A STUDY OF 'STONE CELLS' IN PEARS WITH SPECIAL REFERENCE TO VARIETIAL DIFFERENCES

THESIS FOR THE DEGREE OF M. S.

Lawrence P. Batjer

1930

A STUDY OF "STONE CELLS" IN PEARS WITH SPECIAL REFERENCE TO VARIETIAL DIFFERENCES.

Thesis

Presented to the faculty of the Michigan State College of Agriculture and Applied Science as partial fulfillment of the requirements for the degree of Master of Science

by

Lawrence P. Batjer

1930

Approved June 5, 1930
V. R. Gardner

THESIS

INTRODUCTION

Cellular structures known as stone cells are of general occurrence in the tissue of the plant body, being found most often in the cortex, in the phloem and in the fruit. When present in the fleshy pericarp of the fruit, as in the pear, they are usually termed "grit cells".

Variation in grittiness in the fruits of pear varieties has long been recognized. Early writers made many interesting but not altogether accurate observations concerning them. Some varieties are extremely gritty, and this is partly responsible for their low standing where quality is concerned. The Kieffer tree as an example, is considered correctly as an "easy grower", and if it were not for the very objectionable feature of the grittiness of the fruits it would probably be one of the outstanding favorites of both producer and consumer, perhaps not as a desert pear but for general purposes such as canning and cooking. Since these grit cells do influence the chemical and physical qualities of pear varieties their consideration should be regarded as extremely important.

In-as-much as no detailed study of stone cells, with special reference to time of formation and varietial differences, has ever been undertaken, there seemed to be a need for information on this subject. Therefore a detailed study of stone cells in pear fruits was made. The results of the investigations are reported on the following pages.

Materials and Methods

The plant material used in the experimental work was taken from two pear orchards: the orchard in the Experiment Station grounds, and a commercial orchard in the principal pear-growing section of the state near South Haven, Michigan. Both of these orchards are composed of thrifty, grafted trees with unknown stocks. The trees in the College orchard are all the same age and are growing under sod culture. The South Haven orchard is under cultivation.

Histological Procedure

The young fruits used in the stone cell studies were killed and fixed with chromacetic acid solution, carried through the steps in the paraffin process of embedding, cut transversely into sections 12 thick with a rotary microtone, and double stained with safranin and Delafield's hematoxylin. These sections were mounted in balsam and microphotographs made as required. Cross Sections of spur wood were used in studying the differences in fiber formation in the wood of various varieties. The spurs were sectioned immediately after their removal from the tree with a sliding microtone and stained and mounted in the same manner as the fruit sections.

One method used in arriving at the grit cell content of the fruit was that of taking transverse and longitudinal sections approximately 1/8 of an inch in thickness through the specimen at the center of the core region, and clearing these to the point of transparency. The grits remained opaque and in place. As soon as cut the sections were placed in 50 percent acetone solution where they were left for 24 hours. Following this, they were transferred to 75 percent acetone solution, then to 90 percent and finally to pure acetone, and allowed to remain for 24 hours in each grade of solution. They were then placed in absolute ethyl alcohol, left there for 24 to 48 hours, and finally put into oil of cedarwood where they remained until clear. Photographs were made (all under the same magnification) with the cleared sections suspended in Canada balsam contained in flat glass cells. This is essentially the method used by Kraus (8) the only difference being the use of acetone instead of alcohol. The fresh sections discolored less in the acetone solution than when alcohol was used.

Chemical Methods

The grit cells of the pear are principally lignocellulose in chemical composition, and they constitute practically all of the lignified tissue of the fruit aside from the carpels.

Since this was found to be true, it became possible to determine their amounts quantitatively by making summative analyses which lead to lignin as the final residue.

Fruits to be used in the analysis were selected as to uniformity of size and color, from a large sample taken at random from the tree. A transverse section as wide as the main core was long was cut from the mid-portion of each pear. These discs, varying in thickness from 1/4 to 1/2 inch, were quartered, and the carpels with their contents completely removed. The quarters were chopped into fine pieces, and the whole thrown together into a good size composite sample. The material was then spread out on waxed paper, dried as much as it could be by an electric fan, and next placed in an electric oven at 65 degrees centigrade. The temperature of the oven was raised gradually, as drying ensued, until it reach 95 degrees centigrade, where it remained until there was no further loss of water from the sample. Upon removal from the oven, the dried material was ground to pass a 60 mesh seive.

Two grams of the oven dry material were placed in an alundum thimble and extracted first with benzene (100 cc) for four hours, then with 95 percent ethyl alcohol (100 cc) for eight hours in an extraction apparatus of the type used for rubber insulating materials, and described in Circular 232 of the United States Bureau of Standards. In each case

the extract was transferred to a weighed receptacle, evaporated to dryness on a steam bath, placed in an oven at 95 degrees centigrade for 1 hour, cooled and weighed. The residue was transferred to a 400 cc beaker with 200 cc of distilled water and allowed to set for 24 hours at room temperature. After the mixture had been filtered under suction and washed with water through a double disc of mercerized cotton cloth in a tared Gooch crucible, the residue was kept in an oven at 95 degrees centigrade for 16 hours and then weighed, whereupon it was extracted after the same routine with 100 cc of 2.5 percent NaOH solution. When filtering the alkali mixture, washing was first done with water, then with dilute acetic acid (one part to three parts of water), followed by another washing with water. When difficulty in filtering was encountered, filtering and washing were accomplished with the aid of a centrifuge. The residue from the alkali extraction was transferred to a one by eight inch Pyrex test tube, covered with 10 cc of concentrated (not fuming) HCl and treated with hydrochloric acid gas for 2 hours while the tube stood in cold water. The tube was then removed, closed with a rubber stopper for 24 hours, at the end of which time the contents were diluted, filtered on a tared Gooch crucible, washed thoroughly with water, dried at 95 degrees centigrade for 16 hours and weighed.

The method described above was used as well with samples of pear wood, principally spur wood. Essentially

it is the method devised and used by Dore (3). In prospect the procedure up to the treatment with gaseous HCl, is aimed to accomplish the complete extraction of all other constituents of the material, leaving only the lignin as a residue. While the method is a highly improved one as compared with several others, it is realized generally that at present there is no method for the determination of lignin which is not more or less arbitrary and unsatisfactory. This condition will probably exist until the chemistry of lignin is better established and agreed upon.

Qualitative tests for peroxidase activity were made on a cross section from the center of the fruit cut with uniform thickness (1/8 inch) by means of an automatic meat slicer. As soon as cut the section was immersed in 50 cc of two percent benzidine in 60 percent ethyl alcohol for 20 minutes. When removed from the solution, the section was quickly wiped dry on its surfaces, placed on a glass plate illuminated from below, and photographed. Intensity of peroxidase activity is indicated by the degree to which the tissues which are acid become purple in color, and those which are alkaline redden. Drain (4) employed this method in his work with apples and pears.

Time of Formation

Duhamel (5) in his article on the anatomical study of the pear makes the following comment "It would be useless to search for the grits in the fruit lately set. Those parts of the fruit which should harden appear at this time as only a white mass, compacted 'tis true, but which has not the hardness which it will require later on. This substance seems to be divided by white granules, which then have but little solidity, and which makes up nearly all of the interior substance of the fruit. Finally these grains enlarge and harden little by little in a way that the fruit when they are yet very small are entirely filled with the stones".

Following an exhaustive anatomical study of pear grits, Meyen (9) concludes that they were formed from ordinary parenchyma cells which had been transformed by heavy layer thickening of the walls. He held that they were organic in nature and classed them along with all other types of woody hardenings. Turpin (11) states that the cellular tissue of a very young pear is regular and offers no trace of stony concretions and that it is only after the pear has attained the size of a small nut that these stones are perceivable. He recognized the similarity of these grits to ordinary woody hardenings and perposed the general term "Sclerogene" to denote all unassimilated material of the organism, which separates from the cell sap and incrusts the inner walls of cells.

Burgerstein (2) holds that the rate of lignification is very variable, depending on the conditions of growth and the specific physiology. He obtained evidence of its inception in cells but two days old. Stevens (10) writing on the ontogeny of Sclerenchyma tissue, says that stone cells as a rule are transformed thin walled parenchyma cells whose walls have become greatly thickened and more or less lignified.

In this work three varieties of pears - Kieffer, Bartlett, and Clapp Favorite - were employed in studying the time at which the first appearance of lignification could be observed, and also the anatomical changes which accompanied its inception and progress. Samples were taken twice weekly from the time of blossoming on May 10 up to and including June 12, at which time the pears had attained such a size that embedding and sectioning could no longer be accomplished. The trees from which the samples were taken are all growing under sod culture in the College orchard receiving the same cultural treatments. The sections used for study were prepared according to the procedure outlined under methods.

From May 10 up to and including May 25 no evidence of cell wall thickening could be detected in any of the three varieties. However, three days later, in the Bartlett, stone cell formation was well under way. On this date scattered through the flesh of the fruit were numerous cells whose walls had become characteristically thickened Fig. 1. Their formation was by no means gradual, appearing suddenly and without

warning. While some of these newly formed stone cells were in groups of two or three, the great majority were scattered singly through the flesh of the young pear. Four days later sections of this variety showed cell wall thickening more pronounced and the formation of groups or clusters of these cells, especially around the carpel region (Fig. 3). Later stages showed the following changes appearing in the order listed; 1 - Drawing up of the cell cavity followed by greater cell wall thickening, 2 - more pronounced clustering of stone cells around the core (Fig. 5), 3 - formation of branched pits or canals leading through the lignified wall into the cell lumen, 4 - and finally the formation of parenchymatous ray cells which completely surround the mature stone cells. By June 12, 22 to 25 days after fruit set, the stony formations had reached a stage similar to that found in the mature pear, the chief difference being in the completion of the ray cells (Fig. 7).

In the Kieffer and Clapp Favorite varieties lignification takes place in a similar manner with the exception of the time of first appearance. In every instance the date on which the first signs of cell wall thickening occurred was four days later in these varieties than in the Bartlett. This delay cannot be attributed to differences in blooming date since the Bartlett was more backward than either the Kieffer or Clapp Favorite at this particular time. Larger

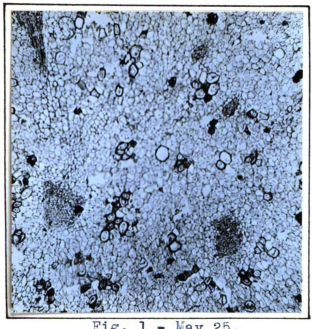


Fig. 1 - May 25.
Beginning of lignification

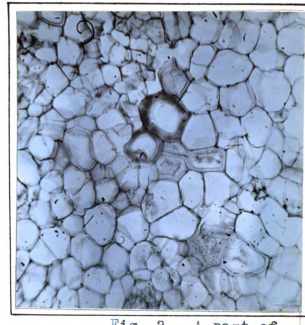


Fig. 2 - A part of
fig. 1 greatly enlarged

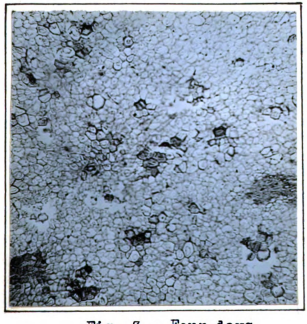


Fig. 3 - Four days
later than fig. 1. More pronounced clustering of stone cells apparent

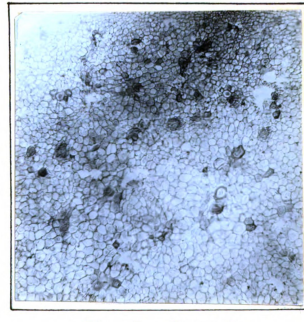


Fig. 4 - Characteristic grouping of stone cells beneath the epidermis. June 12

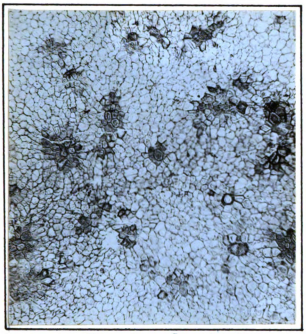


Fig. 5 - Clustering of stone cells around carpel region. Taken from same fruit as fig. 4.

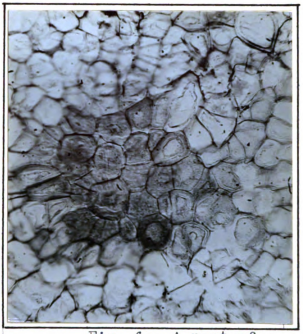


Fig. 6 - A part of fig. 5 under high power.

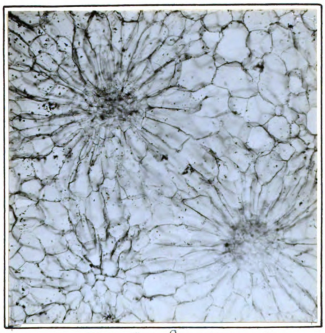


Fig. 7 - Clusters of stone cells surrounded by ray cells. Taken from mature fruit

and more numerous clusters around the carpel region characterized the Kieffer while no difference between the Bartlett and Clapp Favorite could be detected up to June 12.

The series of photographs on pages 33 to 35 inclusive will give some idea of the distribution and differences in the stone cells of the three varieties at various stages in their development.

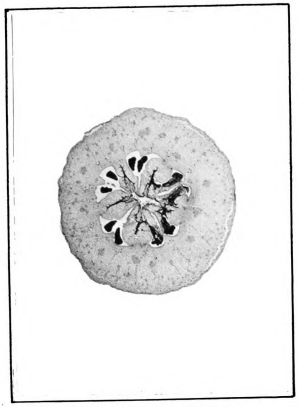


Fig. 8 - Bartlett on May 25. No evidence of lignification

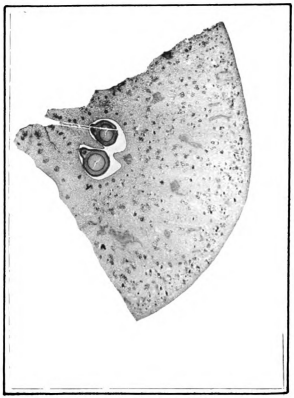


Fig. 9 - Bartlett on May 29. Beginning of cell wall thickening

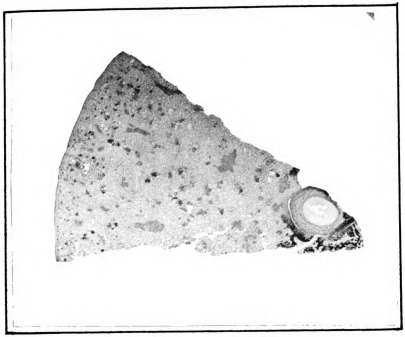


Fig. 10 - Bartlett on June 12. Lignification well under way

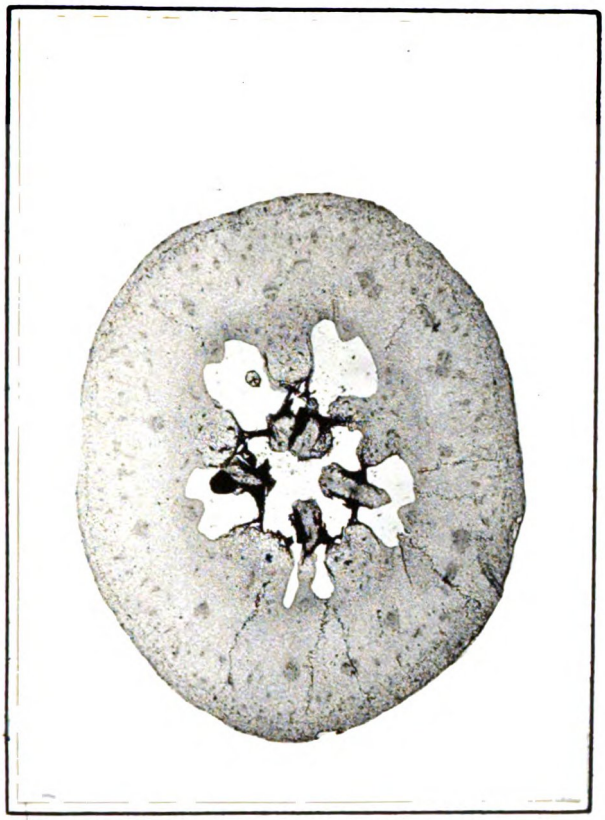


Fig. 11 - Kieffer on May 29. No evidence of lignification

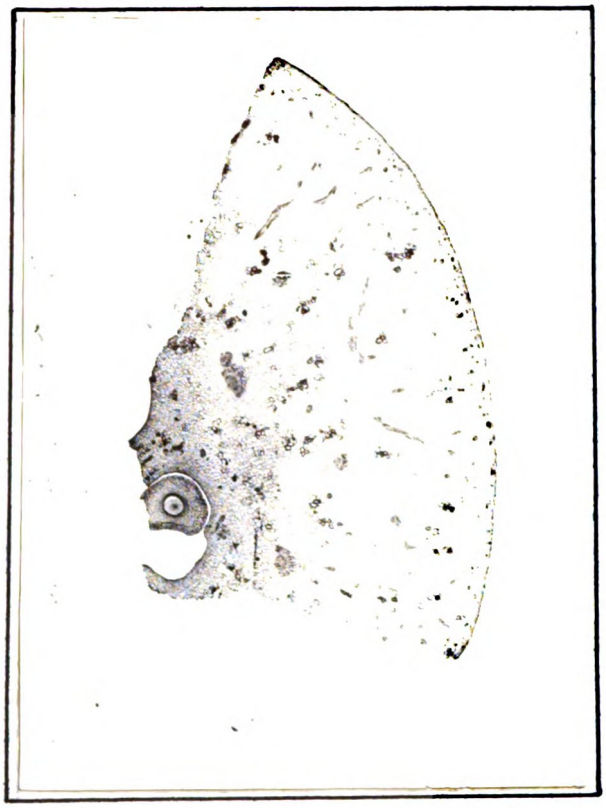


Fig. 12 - Kieffer on June 1st. Cell wall thickening apparent

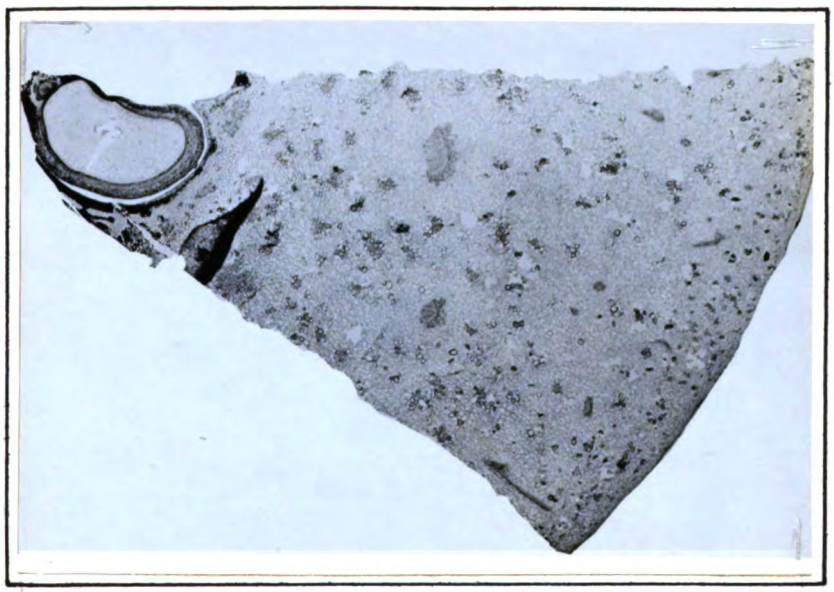


Fig. 13 - Kieffer on June 12 Lignification well under way. Larger and more numerous clusters of grits, around the carpel region characterize this variety

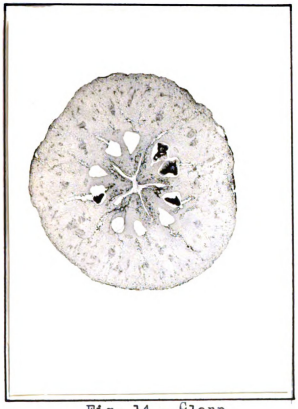


Fig. 14 - Clapp Favorite on May 29. No evidence of lignification

Fig. 15 - Clapp Favorite on June 1st. Cell wall thickening apparent.

Fig. 16 - Clapp Favorite on June 12. Lignification well under way

Variation of Grit Cell Content with Pear Varieties

Regardless of variety there are two fairly definite regions in which grits tend to concentrate. One of these is just beneath the epidermis where they are scattered singly or in small clusters in great numbers. From here they thin out toward the center of the fruit and again concentrate around the carpels where the groups are larger and more numerous than in any other part of the pear. In some varieties where extreme grittiness occurs the grits aggregate themselves around the core as though a solid shelled pit were to be formed. It was this condition that probably led Grew (7) to the belief that the pear is nature's preface or introduction to the plum. The effect of stone cells upon the quality of the pear depends not alone upon their quantity, but also upon the degree to which they aggregate into clusters or nests that become more noticeable when the fruit is eaten. When these two criteria are used, seven varieties examined in the college orchard rank in the following order of increasing grittiness: Bartlett, Flemish Beauty, Anjou, Seckel, Clapp Favorite, Winter Nelis, and Kieffer. (See photographs on pages 17 to 23 inclusive).

It will be seen from the above grouping that Bartlett and Kieffer represent the two extremes while Clapp Favorite is intermediate. Samples of these three varieties were taken monthly from the college orchard and summative analyses were made as described under chemical methods. The results are given in the accompanying table.

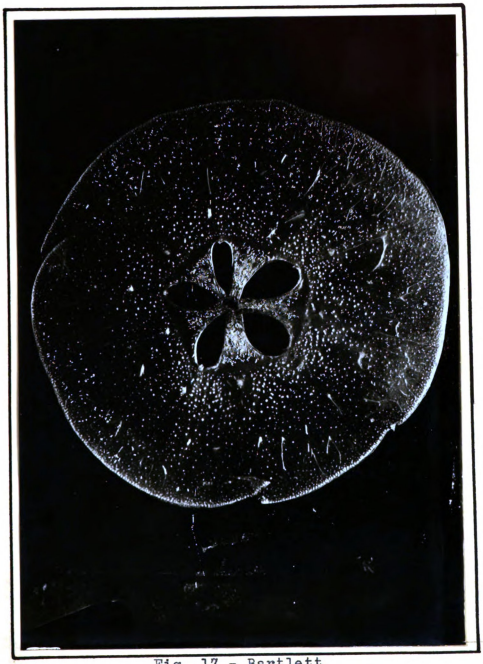


Fig. 17 - Bartlett

Fig. 18 - Flemish Beauty

Fig. 19 - Anjou

Fig. 20 - Seckel

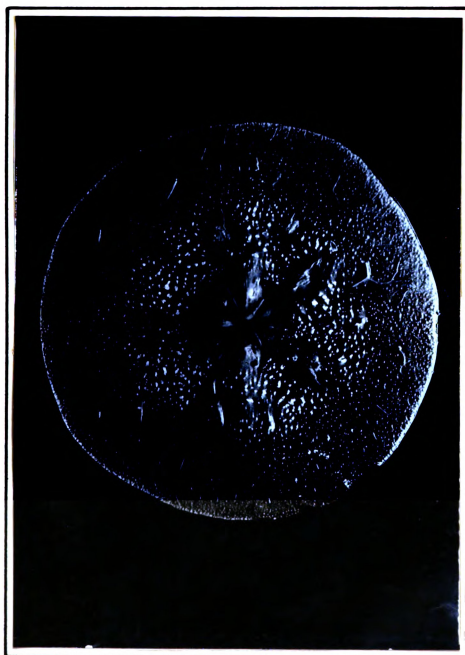


Fig. 21 - Clapp Favorite

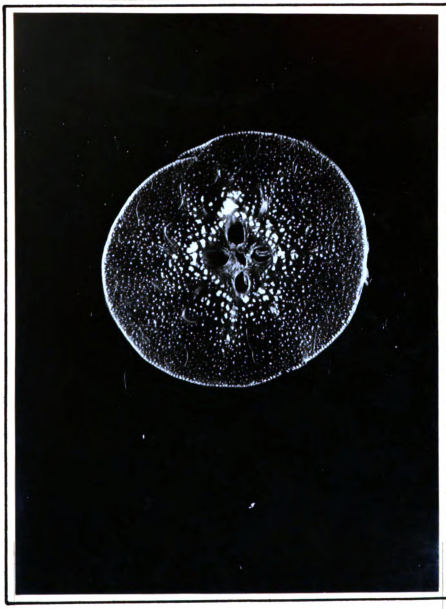


Fig. 22 - Winter Nelis

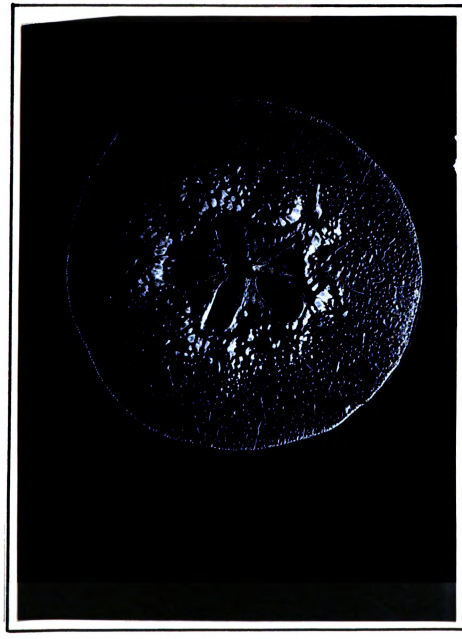


Fig. 23 - Kieffer

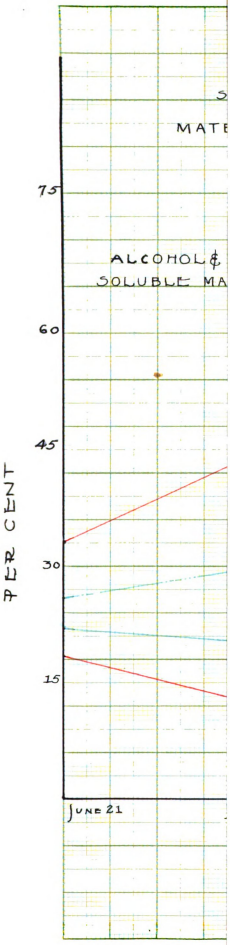
Table 1 - Summative analyses of pear varieties
(Numbers represent percentages of dry weight

Variety	Date	Benzene Extract	Alcohol Extract	Water Extract	Alkali Extract	Ligno Cellulose	Lignin
Bartlett	July 12	1.18	27.94	10.20	17.01	43.58	16.89
Kieffer		1.09	19.76	7.10	18.40	53.65	22.57
Clapp Fav.		1.21	23.71	9.57	21.79	43.72	16.36
Bartlett	Aug. 16	1.12	53.20	8.15	5.81	31.72	7.45
Kieffer		1.02	43.50	5.84	8.11	41.53	12.54
Clapp Fav.		1.10	64.62	4.44	4.91	24.93	10.20
Bartlett*	Sept. 4	1.06	64.49	5.45	5.39	21.61	4.83
Kieffer		.98	60.28	4.76	6.60	27.37	7.50
Kieffer*	Oct. 10	1.25	63.52	10.11	5.08	20.04	6.76

* Picking date

Since pure stone cells are made up largely of lignocellulose and a high percent of this is lignin, these are the two materials which would naturally show varietial differences in respect to grits. The analytical results in Table I bears out the observed grittiness of these three varieties. In all three the peak of lignification had been reached when the first samples were taken (JULY 7). From this time on until the fruit was mature there was a gradual decline. In lignocellulose and lignin the Kieffer is much higher relatively than the Bartlett at successive samplings. The Clapp Favorite, while showing practically no difference from the Bartlett on July 7, drops into intermediate place on August 8. Due to a very light crop and the tendency to drop badly before picking time, it was impossible to obtain a sample of the Clapp Favorite variety at the market ripe stage. The data in Table I show a difference of over 28 percent in lignin between Kieffer and Bartlett at their respective picking dates.

The difference in these two varieties was further verified by a series of analyses run on samples taken from the orchard at South Haven, Michigan. The trees from which the fruit was taken are all growing in the same orchard under cultivation, receiving uniform treatment. The results are shown graphically in the accompanying curves. (Fig. 24). It will be seen from the graph that there is a very significant


MATE
75
ALCOHOL &
SOLUBLE MA
60
45
PER CENT
30
15
JUNE 21

difference in lignin trend throughout the season and at the respective picking dates of the two varieties, the Kieffer is over 32 percent higher. The inverse behavior of alcohol and water soluble material (which is largely sugars) would seem to indicate that low sugar content and high percentage of lignin go together and vice versa.

Differences within Fruits in Continuity with that in the Wood

It has been shown that fruits of various varieties differ greatly in respect to their lignin content. Examinations of cleared sections of a number of varieties showed this to be true, and was further verified by analytical data. Since the fruit is nothing more than a modified stem it seems logical that the wood of these varieties would show corresponding differences in fiber content. While plant fibers are not anatomically the same as stone cells the similarity is probably sufficient to indicate a general class relationship. Eames and McDaniels (6) imply as much in their statement: "In contrast with fibers, stone cells are characterized by having diameters essentially alike, that is, the longer diameters of a stone cell may be much greater even several times greater than the shorter diameter, but not many times greater, as it is in fibers. Many cells will be found, of course, which are transitional in type between typical fibers and typical stone cells. Such may be called short, stone-cell like fibers or elongated stone cells". Since it is highly probable that stone cells in the fruit are nothing more than shortened fibers which have become bent and distorted it would seem that no great error is introduced in making the comparison.

Two, three, and four year old spur wood of Kieffer, Bartlett, and Clapp Favorite was sectioned and prepared according to the procedure outlined under methods. It was

found that it would be impossible to make a detailed study of the fibers due to difficulty encountered in getting the sections sufficiently thin. Since the purse of these spurs did not present this difficulty they were substituted for the anatomical study.

Approximately thirty purses from two, three, and four year old spurs of each variety were arranged in the following classes, depending upon their apparent fiber content:

0 - Fibers totally lacking or if present only sparsely scattered.

1- Groups of fibers more or less continuous for one fourth to one half the circumference of the section

2 - Groups more or less continuous from one half to three fourths the circumference of the section.

3 - Large groups entirely or nearly completely encircling the section.

The number of sections falling in each of these classes is shown in the accompanying table.

Table 2 - Classification of Purses with respect to the relative amount of fiber tissue contained.

Variety	Class	Purses on different aged spurs			Total	Percent
		2 yr.	3 yr.	4 yr.		
Bartlett	0	5	5	3	13	42
	1	5	5	7	17	55
	2	1	--	--	1	3
	3	--	--	--	--	--
Clapp F.	0	1	--	--	1	5
	1	1	2	1	4	28
	2	3	3	3	9	50
	3	1	1	1	3	17
Kieffer	0	--	--	--	--	--
	1	2	2	2	6	20
	2	5	5	6	16	50
	3	4	3	2	9	30

Table 2 shows that 97 percent of the Bartlett sections fall in the first two classes, 33 percent of the Clapp Favorite and only 20 percent of the Kieffer. The two last named varieties both have 50 percent of their sections falling in class two but the former has only 17 percent in class three while the latter is represented with 30 percent. These results show a relatively clear cut difference in the three varieties in respect to fiber content. (See photographs on page 32).

Using these same sections for further study the total number of individual fiber cells were determined by actual count under a low power microscope. Also the number of clusters and fiber cells per cluster was tabulated. Table 3 gives the means, with their probable errors, of the different varieties.

Table 3 - Varietial Differences in Fiber cell content of spurs.

Variety	Av. Number of fibers in purses of different aged spurs			Probable error of differences
	2 yr	3 yr	4 yr	
Bartlett	227 ± 23.4	194 ± 23.8	258 ± 35.27	± 28.5
Clapp Fav.	358 ± 29.81	446 ± 46.8	351 ± 16.45	± 33.4
Kieffer	507 ± 29.07	471 ± 27.92	451 ± 15.92	± 20.3

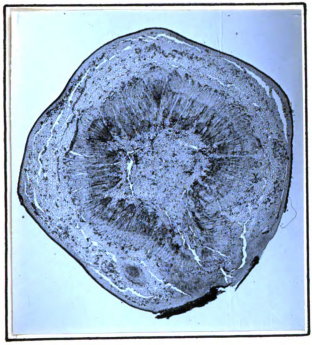


Fig. 25 - Cross Section of Bartlett purse. Very few cortex fibers present

Fig. 26 - Cross Section of Clapp Favorite purse showing groups of cortex fibers scatter half way around the circumference of the section

Fig. 27 - Cross section of Kieffer purse. Cortex fibers nearly encircle the section

From the data in the above table it is readily seen that within a variety the difference in number of fiber cells due to the varying age of the spurs is insignificant. This condition made it possible to throw all three ages into one class and consider them together, thereby increasing the number with which to establish a difference between varieties. Table 4 shows the mean and odds of the three varieties when this is done.

Table 4 - Varietial differences in Fiber content of purse regardless of age.

Variety	Av.No.of fibers per purse	Prob.Err Dif.Bart & Kief.	Odds	Prob.Err dif.Bart & C.F.	Odds	Prob.Err dif.C.F. & Kief.	Odds
Bartlett	226±16.05	±15.53	Unlimited	±17.3	Unlimited		
Clapp Fav.	385±20.03			±17.3		±17.46	
Kieffer	477±16.52	±15.53				±17.46	140 to 1

The data in table 4 show almost unlimited odds supporting varietial differences in wood fibers. The variation in total number of cells per purse is illustrated graphically in (Fig.28).

Not only does the wood differ in total number of fiber cells but also in number and size of groups. This statement is verified in Table 5. (See micro-photographs on page 35).

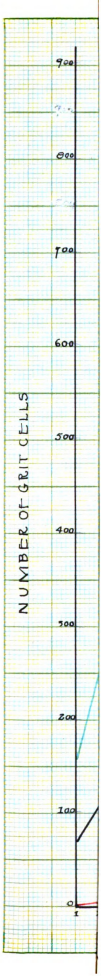

NUMBER OF GRIT CELLS
900
800
700
600
500
400
300
200
100
0
t

Fig. 29 - Grouping of fiber cells in Bartlett purse

Fig. 30 - Characteristic grouping of fiber cells in Clapp Favorite purse

Fig. 31 - Kieffer purse showing larger and more numerous groups of fiber cells.

Table 5 - A comparison of number and size of fiber clusters.

Variety	Av. No. of fiber clusters per spur	Av. No. of fiber cells per cluster
Bartlett	10.12	22.5
Clapp Favorite	14.60	26.38
Kieffer	18.68	25.86

Beside the anatomical study of the wood summative analyses were also made. Wood from both the college and South Haven orchards was used in the analytical work. The same spurs that bore the fruit used for analysis in the preceding section constituted the samples. Tables 6 and 7 give the results.

Table 6 - Summative Analyses of Spur Wood (South Haven)
(Numbers represent percentages of dry weight)

Variety	Date	Benzene Extract	Alcohol Extract	Water Extract	Alkali Extract	Ligno-Cellulose	Lignin
Bartlett (Purse)	June 21	1.62	19.77	16.05	27.06	35.50	13.42
Kieffer (Purse)	June 21	1.97	18.86	14.75	25.68	40.74	16.49
Bartlett (Purse)	July 18	1.38	23.51	10.36	24.58	40.17	12.25
Kieffer (Purse)	July 18	1.17	21.45	9.37	22.28	45.73	14.13
Bartlett, old spur wood	June 21	1.38	12.25	12.36	24.16	49.85	20.73
Kieffer O.S.W.	June 21	1.55	10.45	11.13	16.11	60.76	26.40
Bartlett* O.S.W.	July 18	2.33	24.74	8.49	14.79	49.65	17.32
Kieffer* O.S.W.	July 18	2.28	18.84	8.38	13.54	56.96	23.03
Bartlett O.S.W.	Aug. 16	2.01	12.43	8.55	15.53	61.48	23.45
Kieffer O.S.W.	Aug. 16	1.82	12.30	6.84	15.19	63.85	25.92
Kieffer O.S.W.	Sept.18	1.61	13.68	7.29	13.34	62.08	24.80
Kieffer O.S.W.	Oct. 7	1.59	14.33	8.80	13.47	61.81	22.48

*The bark of the spur wood was used in this determination.

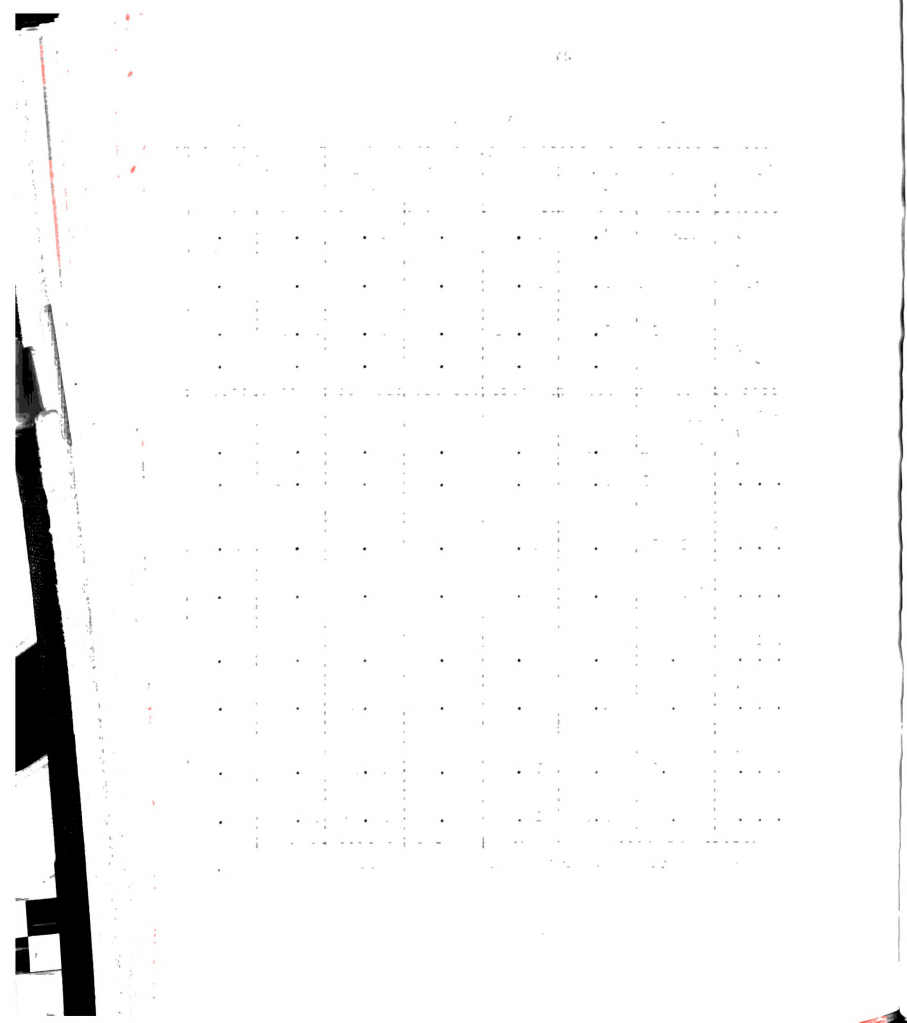


Table 7 - Summative Analyses of Spur Wood (College Orchard)
(Numbers represent percentages of dry weight)

Variety	Date	Benzene Extract	Alcohol Extract	Water Extract	Alkali Extract	Ligno-Cellulose	Lignin
Bartlett (Purse)	July 12	1.99	18.76	12.03	22.25	44.97	15.89
Kieffer (Purse)	July 12	2.11	19.17	13.31	23.34	42.08	17.52
Clapp F. (Purse)	July 12	1.86	21.50	12.26	24.95	39.43	15.23
Bartlett, old spur wood	July 12	1.77	13.78	6.55	17.43	60.47	21.10
Kieffer O.S.W.	July 12	1.64	18.38	4.26	16.18	59.54	24.48
Clapp F. O.S.W.	July 12	1.39	13.3	9.20	17.29	57.82	21.79
Bartlett O.S.W.	Aug. 16	2.04	14.64	6.04	15.30	61.98	20.46
Kieffer O.S.W.	Augu 16	2.12	13.63	5.73	15.65	62.87	22.61
Clapp F. O.S.W.	Aug. 16	2.16	13.62	6.05	15.36	62.81	21.20
Kieffer O.S.W.	Sept. 4	1.60	15.36	9.47	13.25	60.32	20.42
Bartlett O.S.W.	Sept. 4	1.36	15.46	9.60	14.64	58.94	19.27

It will be seen from the data in the above tables that both the purse and the old spur wood show the same general relationship in respect to lignin that has characterized these varieties all the way through. In table 6 where the bark alone was analyzed the differences are even more pronounced. While the differences in some cases may not be termed significant the same general trend is apparent. Table 6 shows a decided contrast in Bartlett and Kieffer throughout the entire season.

In summarizing the results obtained under this head it would seem that there is a high degree of correlation both anatomically and chemically between lignocellulose formations in the wood and amount of grit cells contained in the fruit. In establishing this correlation it has been shown that it makes little difference in the age of the spur or the part used since all show the same fundamental difference. It was also found that larger and more numerous clusters of fibers in the wood were definitely associated with larger and more numerous groups of stone cells in the fruit and vice versa.

Correlation of Grit Cell Content with Peroxidase Activity

Candlin (1), writing on chemical changes taking place during lignification says "Lignified and non lignified tissues differ from one another in many respects. The former contains in addition to cellulose, lignin and hemicelluloses in appreciable quantities, and only small traces of pectins. In the non lignified tissue on the other hand, lignins are absent; hemicelluloses are present only in small quantities, whereas the pectins are present in relatively large amounts. During lignification therefore, the pectins disappear to be replaced by hemicelluloses and lignins." He goes on to say that the disappearance of pectin is due to its decarboxylation yielding among other products, a substance similar in all its properties to the hemicelluloses obtained in relative large amounts from lignified tissue. Since pectins and hemieelluloses are all derivatives of sugar acids such as glucuronic and galacturonic acid and the formation of these products in turn depends upon oxidation processes, it seems logical that peroxidase activity in the pear should be closely correlated with lignin content. Working on this hypothesis a study of a possible relationship between peroxidase activity and degree of lignification was undertaken.

Samples of Bartlett, Kieffer and Clapp Favorite varieties were taken from the college orchard at monthly intervals and sections of these fruits were subjected to the benzidine test in the manner described under methods. The s series of photographs on pages 43 to 45 inclusive show the results. The darkly colored portion of the fruit indicates the region of peroxidase activity.

It can be seen from these photographs that very striking differences exist between Kieffer and Bartlett. These two pears which are extremes in grit cell content likewise react differently to the benzidine test. All varieties behaved more or less alike in respect to localization of peroxidase activity but there was a great difference in the amount of this enzyme as shown by the color intensities in the photographs. The areas of darkest colors, especially in the Kieffer, which indicates the region of greatest activity correspond closely with the regions of greatest grittiness, namely around the carpel region and through the outer part of the cortex. The Clapp Favorite showed a little greater activity than the Bartlett. However, the photographs fail to show very much of a distinction.

Tests made throughout the season on Bartlett and Kieffer varieties from the South Haven orchard showed the same pronounced differences that occurred in these varieties in the college orchard. (See photographs on pages 46 to 49 inclusive).

The writer fully realizes the limitations of a qualitative test for peroxidase activity such as was employed in this work; nevertheless, it proved sufficient to bring out such fundamental differences as were necessary to make the comparison.

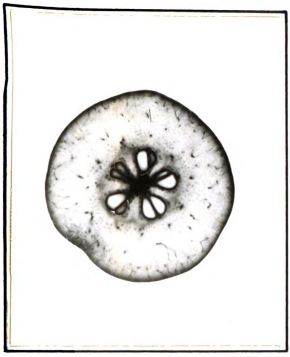


Fig. 32 - July 12

Fig. 33 - August 16

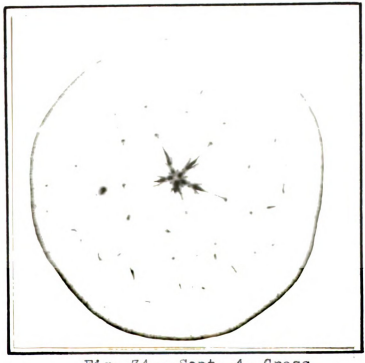


Fig. 34 - Sept. 4. Cross sections of Bartlett fruit from college orchard showing peroxidase activity at monthly intervals. The dark color indicates the region of greatest activity

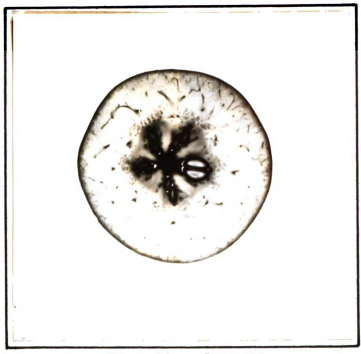


Fig. 38 - July 12

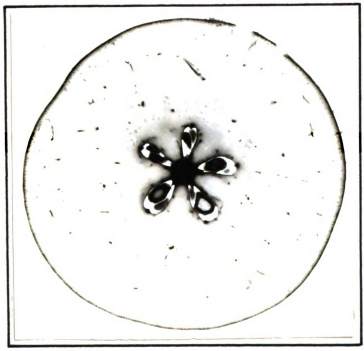


Fig. 39 - August 16. Cross sections of Clapp Favorite fruit from college orchard showing peroxidase activity at monthly intervals. The dark color indicates the regions of greatest activity

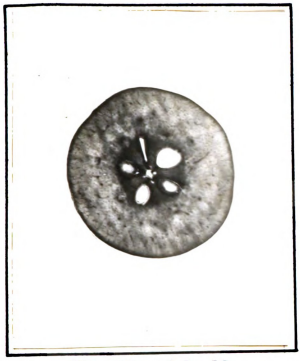


Fig. 35 - July 12

Fig. 36 - August 16

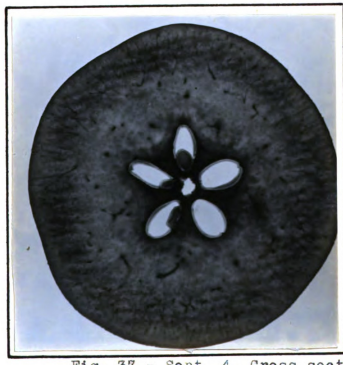


Fig. 37 - Sept. 4. Cross sections of Kieffer fruit from College orchard showing peroxidase activity at monthly intervals. The dark color indicates the region of greatest activity

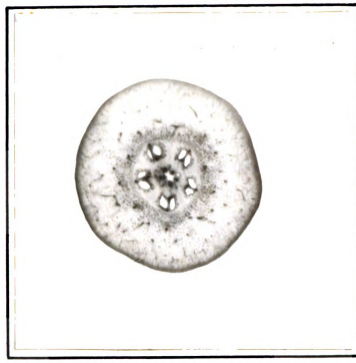


Fig. 40 - July 6

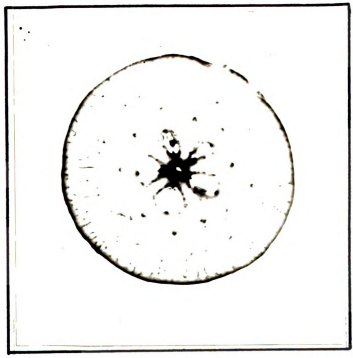


Fig. 41 - July 18. Cross sections of Bartlett fruit from South Haven orchard showing peroxidase activity at monthly intervals (Continued on next page)

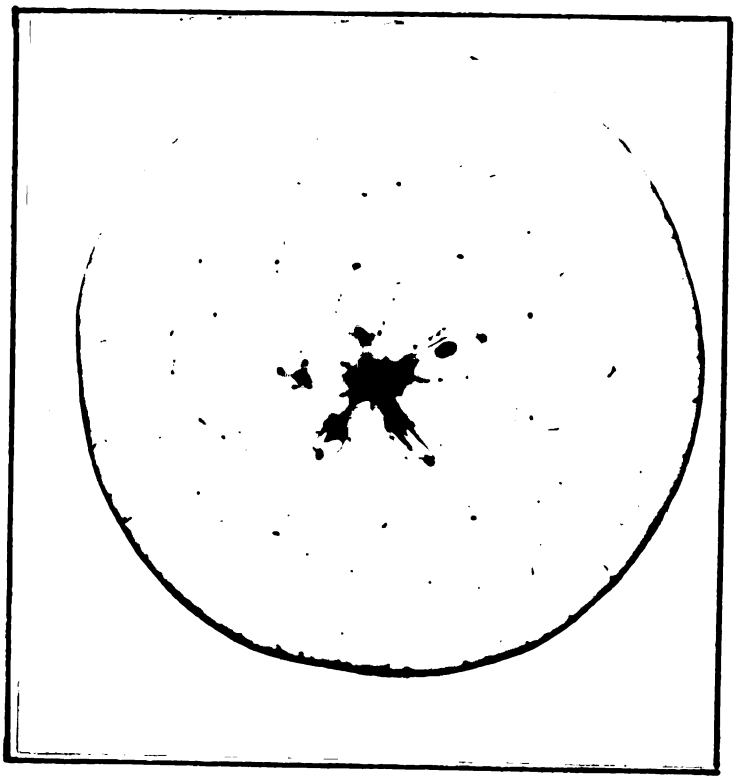


Fig. 42 - August 16

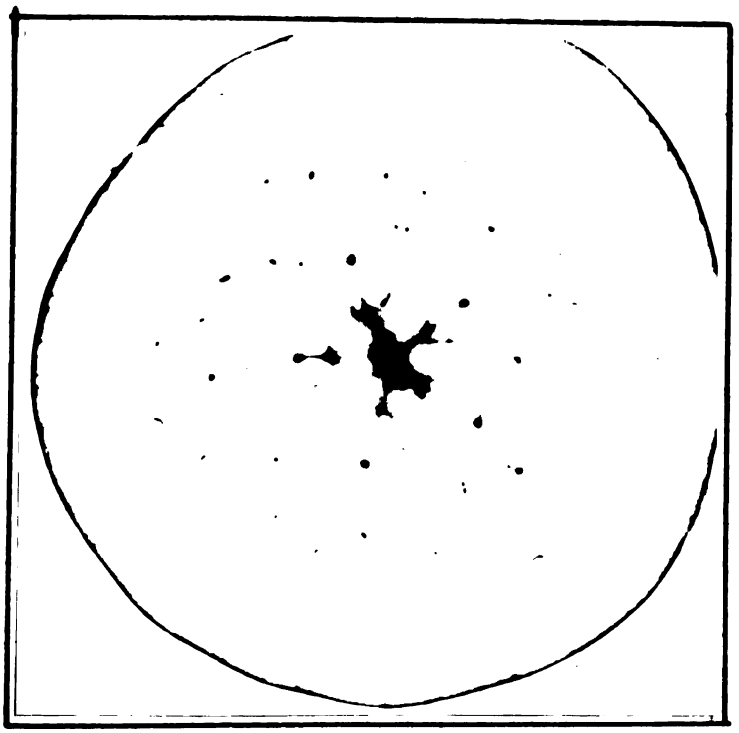


Fig. 43 - Sept. 4

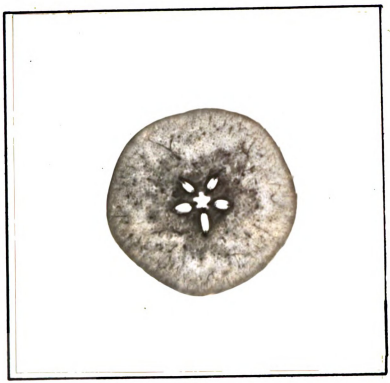


Fig. 44 - July 18

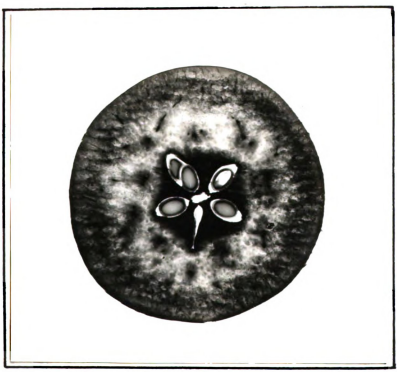


Fig. 45 - August 16 - Cross sections of Kieffer fruit from South Haven orchard showing peroxidase activity at monthly intervals. Continued on next page

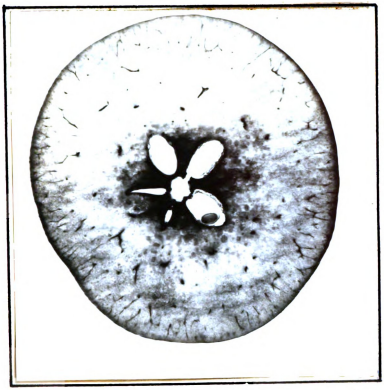


Fig. 46 - Sept. 17

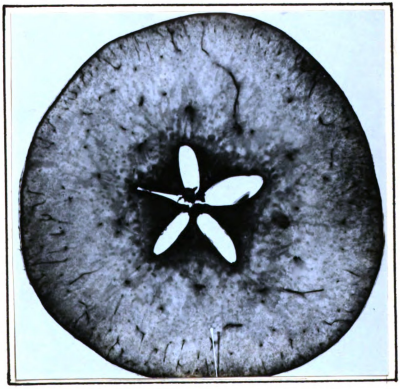


Fig. 47 - October 7

General Discussion

The formation of so called grit cells is characteristic of the fruits of the pear. This distinguishes it from other pomaceous fruits, with the exception of the quince. The various varieties of pears differ as to grittiness, some being much more gritty than others. This variation is not solely a matter of the quantity of grit cells formed and present but also a condition in the distribution and degrees of clustering together of these cells. With the three varieties studied - Kieffer, Clapp Favorite and Bartlett - the quantitative grittiness, as determined by both anatomical and chemical methods, and also the tendency for the grit cells to cluster together into nests were greatest in Kieffer, least in Bartlett and intermediate in Clapp Favorite.

With respect to the time of the beginning of cell wall thickening in the parenchyma cells destined to become grit cells, these three varieties do not differ. The first thickening having become evident in each one twenty-two to twenty-five days after the fertilization of the ovules. However, within a very short time (afew days), differences of the order noted above begin to be obvious, and to become more conspicuous as time goes forward.

Morphologically, the fruit is considered to be a modified stem. Fibers of various types are formed in different parts of the woody stem, and the spur upon which the pear

fruit if borne and developed. Experimental evidence indicates that grit cells are closely analogous, with stem fibers in their chemical compostion, physical structure and manner of formation. These things being true, it was deemed likely that the variety distinctions found in the fruit with respect to the grit cell content would correspond to similar differences in the lignification of the wood and spurs of the trees. This proved to be the case, and hence, grit cell formation in the pear fruit may be looked upon as simply a continuation or an extension of what obtains in the stem, the spur in particular.

It has been pointed out that experimental evidence seems to indicate that pectic substances in plant tissues arise from the oxidation of sugars to uronic acids and the conjugation of these acids with unoxidized sugars; that pectin is converted into hemicellulose by decarboxylation, and that possibly the continuation of this choice of processes leads to lignin and lignocellulose and consequently the structures which are lignocellulosic in character. This suggests among other things, that pear varieties such as Kieffer, Clapp Favorite, and Bartlett differ in grit cell content should vary accordingly in the intensity of their oxidation processes, and the production of uronic acids. Systematic tests in the fruits of these three varieties showed a close relationship between their respective peroxidase activities and their conditions

of grittiness. Other possible antecedents, besides that of peroxidase activity, are open to investigation.

Summary of Results

In these investigations the anatomical and chemical nature of pear grits has been studied. The following conculsions seem warranted:

1. - Lignification in the fruit of the pear begins from twenty-two to twenty-five days after fertilization.

2. - The varieties studied showed that the first indications of cell wall thickening appear very abruptly, there being no evidence of lignification three days previous to when it was first detected.

3. - Early in the development of the young fruit the Kieffer showed a greater tendency to form larger and more numerous groups of stone cells than the other varieties which were studied.

4. - Pear varieties vary greatly in their grit cell content as shown by both anatomical and chemical studies.

5. - There is a high degree of correlation both anatomically and chemically between lignocellulose formations in the wood and the amount of grit cells contained in the fruit.

6. - Peroxidase activity seems to be definitely associated with pear grittiness, the more gritty the pear the greater is its peroxidase activity.

Acknowledgments

The writer is greatly indebted to Dr. J. W. Crist for his assistance throughout the work, from the original planning of the experiment to the completion of the manuscript; to Professor W. C. Dutton for his help with the photographs, and to Professor V. R. Gardner for criticizing the manuscript.

Literature Cited

1. Candlin, E.J. and S. B. Schryver. - Investigations of the Cell Wall Substances of Plants -----. Proc. of the Royal Society (London), Series B. Vol. 103, No B 725 : 365, 1928

2. Burgerstein, Sitz. Kais. Akod. Wiss Wien, 70 : 238, 1874 (Cited by Hass, P. and T. G. Hill. - Chemistry of Plant Products. Longmans, Green and Co., London, 1928.

3. Dore, W H. - The Proximate Analysis of Wood. Vol 11 : 556 1919.
The Proximate Analysis of Hardwoods. Studies on Quercus Agrifolia. Vol 12 : 984, 1920

4. Drain, B D. - Oxidase Activity in Varieties of Apples. Pro.Amer. Soc. Hort. Sci. 125, 1923

5. Duhamel du Monceau, M. - La Physique des Arbes. Paris 1738, 242

6. Eames, A. J., and L. H. McDaniels. - Plant Anatomy. McGraw Hill Co., New York, 1925, 57

7. Grew, Nehemiah, - The Anatomy of Plants ------, W Rawlins, London, 1682, 182

8. Kraus, E. J. - Pollenation of the Pomaceous Fruits -----. Part I, Gross Morphology of the Apple. Oregon Agr. Exp. Sta. Res. Bul. No. 1, 1913

9. Meyen, Pflonzen Physiologie 1841, 274 (Cited by Erdmonu, Julius, Veber Die Concretionen in den Birnen. Amolen der Chemie und Pharmacie. 138 : 1, 1866

10. Stevens, W. C. - Plant Anatomy. P. Blakestons and Co., Philadelphia, 1924, 32

11. Turpin, Par. M. - La Diffelence Ou'offrent Les Tissus Cellulaires De La Pomme et De La Poire ------. 1838