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An Investigation of Apple Twigs.

BYRON D. HALSTED.

With a view of increasing the present knowledge of the minute structure of the twigs, including buds, of various varieties of apple trees the investigations herein described were prosecuted during the months of December (1888), and January (1889). The twigs were either taken directly from trees growing near the College or from a cellar where they had been stored in late autumn. In some cases samples were ordered from other parts of the state and examined upon their arrival. Primarily the investigations were carried out to determine the conditions in which the various food-materials, formed by the trees were stored up for future use; and in the second place to find if there was any marked differences in the amounts and qualities of these reserve food substances or any other constant differences among the varieties subjected to microscopic examination.

An ordinary apple twig of the last season's growth consists of the following parts: (1) A central cylinder of pith which runs the whole length, ending at the tip in the terminal bud. This pith consists of many sided cells or sacks, which are about as long as broad and packed so closely together as to leave but few cavities called intercellular spaces between them. The cells nearest the center are usually largest and the outermost smallest, and have the thickest walls. (2) Next outside of the pith is a ring or zone of wood, which is very thin at the upper end of the twig but gradually becomes thicker as the basal end is approached. This wood consists of long ducts or vessels running lengthwise of the twig and surrounded by slender thick-walled flexible wood cells. The wood is the most substantial part of a twig and makes up the greater part of any tree. In the wood ring of the apple twig are thin plates of pith-like cells which reach from the pith to a thin belt of cells capable of growth situated just outside the wood, and called the cambium layer. These plates of thin-walled cells known as the medullary rays are here specially mentioned because they play an important part in the storage of the reserve food substances formed by the plant.
during the growing season and stored away to be employed during the initial growth in early spring. Besides the vessels, wood cells and medullary rays in the wood ring there are also long rectangular cells in many respects not unlike the pith, which extend lengthwise of the wood and therefore at right angles to the general direction of the medullary rays. These may be styled the wood pith cells and will be referred to again in the body of the paper. (3) The cambium layer, above mentioned, is made up of small thin-walled cells and constitutes the soft layer at which the wood and bark may be separated especially in spring. Boys take advantage of the ease with which the bark may be wrung upon many trees, in making their common whistles. The radiating plates of pith cells which reach from the pith to the cambium, and above designated as the medullary rays, project beyond the cambium and broaden out in the loose cellular part of the bark that lies between the somewhat interrupted ring of bast and the cambium. (4) This bast is the tough fibrous portion of the bast and consists of small bundles of long thick-walled cells which run lengthwise of the twig and are usually midway between the rind and the cambium. (5) Upon the outside of all is a double layer of thick-walled cells. The outer may be styled the cuticle and beneath this is a much thicker layer of firm tissue with the contents of many of the cells colored green. The outer and thinner layer bears much of the coloring matter which gives mature apple twigs their characteristic reddish brown color. Between the rind and the ring of bast is a belt of loose cellular tissue abounding in cavities between the cells known as intercellular spaces. This is the loosest tissue in all the apple twig and may be called the pith of the bark. It however differs from the true pith, in the center of the stem in having the cell contents colored green. It is often and appropriately called the green bark and is the part exposed when the thumb nail removes the rind of a twig but does not pass deep enough to reach the wood layer.

Therefore to recapitulate, the apple twig consists of the following parts beginning at the outside: A double rind or protective covering of thick-walled cells more or less impervious to water and bearing the matter which gives the twig its bright color. Within this is a broad loose belt of green cells and large intercellular spaces which reaches to the tough fibrous band of bast. Succeeding the bast, passing inward, is another loose belt of cellular tissue narrower than the one outside the bast into which the medullary rays project, as they
pass through the cambium layer. Next within this cambium is the wood with its medullary rays which reach to the central cylinder of pith. The above description considers only sections that might be made through the twig at any point between the buds. At the nodes or points where buds are developed there are certain modifications of the structure which need to be considered. At the free end of a mature twig is the terminal bud. This consists of a cone of minute thin-walled cells upon the outside of which the small imperfect leaves, as bud scales, arise in regular order, and overlapping each other inclose the tender growing point of the twig. From the scales, bundles of fibres and vessels descend and form a thin ring of wood around the pith just below the growing point. At this portion of the twig the pith makes up the greater part of its substance. In short, the bud, the stem and for a quarter to a half inch below it are made up of soft tissue easily crushed with the thumb and finger. But below this the large central pith is particularly rigid due to the unusual thickening that has taken place in the walls of the cells. If the reader will bear in mind that the wood zone is very thin at the upper part of the twig, and that the bast is almost wanting it will be evident that the pith alone must give the rigidity, found near the extremity of natural twigs. From the shape of the nearly spherical cells it follows that there can be no very great toughness. Contrariwise brittleness characterizes the upper portion of a well matured apple twig and for two or three inches below the terminal bud it should snap almost like a pipe stem when sufficient side pressure is applied.

The lateral buds in general structure closely resemble the terminal ones but are much smaller and have a somewhat different attachment to the twig. The soft cone of small cells rests upon an abbreviated stem from which the outer scales arise. Where the bud is connected with the twig there is an interlacing ring of fibres and vessels and within this a central pith, which is modified as spoken of while treating of the terminal bud; that is, the cells are very thick-walled having undergone the process known as lignification—a characteristic of the durable parts of most woody or ligneous plants. As the lateral buds are often close to the twig upon one side there is a consequent lack of symmetry. The exposed surface naturally has a greater development of the protective layers, the bud scales being both thicker and more numerous upon the outside than next to the stem. There is also more of the red coloring matter in the exposed parts. Over all parts
there may be a coating of soft down consisting of colorless hairs which grow from the epidermal or outer layer of cells of the bud scales and the body of the twig. As the twig matures this hairiness is quite easily removed so that its presence or absence in midwinter is largely accidental.

At the point of union or junction of two years growth of a twig as of 1887 and 1888, there are but few structural features not included in the description of the twig already given. The point is easily determined from the outside, for the scars of the several scales of the terminal bud of the previous year remain to mark the place, which is somewhat larger in cross section than the twig, an inch or so below and above it. It also is a starting point from which the buds become more distant, proceeding either up or down the twig. If these points are not sufficient, the brighter, fresher red of the last year’s growth will be quite sure to distinguish the place of union. With a cross section of the twig under the microscope, the age in years is quickly determined. The first wood formed on a yearling twig in spring is more porous than that last produced the previous autumn. It is on this account that the wood of an old branch is arranged in evident rings, and if there has been no interruption in the growth of the plant during any summer, there will be a ring for each year, the thickness and porosity of which will vary with the season’s favorableness for growth. Microscopic inspection of the junction does not reveal any very marked modifications of structure. The cylinder of pith is larger here (often twice the diameter) than just above or below; its cells are smaller and thicker walled and inclined to be elongated and somewhat in rows longitudinally, as if to add rigidity to this portion of the twig during the early spring growth, and give general stability at a point where elements of weakness naturally obtain from the union of the wood of two different years. Another service of this pith at the junction will be mentioned when we come to consider the storage of reserve materials. Below the junction the pith contains a cone or thimble, the outer cells of which are usually tinged with brown as if dead, and in shape and position it accords with the exterior of the lignified pith which has been mentioned as beginning a short distance below the base of the terminal bud. With certain chemical re-agents and coloring materials this thimble or cone of pith, surrounded by structurally almost identical tissue, behaves the same as that at the tip of a well matured twig, and easily met with by cutting down through the terminal bud lengthwise with a razor or sharp knife. It is
evident therefore, that as the terminal bud undergoes development in the spring the soft thin-walled cells at its base become lignified and while they are finally apparently the same in structure as the cells below them which were lignified the autumn before the line of separation is not obliterated, but instead may be usually observed, without the aid of a magnifier, by making a longitudinal section through the junction. A fair knowledge of the structure of the point of union as well as the other parts of the apple twig is so important to a satisfactory understanding of what follows that this part of the paper has been extended even at the risk of being tedious, especially to those whose attention has for a life time been almost daily drawn to the subject. The horticulturist will, it is hoped, readily verify many of the statements herein made and he doubtless could add others gathered at the grafting bench or elsewhere, but here omitted because non-essential and to avoid too great length.

**Reserve Food Materials.**

Plants which live from year to year do not use up all the nourishment prepared by the green parts—principally the leaves, during the season of active growth. They lay by a portion of this material to be employed in the vital processes at times when the plant cannot work over the crude substances which are obtained from the soil and the air. In short, the apple tree during the growing season stores some of its elaborated substance in places where it becomes available for nutrition in the early spring while the plant is putting forth its young twigs and leaves. This reserve material as it is termed, may for convenience be divided into two groups: namely, those which are known as carbohydrates; so called because consisting of the elements of water and that of charcoal. In other words the carbohydrates consist of carbon, hydrogen and oxygen united in definite proportions. The leading carbohydrate is starch, familiar to every one as the basis of many foods for animals and man, as found in potatoes, corn and a long list of other vegetable products. Oil is another reserve form assumed by the carbohydrates and abounds in many seeds and other parts of plants. Sugars, which by themselves make up a group of the carbohydrates are often found associated with the other forms of reserve food material above mentioned. There are other forms of carbohydrates but they do not specially interest us in this paper.

The second division of reserve materials, suitable for plant nutrition, is known as the albuminoids, so named from a resem-
blance to the albumen ("white") of egg. Proteine is another term given to the same group of substances, all members of which agree in having nitrogen in their composition—a substance which is absent in the carbohydrates. The group of proteids or albuminoids does not admit of separation into such distinct substances as oil, starch, and sugar under the carbohydrates. They are more complex and less stable compounds than the carbohydrates and are stored usually as amorphous contents of cells. Sometimes however they assume the form of grains (aleurone) or crystal-like bodies (crystalloids) and in these condensed conditions may be met with in seeds, like beans and peas, which are unusually rich in the albuminoids. The proteids are the basis of protoplasm and protoplasm is the substance, usually in a semi-fluid condition, which is invariably present in every living cell. As protoplasm is the complex compound in which life always manifests itself, the importance of the albuminoids out of which protoplasm is made, becomes self-evident.

Doctor Vines in his new work, in treating of the repose of the above compounds says:—"When once deposited the reserve materials undergo no change, or at most the proteids may slowly undergo some alteration, so long as the organ in which they are deposited remains in an inactive condition. An organ in this state is practically dead for the time being, all its metabolic [active living] processes being arrested. It is capable moreover of resisting injurious influences such as extremes of temperature and dessication [drying] which would prove fatal to it—were it actively living. It is obviously in consequence of this property possessed by such organs during what we may term this state of suspended animation that vegetation is maintained in regions in which the cold of winter is severe and in arid tropical regions. The time of the possible duration of this state without permanent loss of vitality varies very widely." It is well known for example that some seeds retain vitality for a long time especially starchy ones. With the quickening influences of warmth and moisture supplied by spring time, the reserve materials undergo changes which convert them into substances that can readily travel to seats of vital activity when they are employed in growth.

STARCH:—Returning to the carbohydrates, they will be taken up in the order of their importance as present in apple twigs. The test for the detection of starch is the very satisfactory one of iodine solution, which turns starch blue while it fails to produce this color in other cell contents of the ap-

*Physiology of Plants, 1886, p. 172.
pie twig. By means of this re-agent, starch is found quite generally in the pith, medullary rays and wood-pith of the various varieties of apple twigs examined, and usually in the form of compound granules the component parts of which separate quite easily. The resemblance of an average grain, in general shape, to that of an apple which has been quartered or cut into smaller parts with each piece somewhat separated from the others is not altogether fanciful. Imagine an apple, as often prepared for a dumpling, and the reader will have, on a greatly magnified scale, the appearance of a minute starch mass as seen with the higher powers of the microscope. These masses or compound granules vary greatly in size and the smaller particles of starch do not exhibit this compound nature. Starch-bearing cells of well-matured twigs may contain hundreds of these granules and then they are as completely filled with the nearly spherical masses as a nutting bag with walnuts after a successful visit to the forest in autumn.

Twigs were supplied for the investigations under the disguise of a number or letter and the evident intent was to have sorts differing widely, in some important particulars, studied at the same time and as parallels. In this way it was impossible for any personal factor on the part of the investigator to enter into and give unintentional coloring to the results. For example No. 1 was Tetofsky, a well known standard variety in the state. It also appeared elsewhere in the investigations under B. Duchess figured in the notes as No. 7, while Red June was No. 9, Ben Davis No. 10, and so on.

As an example of the method observed in the investigation some items from the note book for No. 1, are here given, as they bear upon the amount and distribution of starch. First a thin section made with a razor was taken lengthwise from the center of the terminal bud. This showed no signs of starch. A thin transverse section, i.e., directly across the twig at a right angle to the main axis, was next made one inch below the tip and treated with iodine solution. At this point the pith was proportionally very large and the wood a narrow ring. The pith was well filled with starch as recorded by the drawings made at the time with a camera, that is, an instrument for throwing an accurate picture of the object, under the microscope directly upon a sheet of white paper where it can be traced with a sharp-pointed pencil. The medullary rays also abounded in starch but none was found outside of the wood. A third section was made midway of the twig that is nine inches from the tip. Here the zone of wood was much thicker than in the section taken eight
inches above. The pith for the most part contained starch but there was less in the center where the cells are larger and thinner walled than those nearer the wood. The next section was made near the base of the twig, just back of the thin layer of substance that had dried out while the twig had been stored in the cellar. Starch distribution was about as in the section taken midway of the twig. The last section in this series was made through the dried tissue at the base, and this, although much slower in its action with re-agents, (iodine, etc.), was about the same as the section taken just above it in the green part. The starch was not in this instance removed in the process of drying. A section was next made through the twig and bud (the previous cross-sections having been made midway of the buds) and in this the starch was more abundant upon the side of twig bearing the bud and the pith of the very short stem of the bud consisted of thick-walled cells,—much thicker than those in the pith of the main twig and densely packed with starch. This bud-pith extends upward from its union with the twig-pith at a sharp angle and sections taken directly across the twig cut it midway between the longitudinal and transverse line. It was found best to make sections lengthwise and in the plane passing through the center of both twig and bud. In such a section it was determined that the starch did not extend into the base of the lateral bud beyond the line made by continuing that of the outside of the wood above and below the bud. In short, should the horticulturist with his budding knife cut off a well matured bud and the surrounding bark without touching the wood, he removes no starch but instead leave exposed a cut-surface in the center of the wood of which is a core of dense gritty cells packed with starch. The surrounding wood at this point contains much more starch than the same contents elsewhere, the least being midway between the buds.

Further study of No. 1 consisted in examining sections made in various other directions. For example, in sections obtained lengthwise of the twig some were cut with the surface of the section parallel with the radius of the twig, a section from exactly the middle of the twig would give this, while others were at right angles to this, which may be obtained by longitudinal cutting at about midway between the exterior and the center of the twig. It is evident that in the former or radial section the medullary rays (called silver grain in maple for example) will appear as plates, while, in the section at a right angle to this, the rays will be cut transverse. In this way the length and breadth of all cells may be deter-
mined and their relation to each other in the vegetable fabric better understood.

Also the higher powers of the microscope (including an immersion lens) were employed the details of the results of which are too lengthy to warrant even an outline here. One point of some importance in this connection however may be mentioned. It was with the high powers that a satisfactory demonstration was made of the starch-bearing cells which run lengthwise of the wood and have been previously designated as the wood-pith. These cells vary in length but are usually four or more times as long as broad having many small pits in the moderately thick walls, especially at the adjoining ends. These cells are about the same width but usually longer than the cells of the medullary rays and extend at right angles to them. In this way the wood may become quite thoroughly charged with the starch and under the microscope a longitudinal radial section when treated with iodine appears not altogether unlike a blue mosquito netting, the vacancies in the netting representing uncolored and therefore starch-free tissue.

A parallel study was made of No. 1 (Tetofsky) upon small twigs which were the result of slow growth upon trees less favorably situated than those producing the large finely developed freely grown specimens examined under the laboratory name of "No. 1 large." The differences between the two sizes of the Tetofsky can be quickly stated. The various portions of the twigs bore the same ratio to each other but there was less, that is a lower percentage of, starch in the small than in the large twigs,—a condition of things naturally to be expected when the relations of reserve materials to previous vigor of the plant are considered.

Whitney was the next variety (No. 2) examined and it was carried through the same treatment as No. 1. No starch was found in the terminal portion but elsewhere the central pith and the medullary rays and wood pith contained an abundance. In short no difference in the matter of starch distribution could be seen between this and the Tetofsky. The same can be said of No. 3, which was the Wealthy apple.

After a few varieties had been studied singly sorts were taken up in pairs as for example Tetofsky and Citronat—the latter is one of the Silesian importation of apples. The points of difference can be best brought out by giving a brief outline of the parallel work done upon these two sorts. Twigs of equal length and of as nearly the same size as possible were supplied under the disguise of A. and B.; the former after-
wards proving to be Citronat and the latter Tetofsky. Only
the lower part of each set of twigs was furnished at the out­
set, namely three inch pieces from fifteen to eighteen inches
from the tips. The notes as they were made are here given,
with the omission of such items as do not bear directly upon
the question of starch and its storage.

In a transverse section of twig of Citronat fifteen inches
from the terminal bud, the pith about equals in diameter the
thickness of the wood zone and the cells for the most part are
thin-walled and empty, i. e. filled with air; here and there
yellowish thick-walled cells were scattered through the pith
which when examined in longitudinal section were arranged
in rows and made up the only portion of the central pith bear­
ing starch. The outer part of the pith close to the wood has
its cells small, thick-walled and similar in structure and yel­
owish color to the central ones above mentioned, and are
packed with starch. With a high power of the microscope
each of those starch bearing cells is seen to have, its thick
wall nearly punctured by from thirty to forty small pits or
pores which are so arranged as to meet corresponding pores
from adjoining cells with only a thin membrane between.
This is mentioned to show that while starch-bearing cells in
the apple twig are thick-walled there are numerous thin
places which readily permit of the rapid circulation of their
contents when the starch assumes a liquid form for transpor­
tation. The cells of the medullary rays and the starch bearing
pith cells, which extend lengthwise between the wood fibres
have many of these pits or passages, and the wood itself is
very porous from the presence of a large number of ducts or
vessels. With iodine the medullary rays become deep blue
as also the wood pith cells before mentioned.

The bark is about one-half as thick as the wood and in a
general way is divided into three parts, namely: the rind,
green bark and bast, points which have been treated of under
the general structure of apple twigs.

In B (Tetofsky) the pith was somewhat smaller than in A,
the wood zone much thicker and the bark of about the same
thickness. The diameter of the twig was one-fourth greater
a fact which should be borne in mind in this consideration.
Nearly all the pith cells in B are thick-walled and bear starch
although many near the center are only partly filled. There
is not the contrast between central and outer cells as noted in
A. In a general way it may be stated that in A the central
pith is mostly filled with air while in B. it is well charged
with starch. When empty cells occur in B. they are larger
than the others a fact that is well demonstrated in the longi-
tudinal sections.

The wood zone in B, which makes up a much larger part of the twig than in A, is slightly more solid; that is, camera
drawings of transverse sections, for example of the two vari-
eties show that the Citronat wood has a few more vessels in a
given area than has the Tetoisky. The wood of the latter is
somewhat denser in the outer half, in fact grows gradually
less porous passing from the center to the cambium. The
wood pith cells are more numerous in B. than A. although
the difference is not marked. In the bark the dark red rind
is thicker in B. than A. and the density of the broad layer of
green bark is greater. In A. the bast; that is, the tough fi-
brous portion of the bark, is about midway between the rind
and wood while in B. it is fully two thirds the distance from
the exterior to the wood. The difference in the texture of
the bark is evident from its behavior under the section cutter
(Thome); in A. it breaks readily on a line separating the in-
er surface of the rind, and green bark beneath it, and also
along the belt of bast. The bark of B. separates most easily
along the cambium layer. In other words B. has a tougher
bark, which holds together better than that of A.

Longitudinal sections were made through the lowest lateral
bud of each variety. In A. after treatment with iodine, the
starch is seen to not penetrate into the bud but is so densely
packed in the cells at the base of the bud as to mark a well
defined line separating the starch bearing from the non-starch
bearing tissue. It was very evident that the starch was more
abundant in the wood upon the side bearing the bud. The
corresponding bud in B. has the scales thicker and more
closely placed; but the most marked difference is the abun-
dance of starch in the bud, the basal portion of the cone of
which, is quite highly charged with this substance.

The next sections were made one inch from the tip of the
twigs. In A. the rind is thick and green while the wood con-
sists of a very thin layer and at some places in the ring is al-
most wanting. The pith is without starch in the center but
has an abundance in the angles between the wood bundles
and the medullary rays. The empty pith cracks badly and
is of an ashy color. In B. the rind was not green; wood
much thicker than in A. and the pith and medullary rays uni-
formly containing much starch. The bast is nearer the
cambium than it is in A. A section made lengthwise of a
lateral bud near the tip contained starch but not so much as
the one examined fifteen inches below. No starch was found in a corresponding bud of A.

Fresh material of the Citronat variety was gathered from the college nursery the special point being to examine those twigs which were green at the top and had not at that time (Dec. 6th,) dropped the upper leaves. The notes made upon this portion of the work in abbreviated form are as follows:

Starch abounds in the terminal portion of the twig including the bases of the large bud scales and the stalks of the remaining leaves. One inch below the tip there was but a small amount of starch in the loose pith except next to the thin zone of wood. At a foot from the tip the starch is mostly in the outer layer of pith and the medullary rays. Two feet below the tip starch was found in considerable quantity outside the cambium scattered throughout the loose bark. A section lengthwise of a bud was made at this last point and starch was generally found scattered through all parts of the bud especially in part between leaf scar and bud. Starch was abundant in the pith of the twig at this place. Sections were made through green leaves found upon the upper portions of the twig and starch was found only in the leaf stalk.

It is a source of regret that a parallel study of B. could not be carried out from a lack of fresh material available at the time. Several twigs of B. from the cellar were examined January 24th, about seven weeks after the first investigations upon this variety with the same result, namely the starch was abundant in the buds. In those twigs which were partially dead the starch was wholly or in part absent from the pith; in those in which fermentation had proceeded farther there was very little starch in the wood, and some of the most decayed, with fungus threads all through the bark and forming spores in large pits under the rind or rupturing it, had the starch still in the buds. It would seem from this study that when low forms of life prey upon cut twigs in the cool room the starch is first withdrawn from the large central cells of the pith, afterwards the wood and last from the buds. It will be remembered that in the inspection of No. 1 no starch was found in the buds while a short time after, the same lot of cuttings furnished material under B. which showed lateral buds with an abundance of starch. On January 29, after the above was written, twigs of Tetofsky fresh from the tree were obtained from a distance and in those no starch could be found in the buds. It will be remembered that the Citronat twigs which were examined from the cellar showed no starch in the buds while those collected fresh from
the nursery were immature and had an abundance of starch in the buds. The thought occurs that in case of the Tetofsky with starch in the buds it may be possible that the twigs were cut before they were ripe or else under the condition of a warm cellar there may have been a transfer of the starch after the twigs were stored.

Twigs of the Boiken apple were subjected to an examination similar to that outlined for Tetofsky and Citronat. Fresh material was collected from the College nursery and the twigs had the appearance of being immature, that is, green leaves were remaining, and no well formed terminal buds were upon the twigs under investigation. Starch was found very generally distributed but not densely stored in any part. There was much starch in the soft bark. Lower down the twig, that is, twenty inches from the tip the lateral buds were well formed and contained no starch.

A parallel study of two varieties was carried out under the numbers 8 and 9 representing respectively Repka Keslaja, one of the government importations from Russia and Red June a standard old variety. The lengthy details of this portion of the investigations, which covered a week of time and included the comparison of sections from all parts of the twigs are purposely omitted.

It should be stated that the Red June twigs were the larger, being on an average about one-third greater in diameter. Both sorts had remarkably red bark this being due to a strawberry colored liquid in the cells of the inner rind. The bark of the two varieties is somewhat different. In No. 8 the soft portion called the green bark has large intercellular spaces formed by the cells being arranged somewhat in threads which extend irregularly in the general direction of the circumference of the twig. In No. 9, the bark while proportionally no thicker is somewhat denser, and does not break along the line of the soft green cells with the ease characterizing No. 8. The layer of bast is about half way from the wood to the rind while in No. 9 it is about one-third the distance and the cambium is correspondingly broader in No. 8 than No. 9. There is no observed difference in the amount of starch and its distribution in the pith and medullary rays.

The most evident difference in these two varieties is the freedom of starch from the buds and bark of No. 8 and its presence in these parts in No. 9. The sections of the former when treated to clearing re-agents became clean of the starch-bearing portions mainly the pith and wood were well defined, while under the same treatment the sections of No. 9, showed
the blue-black granules of starch in all parts. If the expression could be indulged, the Repka Keslaja has the appearance of having made a clean cut growth and finished up its work before winter set in. On the other hand the Red June gives evidence of having been caught by the autumn frosts before the twig had “gathered up the loose ends” preparatory to the period of repose.

A double series of observations consisted in studying first a set of three sorts, carrying each through the same treatment followed by a group of three others and afterward comparing these two parallel sets. In the first set the following varieties were considered, namely: Repka Keslaja, Recumbent, and Hibernal—all apples which have proved well adapted to the peculiar conditions which obtain in Iowa. With this set was compared a group consisting of the Red June, Wine Sap, and Ben Davis.—sorts which have been unsatisfactory in the orchards of the state.

The Repka has the smallest and the Hibernal the largest twigs when those of the same age are compared. In Repka the terminal buds are small, larger in Recumbent and largest in Hibernal. Repka had the twigs somewhat wrinkled while in the other two they are plump.

There were no marked differences in structure of the wood or pith, none for example that would not come within the range of variation for twigs of the same sort. The bark of Repka had a loose green layer which breaks more readily while making the section than that of the other two sorts, having the inner bark of a firmer texture. The investigations were of course not sufficiently extended to lead to the conclusion that this difference in the bark was constant under all circumstances. It may be that the Repka is better provided than the others for withstanding those destructive agents which work when the liquids in twigs undergo alternate freezing and thawing. Possibly the Repka permits of a greater drying out of the moisture of the twig and this might have an influence upon the resisting power of the variety to changes of temperature and other surrounding conditions. As all the twigs of the three sorts were cut at the same time in autumn and stored under like conditions in a cellar, there seems to be no external influence causing the drying out and wrinkling of the Repka bark which did not act upon the others.

After treatment with iodine none of the varieties showed any starch in the buds. Longitudinal and transverse sections of Repka exhibited abundance of starch throughout the
whole pith, less however in the center than near the outside. In Recumbent the starch of the central pith is borne only in certain cells and the same was true of Hibernal. As the Repka twigs were smaller than the others it was estimated that there was about the same total amount of starch for equal lengths of the several twigs taken at the same distances from the tip.

The second set of apple twigs, consisting as before stated, of Red June, Wine Sap, and Ben Davis, showed no marked differences in internal structure and agreed very generally in all particulars with the three of the first set. In other words the sections of any variety could not be selected from a mixture of the six, unless it be the Repka, on account of its bark. This holds good particularly of sections made through twigs some distance from the tip. When the extremity of the twigs is considered there are differences and this is true for some distance from the terminal bud. But these differences were due to degree of maturity and not constant between any two varieties. The first set exhibited twigs which were usually well matured and the pith a short distance below the terminal bud had become lignified and uniform throughout the whole section. In many of the Ben Davis twigs upon the other hand there was no well formed terminal bud, the cone of lignified pith had not developed, and starch was not shown in a definite line as seen in ripe twigs. Likewise the Red June in many instances exhibited the same lack of maturity, and in such cases there was general distribution of the starch, as before mentioned for this variety treated elsewhere.

In twigs that had completed their year's growth there was the characteristic lignified starch-bearing cone of pith and no evident microscopic differences between the two sets of twigs. While under ordinary conditions it may be true that there is a considerable difference in the upper parts of twigs of Repka, Recumbent, and Hibernal, and those of Red June, Wine Sap, and Ben Davis, it is evident that in an exceptionally favorable year like the last one may finish their growth and then the microscope does not determine differences which in the light of the present investigations may be considered characteristic of any sort. In other words, the differences which may be found between an immature twig of Ben Davis and a mature one of Hibernal may be found between the mature and unripened twigs of either of those varieties. If one sort is more inclined to extend its period of growth into the late autumn than another it follows that that variety will have less matured wood and the recognition of this fact does not require the use
eye and trained fingers can determine the condition of a twig whether mature or not with a good degree of accuracy. Fresh material of Duchess and Landsberger Rennet was obtained in abundance from the nursery and orchard and the inspection yielded the following results:

Well matured twigs of these two varieties are very much alike in outward appearance, color, shape, size, etc. Terminal buds are large broad nipple-shaped. The Landsberger has the greater hairiness. The interior however is not the same. In longitudinal sections the Duchess has the pith well filled out, but little rusty, brown color, and leaving a smooth surface when cut. In Landsberger the pith is rusty, and large flaws are found in it. About a half inch below the tip of the terminal bud in the Duchess is a well-formed lignified cone of pith which is very rigid, and gives the upper part of the twig the stiffness so noticeable in many sorts. The bark and very thin layer of wood lying over the lignified pith may be easily removed, leaving behind the pith which is so brittle as to snap like a pipe stem when sufficiently pressed to one side. This pith lower down the twig is not so much lignified and being overlaid by a considerable zone of wood there is no demand for this provision for rigidity so much needed at the tip where the wood is almost wanting.

The pith is much less in area at any other point in the twig than at the cone an inch or so below the tip. In the Duchess the cone cells are densely packed with starch, and the only vacant places are a few intercellular spaces of small size scattered through the dense area of thick-walled parenchyma. A foot below the tip the pith is fairly well filled with starch except in the center. But at this part in the twig the wood is of considerable thickness and the starch abounds in its medullary rays.

In the Landsberger the cone of lignified cells is imperfectly developed. At its apex it has no flaws but below the central portion of it the pith is wanting. The whole twig is therefore much less substantial than the Duchess. Transverse sections were made through the two sorts of twigs at one-half, one inch, three inches and one foot from the tip. There is very little difference in the bark, that of Landsberger being less firm. The rind and bast were not materially different. The chief difference was in the wood and the pith which has been noted above. The wood of two sorts as to porosity and other microscopic qualities was practically the same, but the quantity differed greatly. In Duchess the zone was twice as
thick as in Landsberger and the pith correspondingly smaller for the two twigs were of the same size at the place sectioned. More than this, the Duchess pith was perfect at all these places thick walled i. e. lignified and well filled with starch, while in Landsberger the central pith was wanting and only about half of the whole area thick walled and starch bearing. Fresh twigs of two sorts under trade numbers namely, "290" and "984" gave in brief the following results: 290 has the larger twigs with good sized terminal buds approaching the Duchess in general appearance. The upper part of the twig is coated with a felt of hairs not easily rubbed off. The 984 is below medium size with terminal bud medium and abundance of lateral buds close below it and not very hairy. The cone of lignified pith below the terminal bud of 290 is very solid and the pith below colorless and without flaw, while 980 has the cone less well developed and a vacancy in the central pith reaches up to near the tip of the cone. Below, the pith is rusty and full of flaws. The writer should judge, from the specimens, that 290 was superior to 984 as regards the ability to bear the trying conditions which surround the apple tree in Iowa. As to the history of the two sorts, the quality of the fruit and the other elements which serve to give these varieties their rank, the writer has purposely kept himself ignorant that the reader who is familiar with the sorts in hand may be able to judge how far the microscope may be trusted in matters of this kind. Let it be borne in mind that this inspection was made near the close of the investigation and is based upon all the experience which the previous work could give.

Just as the investigations were being brought to a close, a bundle of twigs was received by express from a distance and examined; only one of the twigs had a terminal bud—the bundle being made up of material suitable for scions. This bud was small, poorly coated with hairs and not brittle below. With iodine no sharp line of starch was found at the top of lignified pith. There was an entire absence of starch from the lateral buds. And, judging from the size of twigs, amount of starch and especially from the tip, there was feebleness throughout together with signs of immaturity.

The investigation of the terminal portion was necessarily limited and it may be that the next twig might have given different results. The twigs were of the Iowa Blush—a variety in which some horticulturists of the state are much interested.
From all of these investigations it may be concluded, so far as the question of starch is concerned, that it prevails in all sorts of apples and that it is most abundant in those twigs which have come to maturity. When "water sprouts" and tree-top twigs of any sort are examined the starch in the former is found confined to the outer pith and the medullary rays and in these mostly in the vicinity of the buds, except the terminal bud which may have none within an inch of the end of the twig, and that only scattered in small quantities along the inside of the thin zone of wood. The twig from the top of the tree and fully matured will have all parts well developed, including buds, the pith and wood well charged with starch and the rigid tip of the twig, a short distance below the ripened terminal bud, with its pith sound and composed of thick walled cells filled with starch. As far as the examinations have gone there is nothing in the quantity, quality or disposition of the starch which may be taken as a distinguishing characteristic of any variety. On the other hand it seems clear that the iodine test, is a good one for maturity of the twigs of any sort.

In this connection it may be said that the junction of two years growth is characterized by a large area of pith which is lignified—points which have been developed elsewhere—and in this portion and the surrounding wood there is a large amount of starch. In slow growing twigs there are thence reservoirs of reserve substance at frequent intervals sometimes not more than four or six inches apart. The wood of the main stems of apple trees six or more inches in diameter was examined and throughout starch was found but in less amount than in a yearling or two year old twig. Starch was also found in wood upon the south side of trees where, from injury, it had been dead for several years and nearly overgrown with new wood and bark.

SUGARS:—Tests for sugars were made in a large number of instances; grape sugar was generally present in variable quantity in all lateral buds, but as a rule there was least in the tip of the twig. Cane sugar and dextrine were likewise present in most cases, but in small quantities. Grape sugar was most evident near the growing points and cane sugar, if it appeared at all, was in the fine unlignified tissue at the base of the bud. In view of the fact that starch is readily changed into sugar within the plant under conditions similar at least, to those surrounding the twigs stored in the cellar, (which was not cool) it follows that sugar, which in this connection may be considered as another term for soluble starch,
would be expected and in variable quantities. Twigs gathered directly from trees also showed these sugars in small amounts. Thus in Recumbent the note book records "cane sugar was evident in the tip of the twig above the lignified cone with both Trommer and Fehling tests." On another page the notes contain: "No marked difference between mature and immature twigs."

Flesh of sugar beet and Baldwin apple was employed to serve as a guide and it may be said that the amount of sugar in apple twigs was only a small portion of that which is found in the beet or apple pulp. The conclusion of this portion of the investigation is that during the two months of midwinter apple twigs stored in the cellar, or taken fresh from the trees during an unusually mild winter, have small quantities of sugar in the soft part of the buds especially the lateral buds, but this presence of sugar does not offer any criterion by means of which to judge of the ability of a tree to bear extremes of heat or cold or other unfavorable conditions.

Other carbohydrates are not present in sufficient quantities in apple twigs to warrant a consideration of them here.

ALBUMINOIDS OR PROTEIDS. — This group of complex and very variable substances does not admit of separation into individual sorts but must be treated together. They are present in all living parts of the apple tree and are most abundant near the points of greatest vital activity. In the apple there is little or no albuminoids in the pith at the base of an old twig, very little in the wood zone, an abundance in the cambium layer and the largest percentage in the buds both lateral and terminal. In these last places when the buds are mature the albuminoids make up a large part of the cell contents and to the exclusion of the starch. All of the varieties studied, fully twenty-five, exhibited the albuminoids as brick red particles when treated with the Millon test. (A nitrate of silver compound.) On account of the conspicuous color the presence of the protoplasmic compounds was easily demonstrated. No distinguishing characteristic for any variety could be found, as all, when they had their buds well formed, and autumn growth complete, exhibited about equal amounts of color when treated with the test for the proteid compounds. When the tip of a twig was still soft, spongy and without rigidity from a failure of the pith to lignify, there was only a feeble response. Protoplasmic matter was present but so scattered over a large area of cellular tissue that no distinct line or point of coloration developed as is true of buds which
have become ripe. The lateral buds of rapid growing water sprouts, for example, have but a small amount of protoplasmic matter, in fact such buds are small and scarcely appear above the surface of the twig.

The albuminoids therefore, like the starch, from their behaviour in green and ripe twigs, become a test for maturity instead of a means of distinguishing one sort of apple from another. These complex compounds are the ones out of which protoplasm is formed and afterwards renews its strength. They are the most important reserve materials in the sense that they stand, in composition, nearest to that of protoplasm, which is the acknowledged vehicle of vital activities. It is not unnatural that these compounds, in a resting twig, should be stored in the buds where they are to be needed the coming spring. Starch may therefore be excluded from a well equipped bud because other substances of greater consequence, and accumulated in smaller quantities, are conserved at the points of growth. We can see the tendency of the starch to get as near to the growing point as possible, for in rapidly growing twigs like water sprouts which have made more wood than anything else and a correspondingly small percentage of starch, there will be little or none of this reserve substance midway of any two buds, and the bulk of it lodged at the base of the bud. Even in a slow growing mature twig from the top of a tree there is quite a difference in the percentage of starch midway between and at the buds. In other words there is a manifest tendency for the concentration of reserve materials at points where they are to be used and in the order named; first the albuminoids and next the carbohydrates—first the basis of protoplasm and next the substances protoplasm must use in the building up of new tissue and in other vital processes. Following upon this is the conclusion resulting from the investigations upon the apple twigs, namely: that other things remaining the same, the best conditioned twig is the one having a sufficient amount of reserve material within easy reach, and in the best condition for the use of the plant. A well preserved bud is therefore plump with reserve substance in a comparatively solid condition, being firm but not woody; is well protected from the injurious effects of very sudden extreme changes of temperature, and has close at hand a sufficient amount of starch or other carbohydrate for its most advantageous development. Whether one form of bud, as to its length, breadth and thickness, color, or number and hairiness of scales will prove better than another under any spe-
cial condition is a question more likely to be determined by field trial than by laboratory tests. Plants seemingly equally hardy may have the vital points very differently constructed. No matter along what line the problem has been worked out by the species the chief point is to prepare for hard times, and having once made the preparation keep quiet until the coast is clear for another year of growth. The inherent tendency of one introduced variety to start into growth before another may throw it into the class called tender while another with the same structure but differently disposed will prove hardy. It is possible that the tests for sugar in the late winter may aid in deciding that a difference in this tendency exists, for starch, the chief form in which the carbohydrates are stored up in apple twigs, is changed into sugar before it becomes available for plant nutrition.

**CRYSTALS:**—Vegetable physiologists are agreed that true crystals (not including crystalloids) in the tissues of plants are a form of refuse or left over matter resulting from the processes of growth and put up in a consolidated form to get them as much out of the way as possible. Some one has compared them to the pieces of brick, mortar and other material thrown into boxes or barrels during the construction or repair of a building. Crystals principally of one sort, have been constantly met with in all sections of all varieties of apple twigs subjected to microscopic examination. They were rarely found in the old pith and have not been seen in the wood, but are very abundant just below the growing tips of all buds in that cylinder of tissue connecting the free extremity of the bud with the starch bearing cells—a half inch or so back of the tip. The loose green bark of all parts of the twigs also abounds in these bodies, and they are especially numerous in the cellular tissue that lies between the leaf scar and the bud above it. In this locality a second form is often met with which is smaller and rectangular in shape, while the prevailing sort is an irregularly spherical aggregation of sharp angled bodies which have taken the name of sphæro crystals. These crystals are composed of oxalate of lime (calcium oxalate) and may be dissolved by mineral acids.

From the composition of these bodies; their universal prevalence in about equal numbers, first in proximity to tissues which are the centers of rapid vital processes and secondly in out of the way places, and for various other reasons it is safe to conclude these crystals are no safe criterion by which to judge of the relative resisting powers of plants to the un-
toward influences that may surround them. One would as soon think of deciding upon the stability of a human structure by the number of chips that the builders have made.

The varieties which were more particularly examined for crystals were: Tetofsky, Whitney, Duchess, Citronat, Ripka Kaslaja, Red June, Wine Sap, Berdaus, Boikan and Pirus ringo. But in connection with other observations they were noted in many other varieties.

**TANNIN:**—Like crystals in vegetable tissues, tannin is considered as refuse matter and not with perhaps certain exceptions further active in the vital processes of the plant. Tannin as recognized by salts of iron is abundant in the apple twigs, and in transverse sections is found most common in the rind, and the inner bark close upon the cambium and between it and the zone of bast. There is no great or constant difference in the amounts of tannin between the several varieties. The buds contain more than the other portions. In immature terminal buds the tannin is quite generally diffused through all parts of the soft tissue.

**ASH, MOISTURE, VEGETABLE ACIDS, ETC:**—Determinations of the percentage of ash, water, vegetable acids and allied compounds have been made by Professor Patrick, Chemist of the Station, and by him the results are presented elsewhere in this bulletin.

**LIGNIFICATION, AND THE SO-CALLED “GRIT.”**—This is one of the most important modifications of the ordinary cell wall. It consists of a thickening due to the interculation into the cell wall of a substance called lignin which chemically differs somewhat from cellulose and adds materially to the hardness and durability of the tissue lignified. This power of resisting external influences is gained however at the loss of much elasticity. Lignified cells do not abound in protoplasm, but water passes freely through their walls. In short the formation of lignin in cells is an index of healthy growth. All permanent inactive tissues may become lignified and when this process is fully carried out it yields the durable heart wood so familiar in many kinds of timber. The subject is of interest because it helps to explain the matter of “grit” or “grittiness” so frequently spoken of by grafters and others who cut the twigs of various sorts of fruit trees. The inflexibility of tips of certain varieties is due in most part to the large amount of lignin they contain.

As nearly all the observations described in this paper were made with sections cut either transverse or longitudinal of the twig, it is evident that any marked difference in resistance to
the knife would be observed and this difference associated with any constant accompanying variation in the structure as revealed by the microscope. This resistance to the blade is easily divided into two parts: first, that which is due to the amount and firmness of the tissue and secondly that which comes from the so-called “grit.” With two twigs of the same diameter one with a thick and the other having a thin zone of wood it goes without saying that the former will cut easier than the latter—provided the other parts are the same. The investigations show that varieties of apples do not vary much in the thickness and firmness of the bark and therefore this part of the twig may be left out of the question when considering twigs of the same age and size. As far as it has been possible to determine, the wood fibres and vessels are so much alike that it is safe to say that equal areas of wood give equal resistances. In working upon this point, sections were taken for example across rapidly grown “water sprouts” of the Romanstem, that had attained the length of six feet in a single season, and compared with those of slowly formed twigs from the tip of the same tree.

The wood in the two twigs at a point midway of the year’s growth was so nearly identical in porosity that persons requested to study the sections placed side by side were unable to distinguish any difference.

There only remains the pith, medulary rays and the wood-pith to produce any structural differences. It is in these that the so-called grittiness resides and is due to the thick and hardened cell walls. The fact that starch is most abundant in the small and thick-walled cells adds rather than otherwise to the resistance of the lignified cells. The wood fibres and vessels are also lignified but it must be borne in mind that these cells are long, slender and when the knife is once set between them it is largely a matter of splitting for the blade to pass through. With the pith and its modifications the conditions are different. The cells are nearly of equal diameters in all directions, closely held together and the knife when it strikes them must cut through the thick hard walls. If a thin shaving is made of a tissue consisting wholly of lignified pith the sound and feel of grittiness are very evident. The best place to find clear grit is in the hardened pith about an inch below the tip of a well matured twig. Pear twigs furnish fine examples of grittiness, for the pith is large, the cells are very thick walled, well charged with starch and contain apparently only a small percentage of water. If such a twig tip is stripped of the small amount of bark and wood
that surrounds the pith the location of the grittiness may be quickly demonstrated by shaving the rigid but very brittle central cone with a sharp razor. If the medullary rays and wood pith of two varieties differ in number and in the thickness of their walls it is evident that the one having the larger per cent of this kind of tissue, other things remaining the same, will be the less easy to cut. As has been shown, the same one will also be the richest in starch. In other words the amount of starch in the wood zone becomes an index of the grittiness of the wood.

**Exterior Appearance of Buds**—The whole subject of the exterior appearance of buds including the number, thickness, hairiness etc., of the bud scales has been purposefully omitted from the present investigations with the microscope. Some expert horticulturists claim that they can distinguish the hardy from the tender sorts by these points in gross anatomy and judging from the functions of those parts it would seem that such ability was possible.

Behrens says: “The leaf scales are of great importance to the bud since they render it capable of standing the winter. Closely overlapping one another as they do, they form a sort of armour which protects the tender internal parts from harm,”—many secrete a sticky gummy or resinous substance and thus cement themselves together, forming an effectual protection against cold and damp. One can easily see the extent to which this secretion of resin and gum goes on by glancing at the opening buds of the buckeye which are so thickly coated over with the sticky fluid that they shine when the sun’s rays strike upon them and if touched adhere to the fingers.

**Resisting Cold, Etc.**—Dr. Sachs has shown that loose cell tissue may become ruptured by the formation of crystals of ice in fissures between the cells. Freezing does not necessarily cause death, for readjustment may follow slow thawing. If the thawing is rapid the water instead of passing back into the cells, escapes into the intercellular spaces and the organ is killed. The tolerance of dry or moist organs is due to several causes. Moist parts are usually in an active living condition and the protoplasm is more susceptible to any disturbance of the equilibrium of the cells. The cell sap is more concentrated in the dry organs and their freezing involves a lower temperature while the disturbance is less than in moist parts. Dr. Vines says “That the power of enduring extreme cold is possessed in different degrees by the same organ in different plants. Martin compares each plant to a
thermometer the zero point of which is the minimum temperature at which its life is possible. Secondly we learn that the different metabolic [vital] processes do not all stand in the same relation to temperature; that in any given plant some of the processes can go on at lower temperature than others, each process having its own zero point. Thirdly that the larger the proportion of water in an organ the more liable it is to be injured by frost; hence the zero point for the life of any given organ will vary according to the amount of water which it contains at different times."

As there is a zero point below which vital processes cannot be maintained, there is also a maximum point above which the temperature cannot go with safety. Somewhere between these two extremes is the best mean or optimum point for the activities of each species—the point varying greatly with the species.

Some plants exhibit a remarkable tolerance for high and others for low temperatures. There is, as yet, no satisfactory explanation for this wide difference. Again quoting from Dr. Vines' recent and admirable work on Vegetable physiology: "It is doubtless upon the living protoplasm of the cell that the temperature acts; the effect first manifests itself by a diminution of the metabolic activity of the protoplasm and ultimately effects its disorganization. Plants or parts of plants which have been killed by exposure to high temperatures present the same appearance as those which have been killed by exposure to low temperatures. They are flaccid because their cells are incapable of becoming turgid. This is due to the fact that the protoplasm has become permeable, as a result of death and this may or may not be accompanied by its separation from the cell walls so as to form amorphous masses." * * * In the light of these physiological facts it becomes evident that a variety of conditions effect the power which a plant or organ possesses of enduring exposure to extreme temperatures and it is on account of the influence of these conditions that the results of different observers in any one case are not always in accord." It may be stated therefore in brief, that amount of moisture, age, and the conditions under which the plant has been growing are all factors in the problem of hardiness, and none of these are alone a safe index of the ability to withstand extremes of temperature.

OTHER THAN APPLE TWIGS:—In addition to the work upon the cultured apple twigs, those of the crab apple and Soulard crab have been examined. Very hasty observations
were also made upon the young wood of some other varieties of fruit trees, and also the currant, gooseberry, and grape. The chief point was to determine the amount of starch present and the nature of its distribution in the plant. Twigs of a number of forest trees and ornamental shrubs were also subjected to the starch test and some of the most interesting results are here added as an appendix to the paper.

The wild crab apple (*Pirus coronaria*) is a slow low growing tree with the young side twigs characterized by a lateral upward growth; many of the lower ones upon a three year old branch, for example, terminating in sharp horny points which constitute the thorns of the small tree. The young somewhat zigzag twigs have a very firm and elastic wood with small pith, taper into slender tips ending in small terminal buds. The lateral buds, of good size have scales of a bright red color usually obscured, except at the base, by the thick coating of gray hairs covering nearly equally all parts of the last year's growth.

The microscope reveals the fact that the wood is of a finer texture than in the cultivated sorts of apples, and the bark is also dense, having but few small intercellular spaces between the ring of bast and the highly colored rind. The starch, in cross-section, is seen to be distributed as in cultivated apples but the pith is much smaller and the medullary rays more numerous. There is a remarkably well developed although slender cone at the upper end of the twig beginning about a half inch from the extremity. Longitudinal sections through short fruit spurs when treated with iodine show a large reserve of starch at the base and in the main twig at the junction. Thorns upon older parts of the tree like wise have the same store of starch. There is a good supply of albuminoids in the buds but they are much more abundant in the tip of the lateral fruit spur than in the terminal buds.

The Soulard crab has many of the characteristics of the wild crab. It is a more vigorous grower with branches less induced to be horizontal; thorns generally absent and in their places long spurs with conspicuous terminal buds bearing large loose scales. The long side twigs are nearly straight, lateral buds prominent, and the whole of the last year's growth coated with fuzz. Starch is very abundant in the pith, particularly the lateral spurs, except the upper half inch which is highly charged with albuminoids. In short, the Soulard in fineness of woodfibre, smallness of pith, giving toughness and flexibility is much like what a wild crab apple tree improved by cultivation would naturally be expected to become.
The cherries vary somewhat in the amount of starch but usually there is only a little present and, that forms a narrow line in the outer layer of pith and no well-developed cone near the base of the terminal bud. In the wild cherry the starch was very small, but gum or mucilage was abundant and probably serves the same purpose.

Wild seedling pear twigs from the nursery row had a large amount of starch which was especially abundant in the thorns. Cultivated pear twigs offered the best illustrations of “grit,” and starch was found throughout the large pith. The currant was examined somewhat at length because of a failure to find starch anywhere in the twigs first gathered. But when the plant was torn from the ground and the roots examined, large amounts of starch were found stored in them in all parts, but more particularly in the soft outer part (cotex,) which may be called the bark. Albuminoids were very abundant in the large pith for a foot or more below the terminal bud. Only the smallest quantity of starch was met with in the branches of gooseberry. The roots were not examined because of the difficulty of obtaining them when the inspection could be made.

_Rose ragosa_ had only a small thimble of starch-bearing cells near the terminal bud, and a smaller lodgment of starch in the pith at the base of each lateral bud. In the Elder the starch is almost wholly confined to the pith and wood at the nodes or joints, which bear two opposite buds each with long intervals between the pairs of buds.

The lilac is another shrub with opposite leaves and in this there was a large supply of starch, especially at the joints. In the honey locust the starch kept as close to the buds as possible and was nearly absent from the long intervals between the buds. It was observed that there was more at the junction of a thorn with its branch than anywhere else. The base of each thorn becoming a reservoir for starch. The white oak has a very large amount of starch—especially near the buds. Barberry twigs had starch only at the buds when it is lodged in large quantities. There is none in the prickles of this plant or the rose.

In some plants like the Hercules Club no starch was found until the root was reached when it was abundant. Catalpa had only a very little. The same is true of several willows and poplars examined, and, as was to be expected, no starch was found in dead stems of golden rods, sunflowers, flea bane, stems, etc., designed to live for only one season.
Concluding Remarks.—Any conclusions that may be drawn from the investigations above reported are only tentative, for it must be evident that the subject is not one to be encompassed within the short space of two consecutive months. At least a whole year is required to obtain a satisfactory knowledge of the behavior of reserve materials in apple twigs. Similar twigs from the same tree should be examined during the various seasons of the year. A study of them in early spring, in mid-summer, and again in early autumn would be quite sure to add interesting facts to those obtained by mid-winter inspection.

No constant difference in all structure probably exists among apple twigs by means of which one sort may be unmistakably distinguished from all others. Much less is there any point in minute structure invariably present with those sorts which are classed as hardy and absent from tender varieties. Maturity of twigs is a condition of successful wintering, and therefore the so-called hardy sorts are quite sure to finish their season's growth before autumn frosts arrive. But under favorable conditions—similar to those which obtained last autumn, the so-called tender varieties may complete their growth and pass the winter in safety. When the mature twigs of the latter sorts are compared with those of the hardy varieties no differences of minute structure are observed; at least if any are found between the twigs, they are easily within the range of variation of twigs for the same sort grown under different circumstances.

The problem of tenderness and hardiness needs therefore to be worked out upon other lines than that of differences of cell structure. A problem, which in its solution involves many factors naturally classified under the two groups of those acting within the plant and those without. The former includes all those subtile qualities which are known only by their effects, and may be spoken of here, in part at least, as inherent disposition. That unseen and indescribable influence which in the pear seed determines it shall grow into a pear, and an apple seed into an apple. It is by no means asserted that structural differences do not exist, but these differences for very good reasons are most likely such as would be associated with the composition and arrangement of the theoretical units of minute structure, which under the name of molecules, baffle the microscopist and even puzzle the chemist and physiologist. If plants were simply machines acted upon by forces easily measured it might be no difficult task to attribute differences of activity, to well observed
differences of the component parts, provided such differences could be established. But a plant is a living thing which nourishes itself, and is subject to a hundred influences of light, temperature, moisture, electricity, etc., which are beyond the power of man to measure in their individual relation to a vital organism. These surrounding conditions only determine whether the plant will develop; the nature of the result of growth was determined by ancestral forms through perhaps thousands of years of slow modification by the action of a law known as the survival of those individuals, modified by and, best adapted to the surrounding conditions.

The question of resistance to summer's heat or winter's cold seems therefore more deeply seated than modification of cell structure. If the microscope was sufficiently penetrating it might be that we should see a variation in the grouping of the atoms or ultimate particles of matter which would serve as an index to resisting power. It may be that the protoplasm of the Tetofsky has a different molecular structure than the Red June and that a still wider difference exists between the plastic vital substance of the pear and the apple. Cell structure is but the necessary framework, by means of which the protoplasm is able to live and exhibit its inherent and acquired peculiarities. The whole cellular fabric of a tree may be considered as an excretion from the protoplasm that dwelled within, bearing somewhat the same relation to each other that the shell does to the oyster, or the bony skeleton to the ox. The only hope is to find a peculiarity of product or variation in structure or composition that constantly attends vital peculiarity or a so-called habit of the plant.

These investigations determine no such parallelism between microscopic structural differences and ability to withstand the influences of a trying climate. A test for maturity seems to be found for the apple in the lignified starch bearing cone close below the terminal bud, and the nature of the so called "grit" is determined as consisting of the thick-walled cells of the pith, medullary rays and wood pith. These cells are thickest in well matured twigs, and when well filled with starch, therefore the presence of "grit" is an index of ripeness and the starch-storing capacity. Grittiness is therefore a good sign in the apple but it is not safe to conclude that it can establish the rank a variety should take in any fruit list.