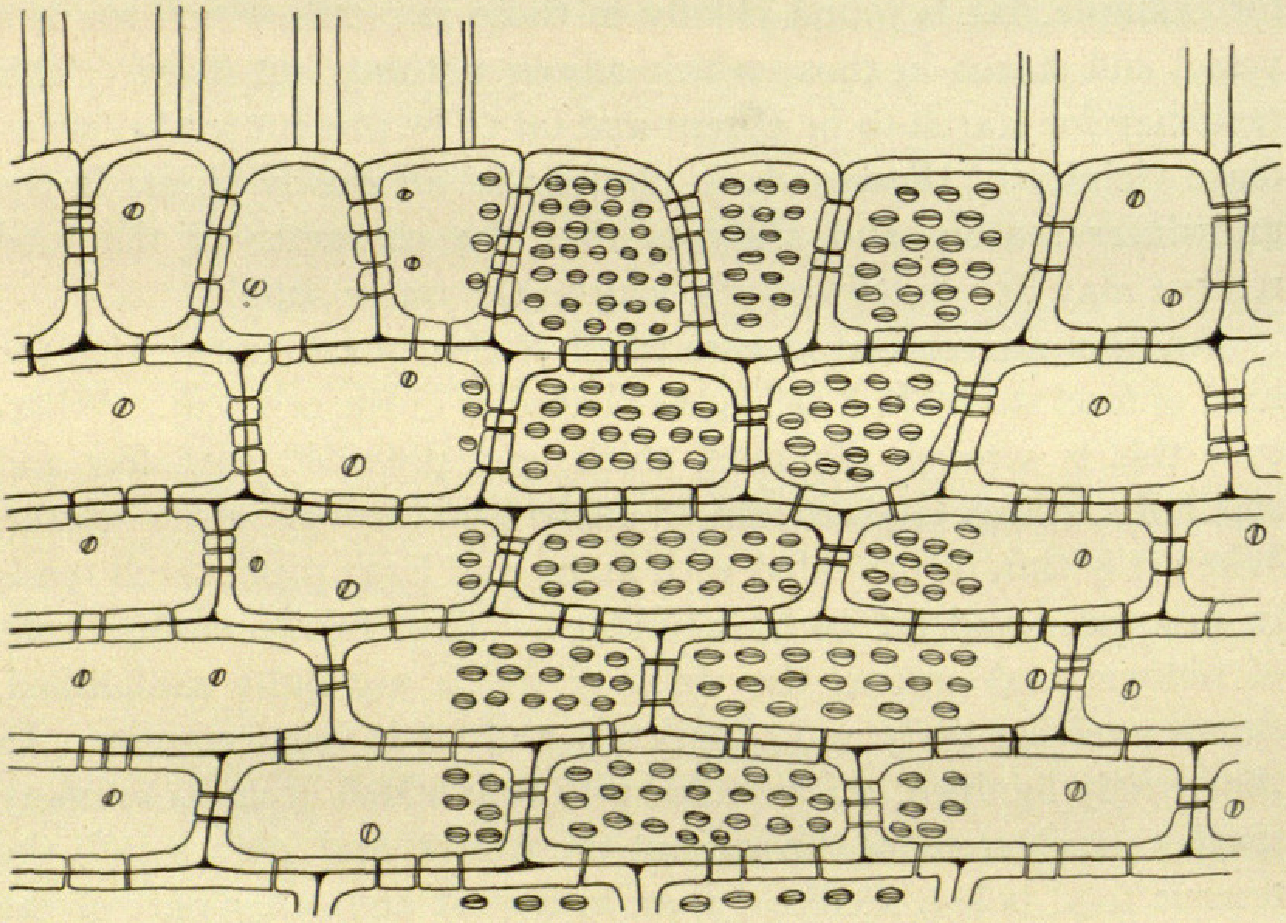

FIG. 1.—*Nyssa sylvatica*, a starch tree: portion of medullary ray of wood seen in radial section as it crosses fibers (at left and right) and a vessel (in center); note thick-walled, squarish ray cells; small pits between ray cells and from ray cells to fibers; and large pits from ray cells to vessel.

Of particular significance are the exceptions already noted to the general rule that there is a connection between hardness of wood and type of food reserve. The hard-wooded pines, for instance, are filled with fat, a circumstance evidently related to the fact that their ray parenchyma and resin canal epithelium (the only seats of storage in the wood) are unlignified and very thin-walled. In *Liriodendron*, *Magnolia*, *Ailanthus*, and *Platanus*, on the other hand, which are soft-wooded but which we have nevertheless observed to contain starch, the rays are made up of thick-walled rectangular cells precisely like those of starch trees. This type of ray cell is here evidently mechanical in its function, since all these species have wide rays which might collapse or be badly crushed were they not built of strong-walled cells. The vertical parenchyma of these soft-wooded starch trees tends to have thinner walls, and in *Liriodendron*, at least, it contains considerable fat.

On the basis of these facts we are forced to conclude that the hardness of a wood affects the type of food reserve indirectly,

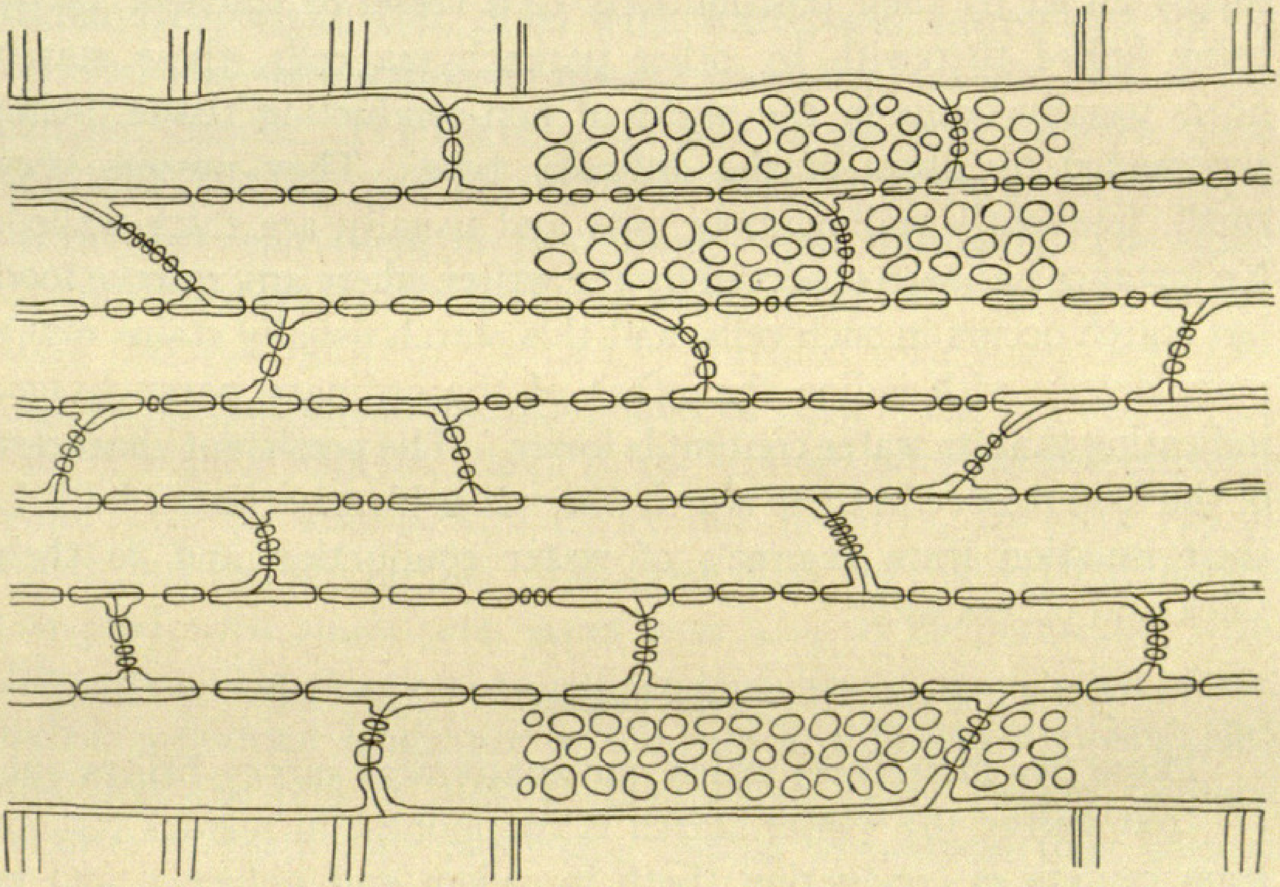

FIG. 2.—*Populus grandidentata*, a fat tree: medullary ray of wood seen in radial section as it crosses fibers (at left and right) and a vessel (in center); note thin-walled ray cells, with slanting or rounded ends, and large pits from ray cell to ray cell and from vessel to (marginal) ray cells.

through its influence on the walls of the storage cells. In certain cases, however, this effect is evidently more direct. The hard-wooded species of *Cornus*, such as *C. florida*, are starch trees, and the soft-wooded ones, such as *C. stolonifera*, are fat trees, although there is no very striking anatomical difference between the parenchyma cells of the two groups. The same fact is noticeable in the hard- and soft-wooded species of *Viburnum* and birches. In these cases it is fair to assume that the high or low degree of lignification of the conducting and fibrous cells is shared by the parenchyma, and that it is the actual hardness of the wall rather than its structure and pitting which is related to the character of the stored food. We shall later suggest a cause for this relation.

Conditions in starch fibers are of interest here. These are fiber-like, starch-containing cells, and are frequently found in the maples, willows, certain legumes, and other trees. Unlike the parenchyma cells, which are definitely connected with the water supply either by their position next to a vessel or tracheid, or by being linked therewith by other parenchyma cells, these starch fibers usually occur in the midst of non-conducting tissue, being surrounded by fibers of the ordinary type. They possess very small, frequently rudimentary, pits and usually are thick-walled. No instance has been observed by the writer where any reserve food but starch occurs in such cells, and this starch usually stains much more deeply with iodine than that of the ordinary parenchyma, indicating that its water content is lower. The persistent character of the food reserve in these starch fibers is evidently related both to their isolation from channels of water conduction and to their thick and pitless walls.

Discussion

These two main facts which our anatomical survey brings out, (1) that during the winter starch is commonest in regions remote from centers of conduction (both in xylem and phloem), and in cells with thick, well lignified, and small-pitted walls, and (2) that fat is most abundant close to vessels or tracheids, in the phloem, and in cells with thin or unlignified walls and large pits, at once suggest that *the character of the food reserve depends primarily upon the ease with which water or substances carried in water have access to the storage cells.* Where access is slow and difficult, the reserve remains in its summer condition as starch. Where access is easy, it is converted to a greater or less degree into other substances, with the consequent appearance of fat.

In storage cells the walls of which are unlignified, as in the phloem and cortex of all species, and the rays and canal epithelium of *Pinus*, starch is quite absent in the winter and fat is very abundant. There is evidently no impediment to thorough diffusion in such tissues, and conversion may take place far from any center of water conduction.

Where the wall of the storage cell has become heavily lignified, however, even though it is provided with pits (which in such cases are usually very small), diffusion seems to be much impeded, and the reserve food remains unchanged throughout the winter. This is well shown by the terminal cell in the medullary ray of a typical starch tree, which cell, filled with starch and surrounded by its lignified wall, abuts directly upon a starchless, fat-filled cell of the cambial region. There are small pits in the wall between these two cells, but the wall nevertheless seems to serve as an effective barrier between them in preventing rapid diffusion. The same circumstance may often be noted where a ray cell touches a vessel. Here the wall between the two is provided with many large pits (fig. 1), so that communication must be easy. The ray cell in this case is usually without starch in the winter and generally contains some fat. Its neighbors at either end, however, are often full of starch but contain no fat. The tangential ray cell walls, although provided with small pits, seem here also to be permeable with difficulty. This leads us to believe that heavy lignification of the wall is a decided hindrance to the ease of diffusion between cells, and that the pits in such a case are for some reason, perhaps because of their very small size, unable to perform their normal functions.

If the wall of the storage cell is less heavily lignified, or is thinner or more abundantly pitted, entrance of water is evidently easier, and we have noted that in these cases starch is more completely converted and fat is more common. In some instances fat may be limited to the cells directly adjacent to a vessel. In others it may extend to adjoining cells, starch occurring only in the more isolated regions. In these cases fat may be observed extending in from the cambial region along the rays for a considerable distance, thus indicating that diffusion takes place between the cells of the phloem region and the ray cells and affording a marked contrast to conditions at the ends of the rays in starch trees. In the true fat trees diffusion is evidently still more easy, for fat occurs throughout the rays and parenchyma, even in regions remote from centers of conduction.

In the cases of *Cornus*, *Viburnum*, and others which we have noted, where the degree of lignification of the walls of the storage cells seems to be a factor which determines the character of the food reserve, it probably operates by rendering easy or difficult the diffusion of water into the cell.

That the starch in starch fibers is never converted into fat is evidently due to the fact that there can be little or no communication between them and the water-conducting elements. The willows are illuminating in this connection. Here the phloem, cortex, and medullary rays contain abundant fat in the winter and very little starch, so that the willows have often been regarded as fat trees. In the last annual ring, however, there frequently occur large numbers of starch-filled fibers, so that some writers have included the willows among starch trees. These fibers are almost absolutely pitless. Diffusion of water among them must thus be a slow process, a fact which probably explains this persistence of starch here where it is lost elsewhere in the wood.

This hypothesis, that the character of the food reserve in a cell is dependent primarily upon the ease with which water or substances carried by water can pass from cell to cell, thus makes more understandable the various facts which have been observed as to the type and distribution of reserves in the stems of woody plants from season to season, and is the contribution of anatomy to the problem under discussion. With this as a basis we may allow ourselves to speculate a little as to just what factors are operative in causing the seasonal changes in the food reserve. That change of temperature alone is quite insufficient to account for these is shown by the fact that they take place in plants kept over winter in the greenhouse. The writer has also observed their occurrence in trees growing in the frostless area of the Gulf states. The changes are doubtless due in the last analysis to the action of enzymes, presumably diastase and lipase. There are evidently two quite distinct series of processes, those concerned with changes in starch, and involving the action of diastase, and those concerned with changes in fats, involving the action of lipase.

Two ways at once suggest themselves through which ease or difficulty of diffusion might affect this enzyme activity. First, the water content of the cells may be modified, those to which water has easy access having a higher content than those which are more isolated or protected. That such a condition actually occurs is indicated by the fact that the starch grains in cells near vessels usually stain more lightly with iodine than the others, showing that they possess a higher proportion of water. Differences in water content doubtless affect the whole physiological activity of the cell and may well determine the type of enzyme action. As to why, on this supposition, there should be such radical seasonal alterations, however, is not clear. There are doubtless changes in the water content of the tissues after leaf-fall and again at the spring awakening, and these changes will of course be felt most by those cells to and from which diffusion is easy. In the case of fat, at least, we know that abundance of water favors lipolytic action, and paucity of water favors the synthesis of fat, facts which probably help to determine the increase or decrease of fat with the seasons.

A second suggestion is that the enzymes themselves are carried by the water as it diffuses through the tissues, and thus effect the characteristic changes in the cells which they enter. That these changes take place in the fall we may perhaps ascribe to the presence of enzymes in the sap which is withdrawn into the tissues of the stem from the leaves before the latter are shed. The enzymes would thus be particularly abundant in the phloem, the ordinary channel of conduction from the leaves downward, and they probably would occur in the water of the vessels. They would be progressively less common in those parts of the plant farthest from the leaves. This would explain (1) why the changes are most marked in the phloem and adjacent regions and around the vessels; (2) why they are more marked in twigs than in branches and trunks; and (3) why in roots they are practically absent.

To determine whether or not lipase is actually present in leaves, a series of experiments was undertaken. The leaves of a number of species of trees were gathered in the latter part of summer, dried, and finely pulverized, and the leaf powder of each was mixed with olive oil and bottled up, a number of bottles being made for each species. The proportion by weight of powder and oil was the same in every case. These were kept at room temperature, and once a week a bottle of each species was taken, the oil was removed from the powder by filtration and was titrated against N/10 sodium hydroxide. The rate of increase of acid measured the strength of the lipolytic action and hence, probably, the amount of lipase. Results showed the ferment to be most abundant in the leaves of those species in which fat was commonest in the stems in winter.

To determine the exact method by which these seasonal changes in the food reserve are effected, however, is beyond the scope of the present paper, the purpose of which is to emphasize the important part evidently played by the minute anatomy of the stem and root in determining the ease of diffusion of water or solutions throughout their tissues and thus affecting the character, distribution, and seasonal alterations in the stored foods, and doubtless in other functional activities of the plant. We may point out that in any such physiological problem as this one a thorough knowledge of the structures concerned is absolutely essential before sound conclusions can be reached.

Summary

1. Previous observations upon the character, distribution, and seasonal changes of the food reserves of woody plants in temperate regions were in general confirmed by the present investigation and were considerably extended.

2. A study of the minute distribution of the food reserves in the tissues of the stem (twigs and young branches) during the winter shows that (1) starch is commonest in regions remote from centers of conduction and in cells with thick, well lignified, small-pitted walls; and (2) fat is most abundant in and near the phloem, close to vessels, and in cells with thin or unlignified walls or large pits.

3. These facts indicate that the character of the food reserve in any cell depends primarily upon the ease with which water or substances carried by water have access to the cell. Where the movement of liquids is apparently slow and difficult, the reserve persists as starch; where such movement is easy, starch disappears at the beginning of winter and fat is produced.

4. This suggests that differences in the type of food reserve may be due to (1) differences in water content of the various storage cells, resulting in modification of enzyme activity, or (2) differences in the ease with which enzymes have effective access to the storage cells.

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