VEGETATIVE PROPAGATION OF THE BLACK WALNUT

With Special Reference to the Factors
Influencing Callus Formation and
Union in Grafting.

Thesis

Submitted to the Faculty of the Michigan State
College of Agriculture and Applied Science
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by

Benjamin Gaillard Sitton

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V. R. Gardner

VEGETATIVE PROPAGATION OF THE BLACK WALNUT

With Special Reference to the Factors Influencing Callus Formation and Union in Grafting. [1]

B. G. Sitton, Associate Pomologist,
Nut Culture Investigations,
United States Department of Agriculture.

INTRODUCTION.

Vegetative propagation of the black walnut (*Juglans nigra*) is usually attended with uncertainty and difficulty. In some cases very satisfactory results have been secured, while, at other times, although the same operator apparently used the same methods and care, results showed very unsatisfactory percentage of successful grafts.

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1. Submitted to the Faculty of the Michigan State College of Agriculture and Applied Science as a thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Work reported herein was begun in 1926 while the author was in residence at Michigan State College, and was continued there until 1929. The officials of the United States Department of Agriculture kindly permitted the continuation of the work during 1930 and 1931.

It is desirable to know why the walnut is so difficult to propagate vegetatively and to find some means of increasing the degree of success, if possible. With these considerations in mind, the work reported herein was undertaken.

REVIEW OF LITERATURE.

Much of the literature on asexual propagation of the Juglandaceae is concerned with the probably unimportant details of various methods which differ very slightly, or not at all, in the essential points. Most of it relates to vegetative propagation of Juglans regia, the so-called English walnut. One group of writers favors the cleft graft to the exclusion of all other methods, while others think variously that the flute graft, the goat's foot graft, or the saddle graft, is the only one that should be followed.

Among the articles which favor the flute graft is the earliest account regarding asexual propagation of the Persian, or English walnut (Juglans regia), which has come to the writer's attention. This is found in Theatre d'Agriculture by Oliver de

Serres(51), as printed in 1805. Leroy (31) comments on the method described, apparently having the first edition available.

De Serresstates that the walnut is propagated by the "Canon greffe" upon the new shoots of the preceding year in the spring or summer. This method is variously called canon (cylinder), cornachet (small horn), tuian (tubular), sifflet (whistle) and flustean (flute). The cion and stock are matched as to diameter so that the cion may fit tightly on the stock. The shoots for the cylinder (bud) are chosen and gathered at the ends of the principal large and vigorous branches, avoiding slender shoots. The leaves and the distal portion of the stem are cut away. A portion of the cion two or three fingers long is rotated between the fingers gently to separate the bark from the wood, cut and started from the wood by a slight twist. On the stock, a branch equal in size to the cion is cut, the bark split lengthwise in three or four places and separated from the wood for a length equal to, or slightly longer, than the cylinder. The cylinder is removed and slipped on the stock. The cylinder must join the wood perfectly.

In discussing this method of propagation, Poiteau (44) says that, though it is desirable to have the stock and the cion as nearly as possible the same diameter, this is not necessary. When the flageolet (cylinder) is too tight, it may be split longitudinally on the side away from the bud, and a strip of bark of the stock used to fill the gap. If the circumference of the cylinder is too great, fitting is attained by removal of a longitudinal strip. All parts of the cylinder should be pressed close to the wood of the stock; usually a ligature is necessary to accomplish this.

Gagnaire (24), Mortillet (39), and others (3), say that the flute graft is the only method practicable for use in the nursery. Martin (34) says this method is commonly used in southern France. Mortillet (39) reports that the most experienced workers succeed with only one-third of the flute grafts set. The operation demands skill and care to remove the cylinder without injury to it. He states that the operation should be delayed until danger of spring frosts is past.

In France about 1830, and in fact much later, it was the practice to graft walnut trees only after they began to bear, if their fruits were not desirable (38, 39, 44). Poiteau (44) discussed the advisability of growing and grafting seedling trees in the nursery and then transplanting to their permanent places.

Various writers (1, 3, 6, 15, 16, 17, 26, 33, 34, 35, 38, 43, 57) have favored the cleft graft and have described their procedures, which do not differ essentially from the method followed today. Baltet (7) advises the cleft in a fork.

Besides the cleft graft, Knight (26) recommends the saddle graft. Baltet (6) recommends the bark graft, which is later described by Morris (37) as "New Method in Grafting", and also by Biederman (11), Leroy (31) and others (2, 25) mention the claw graft, side graft, or goat's foot graft, as it is variously called.

Various authors, Baltet (6) and others (10, 55) have said that the English graft, or as it is known in America, whip graft, is very suitable for vegetative propagation of the Persian walnut. Some recommend the plain whip and others the whip with the tongue to hold it more closely together.

In addition to the flute bud, other methods of budding have been described and advised. Some have said that the shield bud, as commonly used with the apple and the peach, is also applicable to the walnut (4); others (34) have denied its suitability for the walnut.

The flute bud, whereby a long tube of bark carrying buds was slipped off the cion stick and onto the stock laid bare by peeling off the bark, has evolved by various steps into the patch bud. It first was modified into the ring bud, consisting of a piece of bark about an inch long carrying a bud being removed from the cion as a ring and placed on the stock where a similar ring had been removed. This was considered more satisfactory because it did not necessitate the removal of the top of the stock. Usually, however, when the bud failed to unite, the top of the stock died. To avoid this, it became the practice to leave a strip of bark of the stock intact. This latter was then called the "patch bud" and is used very extensively in vegetative propagation of the pecan. This method has been advocated for the walnut by Kraus (28), Lewis (33), Lake (29), Oliver (42) and Batchelor (10), and for the pecan by Blackmon (9), Reed (49, 50), Evans (23), Woolsey (61), and Woodard, Romberg and Willman (62).

Kraus (28), Lake (29) and Oliver (42) suggested a modification of the patch bud. Instead of a path of bark being removed from the stock, an "I" cut was made and the cion patch inserted under the two flaps thus formed.

According to Leroy (31), Batchelor (10) and others (2, 3 and 33), grafting in the crown, or root, level with ground, or slightly below, offers much chance of success. They suggest the use of the claw-foot, or the whip graft. When the graft is made, soil is mounded to near the top of the cion. It is difficult to evaluate reports on crown grafting for some writers (3, 4, 6, 40) refer to grafting in the main branches of larger trees as crown grafting, while some, as Leroy (31), refer to the graft placed at the surface of the soil by this term.

Carriere (15) described a method which he did not claim as original and, 27 years later (16), which he ascribed to Treyve as being new and original. In the spring before the development of leaves, January to March, young trees are taken up, their tap roots cut off and the trees placed in 8 to 10 cm. pots, which are then plunged in a cool place in the garden. If no potted trees are available at the time of grafting, the trees are grubbed, grafted and then potted. It is not fully clear, from his description, at just what time of the year the actual grafting is done, but others (8, 35) give the time as the first part of March. The stocks are cleft grafted near the crown, the grafts bound and covered with wax. A bell jar is then placed over them for three weeks or a month. When they begin to grow, they are given air gradually to harden them off.

Others (6, 8, 17, 35, 56, 60) have described this method; some advised the use of whip graft instead of cleft, some adding minor points and some to explain certain steps. Witt (60) said if bottom heat of 70° to 75° F. is maintained, union is effected more rapidly. Knight (26) used a method quite similar except that he does not mention the use of a bell jar or similar apparatus. His idea was primarily to retard the growth of the stock until cion wood was suitable for use. He used cion wood of the current season's growth.

Witt (60) states that the walnut can be propagated by soft wood grafts. Potted plants in the greenhouse are grafted with wood which is about half ripe. Cions are either the terminal portion or a piece with two eyes, having half of the leaf surface removed. The grafting is done by the saddle or strap method. He also says that soft wood cuttings occasionally strike root.

Various types of cion wood have been used and advised, in many cases apparently from personal preference based on interpretation of experience.

De Serre (51) and others (24, 34, 38, 44), advising the use of the flute bud, recommend cion wood of the past season's growth, cut after growth had begun in the spring and used immediately. This, of course, was necessary in the flute bud. Knight

(26, 27) used wood of the current season's growth collected after buds were well formed. He also used wood of the past season's growth, allowed the buds to swell and removed them, using the small secondary buds for cions. He also used the small buds from near the base of one year wood.

Poiteau (44) recommended cutting cion wood in February and burying it in soil to keep it dormant. Carriere (15) advised the use of cion wood of the past season's growth, cut in January, as did Mortillet (38). Michelin (35) reported cions should be cut early in March.

Leroy (31) stated that his first success in grafting the walnut was obtained by use of wood two or three years old. He said that one year wood had relatively more pith than woody portion, while the older wood had very little pith as compared with wood. Baltet (6) reported one year wood with a portion of two year wood at its base to be satisfactory for use as cion wood. Another writer favored the wood at the junction of one and two year wood, and Gaucher (25) shows a figure illustrating this practice.

In the United States, Cooper (20) described good cion wood as being of the past season's growth, cut in late winter from upright growing branches. Lake (29) gave the following description of desirable cion wood: "wood of past season's growth which has little pith, strong but not large buds and firm wood, 1/4 to 3/8 inch in diameter, with short internodes, taken from mid portion of trees. Cut two or three weeks before growth starts. Dip cut ends in wax, wrap in moist paper".

Evidence on the effect of temperature on graft unions is more extensive than definite. The better results attending late, as contrasted with earlier, grafting, rather frequently reported, may be construed as unconsciously given evidence of favoring effects of high temperature. The success of the Treyve method may be similarly interpreted.

Oliver (41) described a method followed by W. P. Corsa. Stocks, two years old, are cut off just above the junction of stem and roots. Cions are put on by any convenient method, usually tongue or splice, and securely tied. The grafts are packed in layers of spaghnum and placed where the temperature is maintained from 75° to 80° F., usually for three weeks. The grafts are then potted and, when a few leaves have been made, the potted plants are hardened off, and planted in a frame where they pass the first year. By this method, about 75% "take" was secured. In this case also, the temperature was one of the possible factors.

Ravaz (48) reported experiments on bench grafting of the grape. He found that below 20° C. union forms very slowly; at 22°, it is more rapid; and that it is extremely rapid in the neighborhood of 30°. He quoted Mazade to the effect that the best temperature seems to be from 30° to 35°. At this temperature, the evolution of shoots is prompt, the tissues unite in 8 days, and the grafts can be removed from the moist material. Humidity should be kept high.

Most writers emphasize the fact that the walnut is very difficult to propagate asexually. Mortillet (39) stated that a skillful operator can generally count on one-third to one-half of the cions set living. Knight (26, 27), Carriere (16), Michelin (35) and others, reported that asexual propagation is only rarely successful.

EXPERIMENTAL METHODS.

Work on the vegetative propagation of the black walnut was begun in 1926. At the outset, since slow callus formation seemed to characterize the walnut, the condition of the reserve materials in the cion and stock was thought to be primarily responsible for the wide variations in the degree of success obtained at different times. Consequently, the first work consisted of an attempt to modify the stored food materials within the stem, with the aim of obtaining differences in behavior in grafting.

Early in the work, it became apparent that other factors besides the stored food materials were operative and possibly of more importance. The work was therefore modified to include other lines of investigation. These lines are reported separately in the following order: (1) The influence of reserve food supply, especially of carbohydrates and nitrogen; (2) The effect of treating the cions with chemicals; (3) Propagation by budding; (4) The influence of anatomical characteristics; (5) The influence of gross anatomy; (6) The influence of atmospheric humidity; (7) The influence of temperature.

1. THE INFLUENCE OF RESERVE FOOD SUPPLY.

Cion Wood.

For this series of experiments, cion wood was obtained from a block of seedling trees in the nursery of the Forestry Department of the Michigan State College at East Lansing. These trees were about twelve years old at the beginning of the experiment, 10 to 15 feet high, moderately vigorous, but not uniform. Cion wood was selected from those in the best state of vigor.

To secure varied chemical conditions in wood destined for cions, shoots were treated in five ways.

A part of the shoots was left in the natural condition, without any treatment. This was divided into two lots; one lot was gathered in the fall of 1926 and the other lot in the spring of 1927. These are designated "natural-fall" and "natural-spring", respectively. Each of these lots was subdivided into five classes: 1, apical portion of one year wood; 2, basal portion of one year wood; 3, apical portion of two year wood; 4, basal portion of two year wood; and 5, apical portion of three year wood. Class 4 corresponds to the lot "Above girdle" and class 5 corresponds to the lot "Below girdle", as mentioned below.

A third lot was prepared in August, 1926, by twisting a wire tightly around the stem at the base of the two year wood. The six inch portion just above this wire girdle was used as cion wood and called "Above girdle". The six inch portion just below the wire girdle was used as cion and called "Below girdle".

About the same time a fourth lot of cion wood was prepared by partly defoliating. The rachis of the compound leaf was severed so that approximately half of the leaf area was removed. This lot is called "Defoliated".

Trees from which a fifth lot of cion wood was collected received an application of sulphate of ammonia about the middle of September, 1926, after growth had ceased. This did not cause a resumption of growth, but the trees retained their leaves later in the fall than those not receiving the treatment and began growth earlier the following spring. This treatment is designated by the word "Nitrate".

For the cion wood which was used in the 1928 grafting, treatments were begun earlier in 1927. Nitrate of soda was applied on July 11 for the "Nitrate" cions and branches were girdled with wire on July 18 for the "Girdled" cions.

Cion wood from the "Natural-fall" class was gathered December 11, 1926, and stored, and cion wood for the "Natural-spring" and all other classes was gathered April 6, 1927, and stored until needed.

Cion wood for use in 1928 was gathered early in May, packed in sawdust, and placed in cold storage. The storage did not work well and temperatures were too high, causing many of the buds to swell.

Samples for chemical analysis were collected at the time the cion wood was cut.

STOCK.

Seedling trees that were used for stocks onto which were grafted the cion wood previously described were located in the nursery of the Forestry Department. They were about five years old at the time the experiment was begun, and about four feet high. Although as a whole they were not uniform, it was possible to select trees that were fairly uniform. Some of these trees were girdled, some defoliated and some fertilized as described for the cion wood, except that in this case the wire girdle was placed around the main stem near the ground.

GRAFTING.

In 1927, grafting was begun April 25 and completed May 4, and in 1928 it was begun May 14 and completed May 25. Various combinations of the treated and untreated cions with the treated and untreated stocks were made. These combinations, with the percentages of successful unions are presented in Tables 1 and 2. The whip, or tongue graft, made in the usual way, was used in 1927; the simple splice graft and a few cleft grafts were used in 1928. The cion was fitted to the stock tied with a cotton string and the entire union and tip of cion covered with a brush wax, made according to a common formula, (14). The entire cion and union were covered with a kraft paper bag.

CHEMICAL.

Carbohydrate Analysis.

Samples for chemical analysis were cut into half inch pieces, weighed, and preserved by drying at 90° C., ground to pass a 60-mesh sieve. An aliquot of the dry ground material was extracted with five 80 ml. portions of 80% alcohol. After the last extraction, the residue was washed several times with 80% alcohol.

The alcohol was evaporated at reduced pressure at 50° C. and the residue taken up with about 150 ml. water. The extract was clarified with lead acetate and deleaded with dibasic sodium phosphate. The reducing power of an aliquot was determined and expressed as dextrose.

The Shaffer and Hartment iodine titration method was used to determine the reducing power. Duplicate determinations were made which checked to 0.1 ml. thio-sulphate.

An aliquot of the clarified extract was hydrolyzed with 1.5% HCl for 10 minutes at 70° C., and the reducing power determined and expressed as total sugars.

The residue from the alcohol extraction was digested with taka-diastase for 36 hours at 38° C., filtered, and the filtrate hydrolyzed with 1.8% HCl for 2½ hours. The reducing power was determined without previous clarification. From this the starch content was calculated.

The residue from the taka-diastase digestion was hydrolyzed for 2½ hours, with 1.8% HCl, cooled, filtered, and the reducing power determined. From this, calculations were made for acid hydrolozable polysaccharides other than starch, presumably hemi-cellulose.

NITROGEN ANALYSIS.

Soluble, insoluble and total nitrogen were determined by the Experiment Station Chemist, using the Keldahl-Gunning method.

PRESENTATION OF DATA.

The influence of the age of cion and the time of collecting cion wood upon the per cent of "take" in black walnut are presented in Table 1. The cion wood was natural or untreated, part of which had been cut in late fall of the preceding season and part cut a short time before growth commenced in spring. These were divided into the ages and portions as shown in the table. The stock was natural or untreated.

The data of this table clearly indicate that cion wood collected in the spring is much superior to that collected in the fall. This is probably due to the length of the storage period; possibly some change in the stem due to continuation of respiration without replenishment of materials is responsible. Some further observations indicate that loss of water from the stem is also a factor even though the shoots were packed in moist material.

With both fall collected and spring collected cions, the per cent of "take" increases with increase in age of wood. The greater success with older cion wood might appear to be due to the size of the buds and the readiness with which they begin to grow. At each node on the walnut usually three buds develop, the more distal one being large and the proximal one very small, often invisible to the unaided eye. Another bud located between these is an intermediate size. Near the distal end of the one year wood, the distal bud develops to an extent that makes it very prominent and, during the following growing season, may grow into a shoot. The larger bud at each node which does not produce a shoot during the second season usually abscises and occasionally the medial bud also abscises, especially if it developed to a noticeable size. On bearing trees, this medial bud frequently produces staminate flowers. The third or smaller bud at each node remains dormant until forced into growth by amputation of the shoot beyond, or some similar stimulus.

The large buds on the one year wood, especially those on the apical portion, force into growth readily when this portion of the shoot is used for cion. The resulting growth exerts a demand for stored materials which may deplete them to such an extent that callus formation does not take place at the cut end.

In this particular experiment such growth did not occur, in fact, the cion died before any growth took place. Later experiments indicate that the greater xylem and phloem formation is largely responsible for the better results with older (and larger) cions.

This superiority of two and three year cion wood was evident in all subsequent experiments where one, two and three year shoots were used.

Data showing the influence of girdling the shoot, defoliating and application of nitrogenous fertilizers are presented in Table 2. In this table, the data are arranged to show the influence of these treatments on the cion and, in Table 3, the same data are arranged to show the influence of the treatments on the stock.

The percentage of "take" is low in all instances but there are some wide variations. In most cases, these variations did not hold consistently for the two years nor were they consistent within any one or more classes of cion or stock.

Using the combination of natural cion on natural stock as a basis of comparison, there is some evidence of a positive influence of the treatment. Three combinations of cion on stock resulted in an increase in per cent of "take" over the natural-natural both years. These are: nitrate cion on above girdle stock, above girdle cion on above girdle stock, and above girdle cion on nitrate stock. Six combinations gave better results than the natural-natural one year and poorer results the other year. These are: natural-nitrate, above girdle-below girdle, above girdle-natural, below girdle-above girdle, below girdle-below girdle, cion-stock, respectively. All other combinations were poorer both years, or the combination was used only one year.

Of all classes of cion and stock, the most outstanding results are with the above girdle cion and the defoliated cion. The above girdle cion was used in four combinations and with two, the results were better both years, and with two, the results were better one year when compared with the untreated cion. The defoliated cion was used only one year but, in every combination, results were poorer than the natural cion gave. In three of four combinations, the nitrate cion gave poorer results both years and in the other better results both years than the natural cion.

Carbohydrate and nitrogen analysis of the shoots used for cions and of the stock are presented in Table 4, together with the per cent of "take" secured when used for grafting. In most cases, the latter are averages of the results of each class of cion on all classes of stock used and of each class of stock with all classes of cions.

Data for natural cion show a general increase in per cent of success with an increase in total carbohydrate-nitrogen ratio, but there is not a definite correlation. Data for stock show a general inverse relationship,that is,a decrease in success with an increase in the ratio.

TABLE 1. INFLUENCE OF AGE OF CION AND TIME OF COLLECTION ON RESULTS OF WALNUT GRAFTING, 1927. NATURAL CION ON NATURAL STOCK.

Age of cion Years	Portion of annual growth.	Percentage "take". Fall collected.	Spring collected.
1	Apical	0	0
1	Basal	0	21.7
2	Apical	20.0	33.3
2	Basal	9.1	31.6
3	Apical	30.8	52.7

TABLE 2. INFLUENCE OF GIRDLING, DEFOLIATING AND NITROGENOUS FERTILIZERS UPON THE WALNUT CION WHEN USED IN GRAFTING.

Cion	Stock	Percentage "take" 1927	Percentage "take" 1928	Relative Behavior		Order two years combined
Natural	Natural	39.2	32.0	100.0	100.0	4
	Nitrate	36.8	46.0	93.8	143.7	5
	Above girdle	-	12.2	-	37.5	18
	Below girdle	-	30.4	-	95.0	14
Nitrate	Natural	37.5	30.4	95.6	95.0	11
	Nitrate	33.3	26.0	85.0	81.2	12
	Above girdle	58.8	32.0	150.0	100.0	1
	Below girdle	-	17.4	-	54.4	16
Above girdle	Natural	26.7	34.8	70.0	108.7	7
	Nitrate	40.0	32.2	102.0	100.6	3
	Above girdle	40.0	41.6	102.0	130.0	2
	Below girdle	61.6	24.0	157.1	75.0	6
Below girdle	Natural	37.5	4.2	95.6	13.1	13
	Nitrate	-	55.5	-	173.4	10
	Above girdle	15.4	44.0	39.3	137.5	8
	Below girdle	20.0	43.5	50.9	136.0	9
Defoliated	Natural	33.3	-	85.0	-	15
	Above girdle	12.5	-	32.0	-	20
	Below girdle	13.0	-	33.1	-	19
	Defoliated	20.0	-	50.9	-	17

TABLE 3. INFLUENCE OF GIRDLING, DEFOLIATING AND NITROGENOUS FERTILIZERS UPON THE STOCK OF WALNUT WHEN USED IN GRAFTING.

Stock	Cion	Percentage "take" 1927	Percentage "take" 1928	Relative Behavior on basis of natural 1927	Relative Behavior on natural 1928
Natural	Natural	39.2	32.0	100.0	100.0
	Nitrate	37.5	30.4	95.6	95.0
	Above girdle	26.7	34.8	70.0	108.7
	Below girdle	37.5	4.2	95.6	13.1
Nitrate	Natural	36.8	46.0	93.8	143.7
	Nitrate	33.3	26.0	85.0	81.2
	Above girdle	40.0	32.2	102.0	100.6
	Below girdle	-	55.5	-	173.4
Above girdle	Natural	-	12.2	-	37.5
	Nitrate	58.8	32.0	150.0	100.0
	Above girdle	40.0	41.6	102.0	130.0
	Below girdle	15.4	44.0	39.3	137.5
Below girdle	Natural	-	30.4	-	95.0
	Nitrate	-	17.4	-	54.4
	Above girdle	61.6	24.0	157.1	75.0
	Below girdle	20.0	43.5	50.9	136.0
Defoliated	Defoliated	20.0	-	50.9	-

TABLE 4. CARBOHYDRATE AND NITROGEN ANALYSES OF CION WOOD AND STOCK IN PERCENTAGES AND FIELD RESULTS, 1927.

	Dry matter	Total sugars	Starch	Hemi-cellulose	Total carbo-hydrates	Total nitrogen	C/N	"Take"
Natural-Fall								
Apical- 1 yr.	49.6	4.92	1.95	13.05	19.92	1.29	15.46	0
Basal- 1 "	51.8	4.69	1.46	11.93	18.08	.80	22.60	0
Apical- 2 "	54.0	4.45	1.40	11.79	17.64	.62	28.45	20
Basal- 2 "	54.8	3.90	2.48	9.83	16.22	.74	21.92	9
Apical- 3 "	-	6.95	1.48	14.41	22.84	.70	32.63	31
Natural-Spring								
Apical- 1 yr.	48.2	2.91	1.18	13.72	17.81	1.34	13.29	0
Basal- 1 "	54.1	2.50	.38	11.81	14.69	.77	19.08	22
Apical- 2 "	57.4	4.30	1.91	13.05	19.26	.73	26.38	33
Basal- 2 "	54.4	3.67	.97	12.04	16.68	.75	22.24	32
Apical- 3 "	52.7	2.79	1.15	11.25	15.19	.64	23.73	53
Above girdle	51.9	2.35	2.75	12.49	18.59	.72	25.82	41
Below girdle	52.9	2.57	.91	11.81	15.29	.63	24.27	25
Defoliated	54.8	3.20	.98	12.38	15.56	.52	31.84	19
Nitrate	54.1	2.97	.98	11.74	15.74	.87	18.09	42
Stock								
Natural	57.6	5.08	1.56	12.38	19.02	.64	29.72	39
Nitrate	53.1	3.52	1.91	13.61	19.04	.64	29.75	37
Above girdle	58.1	6.25	1.65	14.29	22.19	.60	36.98	32
Below girdle	57.1	4.53	1.56	14.41	20.50	.56	36.61	32
Defoliated	56.2	5.41	1.12	12.04	18.57	.53	35.02	20

The cions treated by girdling, defoliation and nitrogenous fertilizers do not show a correlation either with, or inversely to, the carbohydrate ratio.

In respect to nitrogen analysis, the natural cion shows success inversely to the per cent nitrogen. The treated cion and the stock show success increasing with the increased per cent nitrogen.

In respect to total sugar, starch, hemi-cellulose and total carbohydrates, no definite relationship to the per cent of success can be discovered.

These data do not show any outstanding influence upon success in grafting, nor in carbohydrate-nitrogen analysis, as a result of the treatments of the shoots.

The method of analysis for carbohydrates is open to criticism in that the sample was dried at a temperature which was too high. The time of sampling is also at fault in that samples were taken at the time the cion wood was collected. Behavior of cions upon grafting indicates that the sample probably would have been more nearly representative had it been taken at the time the grafting was done.

EXPERIMENTS OF 1931.

Further work on the influence of stored food materials was postponed until certain other factors could be worked out, principally the influence of temperature. Having this worked out, it was decided to repeat a portion of the earlier experiments.

September 3 and 4, 1930, cion wood on walnut trees of Mr. W. J. Fullilove, Shreveport, Louisiana, was prepared. Branches were ringed in three year old wood and the wound was covered to protect it from drying out. This cion wood was collected February 2, 1931, and packed in moist spaghnum moss, and was later used to make grafts, being placed on two year old seedling roots near the junction of the stem and root. The grafting was completed on February 7.

One hundred grafts of each of the following series were made: (1) basal portion of one year wood from girdled stem, (2) two year wood from girdled stem, (3) three year wood from girdled stem, which was cut so that the bevel was just above the girdle usually cutting through a portion of the enlarged stem which was stimulated by the girdle, (4) basal portion of one year natural stem, (5) two year wood of natural stem, (6) three year wood of natural stem. Fifty grafts were made of the portion immediately below the girdle.

These grafts were packed in moist peat and placed in boxes with temperature controlled at 26°-28° C. They were left in the boxes for three weeks and then examined. After the examination, the grafts were again packed in moist peat and placed on the south side of a building, with the temperature uncontrolled, to allow them to grow. They were examined on April 25 and count made of those actually growing. Results are presented in Table 5.

TABLE 5. INFLUENCE OF AGE OF SHOOT AND GIRDLING UPON THE BEHAVIOR OF CION WOOD OF THE WALNUT UPON GRAFTING. GRAFTS EXPOSED TO TEMPERATURE OF 26°-28° C. FOR 21 DAYS.

Class of cion wood	Callused February 27, 1931.	Growing April 25, 1931.
1 year natural	89	76
2 year natural	90	89
3 year natural	87	90
1 year above girdle	81	77
2 year above girdle	92	86
3 year just above girdle	88	88
Just below girdle	82	70

There is a strikingly higher per cent of grafts growing with all classes of cion wood than had previously been secured when the temperature was not controlled.

With both the natural shoots and the girdled shoots, there is an increase in the per cent growing with increase in age, but this increase is not as great in the girdled wood as in the natural wood. Cion wood from below the girdle gave very markedly poorer results than any other class.

2. TREATMENT OF CIONS WITH CHEMICALS.

Treatment of cuttings with chemicals has been found by Curtis (22) and others (21 and 59) to increase the percentage of rooting in some species. Though it is realized that rooting of cuttings and callus formation are not usually associated, it was thought that similar treatments might increase callus formation and the uniting of grafts. Cooper (20) states that dipping cut cions into, and washing the cut stock with, a solution of four teaspoons of sulphate of quinine to a gallon of water will result in 90% walnut grafts growing.

On May 25, 1928, natural cions were cut as for grafting and some were immediately placed in beakers containing respectively: a 0.5% solution of potassium permanganate, a 0.5% solution of sucrose, a 2% solution of sucrose, a 2% solution of dextrose, a 5% solution of vinegar, a 2% solution of potassium nitrate, a saturated solution of quinine sulphate, about 0.14%. One lot was placed in distilled water, one lot exposed to fumes of ether, and one lot left untreated and cut and immediately grafted or placed in the greenhouse bench.

The cions were allowed to remain in these solutions for 24 hours with the exception of the quinine solution in which they remained two hours. A portion of the treated cions, with check, was placed in clean sand in the greenhouse bench and the remainder were grafted by the whip graft method onto stocks in the nursery as described earlier.

Callus formation on cions in the greenhouse was not materially improved; 13% of the check and 19% of those treated with potassium permanganate and 21% of those treated with 2% sucrose formed callus, while all other cions failed to form callus. The results of the grafting were similar; 25 per cent of the checks, 15 per cent of those treated with potassium permanganate, and 16 per cent of those treated with quinine formed successful unions while all others failed.

These results were from a rather limited trial, but they indicate that none of the treatments tried gave enough improvement in callus formation or union in grafting to warrant their use.

In another series of experiments, the beakers containing the solutions and cions were placed in a chamber which was evacuated by use of a filter pump. This removed air from the cion and the solution was drawn up into the stems. Considerable quantities of the chemicals were probably forced into the stems. Results from this series were in general agreement with the first experiment in that no increase in per cent of successful unions was obtained.

3. PROPAGATION BY BUDDING.

In May, 1927, about 150 buds of named varieties were set and in June, 1928, about 500, some of named varieties and some from girdled and defoliated cions were set.

The patch bud method described by Kraus (28) was used in setting some and that described by Stucky and Kyle (53) for pecans used in setting others. A modification of the budding knife described by the latter under the name of "Texas Agie" was used in all the budding. The modification consisted of the removal of the handle provided, making a tool that could be held in the palm of the hand and therefore was more convenient to handle.

This budding was not done on a controlled experimental basis, but the results are rather interesting. Tables 6 and 7 present the results of the 1928 series of budding.

The bud wood of the Stabler variety was cut about six weeks before the time of budding, while that of the other varieties was cut just before growth started, about two weeks before it was used. The Stabler and Ohio bud wood was not in the best of condition and it was difficult to get the patch with the bud separated from the bud stick without injuring the growing point of the bud. The other varieties were not ideal in this respect, but they were much superior to the Stabler and Ohio. The effect of having suitable bud wood is clearly evident in the results.

Another point of interest is the difference in the take of Stabler on black walnut stocks No. 1 and No. 2. The nuts from which stock No. 1 were produced germinated poorly and the seedlings have grown slowly, showing all appearance of poor vigor. Those seed from which stock No. 2 were produced germinated well and a stand of about 90 per cent was secured. These seedlings have grown well and appear vigorous. The percentage of living patches is smaller on stock No. 1 and those which did live were slower in producing shoots than on stock No. 2. The average length of growth on stock No. 1, on August 8, 1928, was about six inches (Fig. 1), while that on No. 2 was about 18 inches (Fig. 2). Furthermore, a greater proportion of the living patches produced shoots on stock No. 2. Figure 2 shows the Kinder variety but is typical of all varieties

TABLE 6. WALNUT BUDS SET AT GRAHAM STATION, GRAND RAPIDS, JUNE 1, 1928. RECORD AUGUST 8, 1928.

Variety	Stock	Buds set	Producing shoots %	Patch living not producing shoots %	Total patches living %
Stabler	J. nigra No. 1	49	10.2	18.4	28.6
"	" " No. 2	50	30.0	20.0	50.0
"	J. cinera	12	25.0	33.4	58.4
Stabler girdled	J.cordiformis	32	21.9	18.7	40.6
Thomas	J. nigra No. 2	20	60.0	35.0	95.0
Kinder	" " No. 4	25	76.0	4.0	80.0
Ohio	" " No. 4	15	46.0	6.7	54.4
Miller	" " No. 4	21	57.2	14.3	71.5
Total Stabler and Ohio		158	23.4	18.9	42.4
Total other varieties		94	61.7	15.9	77.6

TABLE 7. J. CORDIFORMIS BUDDED ON J. NIGRA AT EAST LANSING IN 1928.

Bud Wood	Buds set	Producing shoots %	Patch living not producing shoots %	Total patches living %
Natural	13	61.5	23.0	85.0
Girdled	14	15.4	50.0	64.4
Nitrate	13	46.2	15.3	61.5
Nitrate girdled	26	23.1	19.3	42.4

on this stock.

Table 7 gives the results of budding done on black walnut using cions of Juglans cordiformis which had previously been treated to modify their chemical composition. Half of a row of two year old seedlings were fertilized heavily with nitrate of soda on July 11, 1927. On August 9, alternate trees of both the fertilized and the unfertilized were girdled just at the surface of the ground by twisting a wire tightly around the stem. These treatments gave four classes of cion wood: 1, natural; 2, girdled; 3, nitrated; and 4, nitrated and girdled. Buds from these cions were set in black walnut stock.

Results show that the untreated cions gave best results and that any treatment seems detrimental. Girdling depressed the percentage of living patches and delayed the shoot growth. Presumably, the girdling and fertilizer treatments result in development of the bud trace and growing point to the point that removal of the bud from the bud stick often injures the growing point of the bud.

In the spring of 1929, a third series of budding was done. On April 5, 1929, one year old and two year old cion wood was cut and divided into three lots each. One lot was covered completely with melted paraffin, a second lot had the cut ends only waxed, whil the third lot was not waxed. On April 30, a second lot of cion wood was cut and treated similarly. On June 1, a lot of cion wood was cut fresh and buds from it and from all of the treated cions were set on that date.

Cion wood waxed completely is difficult to handle. Patches of bark are hard to remove from the stem because cambial activity is depressed and because the wax makes it difficult to secure a firm grip on the cion. Cions which had not been waxed had suffered slightly from desiccation and the bark did not slip as readily as is desirable. Cions which had the cut ends only waxed were in excellent condition. The bark was plump and fresh looking, and it separated readily from the wood.

In all cases except one, the cion wood which had only the cut ends waxed gave much better results than the other classes. Cions waxed entirely gave slightly better results than the cions not waxed but the additional time required to loosen these buds from the bud stick will more than offset the advantage gained.

Cion wood cut June 1 had started to grow slightly and it was difficult to remove the patches of bark without injuring the growing point. Probably had this wood been taken a week earlier better success would have been secured.

TABLE 8. RESULTS OF BUDDING DONE IN SPRING OF 1929.

Date cion cut	Age of wood years	Waxing	Per cent living
April 5	1	none	91
	1	ends	90
	2	none	36
	2	ends	75
	2	all	60
April 30	1	none	69
	1	ends	100
	1	all	87
	2	none	64
	2	ends	82
	2	all	75
June 1	1	none	44

These more or less preliminary trials indicate that the time of budding is very important. Budding done soon after growth began did not give nearly as good results as budding done three weeks later, when the leaves on the stock were about one-third mature. The condition of the bud wood is also important. The cambium must be in an active condition to permit the bark to"slip" from the stock, but the bud must not have become active to the extent that the growing point is likely to be injured during the budding process.

If the bud trace of the bud selected developed to the point that any great portion of it becomes lignified, it will be difficult to remove the bud without injuring it. This is the condition of most of the larger buds on the distal half of a twig of the preceding year's growth. The best buds for use are usually found at the proximal end of the preceding year's growth, or at the proximal end of two year old wood. On the two year old wood, the buds may often be so small as to be almost invisible. These buds are the ones that were formed last of the two or three that occur in each leaf axis, the larger ones formed earlier having shed. Such buds are usually satisfactory for use in propagating by the patch method.

The method described by Kraus (28) is unsatisfactory in the black walnut for several reasons. To secure good results by budding, it is necessary that the bud be pressed firmly against the wood of the stock. The two flaps of bark from the stock seriously interfere and pressure of the bud against the stock is not secured, which means that the union between the growing point of the bud and the stock will be delayed and may entirely fail (Figs. 3 and 4). A second objection is that the vertical cut on the stock usually comes directly under the growing point of the bud. The injury to the cells on the stock may prevent the formation of callus tissue from the stock and thus delay or prevent union.

Figure 5 shows the normal uniting of the regular patch bud and Figure 6 shows a case in which the shield united well but the bud failed to grow because the growing point was injured in the transfer.

4. THE INFLUENCE OF ANATOMICAL CHARACTERISTICS.

Sections were made from successful and unsuccessful grafts and from pruning wounds which had begun to heal. Fresh material was sectioned on a sliding microtome set to cut 20 microns. They were stained by a modification of a method described in Turtox News: sections were placed in an alcoholic solution of safranin for 30 minutes to several hours. They were destained in acidified 50% alcohol and transferred to 50%, 70%, 95% and absolute alcohol, remaining in each only a few minutes. They were passed thru 75% xylol in absolute alcohol into xylol in which a small amount of gentian violet was dissolved, allowed to remain there until the cellulose tissue was stained, then transferred to pure xylol and mounted in balsam.

The most striking aspect of sections of walnut grafts (Figures 7 and 8) is the large size and number of vessels, and of the large proportion of the old xylem tissue which is dead. The vessels had not become plugged with gum as is common in the tissue of the pear (Figure 9) and apple (Figure 10), possibly because the wood died too rapidly. Rapid killing of apple wood is characterized by absence of wound gum.

Sections of pruning wounds on walnut stems (Figs. 11, and 12) show some degree of closure of vessels but a much greater closure of tracheids and vascular ray cells. The rays and tracheids seem to be closed by plugging with some gum like substance, the nature of which has not been determined (Fig. 13). Some of the vessels are closed by tyloses (Fig. 14) which occur in wood formed after the wound is made as well as in wood already present at the time of the wound. In the sections examined no tyloses have been found in wood older than two years. Figure 14 shows relatively greater proportion of vessels having tyloses than is typical for tissues not stimulated by wounds.

Apparently, the uniting of walnut grafts is not hindered or limited by the inability of the new growth to change directions. Figures 11 and 15 show the change in orientation of new growth. Figure 11 is a radial section of a stem of walnut which was amputated to a lateral branch. The new growth is supplied by the lateral branch at an angle of 90° from the plane of the section. The vessels in the new wood are cut almost transversely, indicating that the direction of flow of sap had changed. Figures 7 and 8 show similar reactions in the growth succeeding the union of a graft.

The difficulty in grafting walnuts, from an anatomical standpoint, seems to be due to the fact that active division of the cambium cells with the resultant formation of wound parenchyma is limited, especially in the cion. This delay in the formation of wound parenchyma seems to be due to a large extent to the failure of the vessels to become plugged, thus allowing excessive loss of water. In addition, the ray cells and tracheid cells do become plugged making translocation of stored materials and water to the cambium difficult. Priestley (45) and Priestly and Swingle (46) advance the theory that a high water content in the cambium and the adjoining cells is necessary before active growth can take place. This theory seems to help explain the slowness of the walnut to form wound parenchyma.

The union of stock and cion in grafting is the result of the meeting and, when sufficient pressure is exerted, the fusion of the wound parenchyma arising from each. If either the stock or the cion does not form wound parenchyma (callus), regardless of the amount formed by the other, no union can take place. Since the cion is usually severed from its water supply some time before it is placed on the stock, it suffers more from the loss of water than the stock does.

The walnut occupies a position intermediate between the apple and the peach with respect to vegetative propagation. The apple can be propagated with ease by budding and by grafting on both young and old trees. The peach is very difficult to graft successfully, but can be budded with ease on young trees. The walnut can occasionally be grafted with a fair degree of success and can usually be budded, with good cion wood, with reasonable success. Why such variable responses? It seems that the reaction of these plants in an attempt to heal wounds offers some clue to the situation.

If the bark of the apple is peeled off when growth is active, the cells left on the wood multiply rapidly, under favorable conditions, forming wound parenchyma or callus directly on the surface of the wound. Later a cambium layer is regenerated and xylem and phloem are differentiated (Fig. 16). This type of healing is usually called regeneration. If the injury is such that the young cells on the surface of the wound are destroyed, regeneration healing does not take place, but an entirely different kind of healing does occur. The cambium layer bordering the wound produces a profusion of parenchymatous cells, which tend to push over the wound. Finally, a layer assumes typical cambium characteristics and produces xylem elements on the inner side and phloem elements on the outer side. The cambium tends to cover the wound by a lateral growth. Such healing is called walling-over and is illustrated in Figures 9, 10 and 12. If the over-walling tissues from the opposite sides of the wound meet with sufficient pressure, either before the cambium layer is formed or after, they unite, forming a continuous layer of tissue over the wound. This has occurred in Figures 16 and 17. Both types of healing occurred in 16 and 17.

The walnut has power to heal by regeneration under very favorable conditions and also to heal by over-walling, but to a lesser degree than the apple. The peach forms regeneration tissue freely on young trees but on old wood this type of healing is not so common.

The production of wound gum, distinct from that which exudes from wounds on Prunus species, by which the vessels, tracheids and ray cells become plugged, seems to be an important factor in the success or failure of grafting. Swarbrick (54) finds that such plugging occurs to the extent of 0.1 to 0.3 mm. further than the knife wound in ringed apple stems. Bradford and Sitton (12) consider gum formation, especially the plugging of the vessels, to be important in the healing of wounds and in grafting. The walnut apparently does not react to wounds in the same manner as does the apple. The walnut tissues immediately below the wound die for a considerable distance from the wound before gum formation takes place (Fig. 11, 12, and 15).

5. THE INFLUENCE OF GROSS ANATOMY.

In February, 1930, cions were secured from a single large seedling tree on the United States Pecan Field Station, Shreveport, Louisiana. These were grafted on two year old seedling root stocks, the tap roots of which had been shortened to about one foot.

As the cions were being prepared, about 240 were measured at the point where the bevel was made on the basal end of the cion. These measurements were (1) diameter of pith, (2) width of xylem, i.e., from pith to cambium, (3) width of bark, i.e., from the cambium outward, and (4) total diameter of the stem. The cions were tagged with serial numbers and the measurements recorded by number. The age of wood and the total length of the annual growth from which the cion was secured were also recorded.

The grafts were prepared by the whip method, waxed and packed in moist spaghnum in constant temperature boxes, as described later. Eighty grafts were exposed to each of the following temperatures, 24°, 28° and 32° C. The grafts were allowed to remain in the boxes for four weeks, at the end of which time they were removed and the callus formation determined. The grafts were then planted in the nursery row but unfortunately, due to the extremely dry weather which followed, only one graft lived. The average callus formation of all grafts was 68.5 per cent.

These data have been arranged on the basis of age of cion, length of annual growth from which the cion was secured, diameter of the pith, diameter of the cion, ratio of the diameter of the pith to the diameter of the cion, width of xylem, width of bark, width of vascular tissue.

TABLE 9. INFLUENCE OF AGE OF CION ON CALLUS FORMATION.

Age of cion	1 year	2 year	3 year	4 year and over
Per cent callused	54	79	80	75

Wood two or three years old seems to provide better cions than either younger or older wood. There is very little difference between two and three year old wood; in fact, there is much greater variation within either age than is here shown between the two ages.

TABLE 10. INFLUENCE OF LENGTH OF ANNUAL GROWTH ON CALLUS FORMATION.

Length of annual growth (cm.)	10	15	20	25	30	35	40	45
Per cent callused	74	65	70	54	50	40	100	82

The data for lengths of 35 cm. and over represent results of a small number of grafts, therefore, should not be given too much weight in analyzing the results. The length of the annual growth, per se, probably is not responsible for success or failure in grafting, but generally the longer growth is associated with a greater development of the shoot in all characteristics, particularly in increase in diameter. Data in Table 11 seems to indicate that increase in diameter is associated with better callus formation.

TABLE 11. INFLUENCE OF TOTAL DIAMETER OF CION ON CALLUS FORMATION.

Diameter of stem (mm.)	6	8	10	12	14
Per cent callused	0	53.0	65.0	82.5	82.5

These data indicate that any stem having a diameter under 10 mm., or 3/8 inch, is unsuitable for cion wood. The average lead pencil is about 8 mm., or 5/16 inch, in diameter, so that the statement often made that wood the size of a lead pencil, or 1/4 inch in diameter, is suitable for cion wood is not strictly correct in the black walnut. It would be nearer the truth to say that cion wood should be 3/8 to 5/8 inch in diameter. The data do not show that wood larger than 5/8 inch is unsatisfactory from the standpoint of callus formation, but wood above that size is very difficult to work with and, in actual practice, preference should be given to wood 3/8 to 1/2 inch in diameter.

A good type of cion wood is shown in the group to the right in Figure 18. This is two year old wood of a diameter between 3/8 and 5/8 inch. The large primary buds have abscised and those remaining are small secondary buds which will push into growth readily when forced. Next to the left is a group of one year old shoots. The basal portion of these shoots is suitable for cion wood but not quite as good as the two year wood. On the extreme left is a group of small one year shoots and next to it is a group of small two year shoots. Both of these groups are unsuitable for cion wood.

TABLE 12. INFLUENCE OF DIAMETER OF PITH ON CALLUS FORMATION.

Diameter of pith (mm.)	2	2-2.4	2.4-2.8	2.8-3.2	3.2-3.6	3.6-4.0
Per cent callused	68.5	67.2	76.6	65.7	71.0	78.0

From these data, it appears that there is very little, if any, relation between the size of the pith in a walnut stem and the callus formation. The opinion has often been expressed that such a relation does exist; that callus formation and success in grafting was in inverse ratio to the size of the pith.

TABLE 13. INFLUENCE OF RATIO TOTAL DIAMETER OF STEM TO DIAMETER OF PITH ON CALLUS FORMATION.

Ratio stem/ pith	2	2.5	3	3.5	4	4.5	5
Per cent callused	0	73	68	82	91	92	64

These data are somewhat irregular, but the per cent of callus formation increases with increased ratio of total diameter of the stem to pith. The data represent results from cions one, two, three and four years old. It is probable that the size of the pith does not change materially after the first layer of secondary xylem has matured, certainly no change occurs after the first year's growth is complete. Data of Table 11 show clearly that the per cent callus formation increases with increased diameter of the stem. Increased total diameter is accompanied by approximately unchanged pith diameter, therefore, it must necessarily follow that the increase in ratio, up to a certain point, would be associated with increased per cent of callus formation. The data indicate that the maximum is reached with a ratio of 4.5 but, since shoots with total diameter greater than 14 mm. were not used, and data were not secured on ratios above 5, it is uncertain whether the decrease with the ratio of 5 is actual or merely accidental. Observations, without actual measurements, indicate that cions larger than 20 mm. in diameter form callus more slowly than the somewhat smaller ones.

TABLE 14. INFLUENCE OF WIDTH OF XYLEM ON CALLUS FORMATION.

Width of xylem (mm.)	1.6	2.0	2.4	2.8	3.2	3.6
Per cent callused	55	60	65	55	74	77

With the exception of the xylem width of 2.8 mm., the per cent of callus formation increases with increased width of xylem.

TABLE 15. INFLUENCE OF WIDTH OF BARK ON CALLUS FORMATION.

Width of bark (mm.)	0.6	0.8	1.0	1.2	1.4+
Per cent callused	50	57.5	69.5	78.5	81.5

Here the per cent of callus formation increases with the thickness of the bark.

Early in the progress of the work, it was observed that cions with thick bark gave better results than cions having thin bark. It was at first thought that the amount of cork formation was largely responsible for this difference, being effective through its resistance to desiccation.

Later experiments indicate that the thickness of the bark is not solely responsible, for stems having thick bark also have well developed xylem and the influence of both xylem and phloem contribute in the same direction and their influence cannot be separately evaluated. Considering these facts, it seems probable that the benefits of the thicker vascular tissue must be at least two fold; first, the cork formation is more efficient in conserving moisture and second, the thicker vascular tissue gives greater storage of reserve foods upon which to draw for the formation of wound parenchyma and new tissue.

TABLE 16. INFLUENCE OF WIDTH OF VASCULAR TISSUE ON CALLUS FORMATION.

Width (mm.)	2.0	2.4	2.8	3.2	3.6	4.0	4.4	4.8	5.2	5.6
Per cent callused	33	47	64	61	62	68	76	77	90	100

With the exception of the width of 2.8 mm., there is an increase in the percentage of callused grafts with increasing width of vascular tissue. This seems to signify that callus formation takes place more rapidly where the vascular tissue is well developed, presumably meaning that there is more storage space for reserve foods and that a better system for their translocation is provided.

Winkler (59) reports that grape cuttings with high starch content produced a higher percentage of rooting than cuttings with medium and low content. He does not show comparative measurements of diameter, but his figures show that the stems classed as having high starch content had considerably greater diameter than the stems of medium and low starch content.

5. INFLUENCE OF RELATIVE ATMOSPHERIC HUMIDITY.

Apparatus.

Six boxes were constructed, each having dimensions of 16 x 20 x 12 inches. These were made of 12 inch lumber with one 16 x 20 side left open and the other covered by glass. Each box was provided with a heating coil and a thermometer. Six of these boxes were constructed and the heating element so adjusted and wired in series that all were controlled by one theremoregulator. The total resistance was such that the heating elements would glow only faintly when the full current was on. This provision was made to avoid blistering the cions near the heater. The thermoregulator was adjusted so that the temperature within the boxes was 28° to 30° C. Cions were grafted on shortened root stocks which were planted in moist peat in a greenhouse bed. The grafts were allowed to project through double thicknesses of tar paper which was spread over the top of the peat. The holes through which the grafts projected were sealed with grafting wax. The box was then placed over the grafts, glass side up.

Relative Humidity.

The relative humidity of the air was controlled at (1) almost dry, (2) 25%, (3) 50%, (4) 65%, (5) 75%, and (6) saturated.

According to Wilson (58) a sulphuric acid solution of a given percentage composition has a constant vapor pressure and, when exposed in a confined space, maintains the atmospheric humidity at a constant degree for a constant temperature. Therefore, the sulphuric acid solutions were utilized to maintain the atmospheric humidities.

Evaporating dishes were placed under each box and kept filled as follows:

(1)	Dry atmosphere			Calcium chloride, changed frequently	
(2)	25% relative humidity-		55.9%	sulphuric acid,	changed frequently
(3)	50%	" "	43.4	"	"
(4)	65%	" "	36.0	"	"
(5)	75%	" "	4.0	"	"
(6)	Saturated	" "		distilled water	

It is recognized that, under the conditions of this experiment, it is impossible to maintain the atmospheric humidities accurately because the portion of the stem exposed is constantly giving off moisture, and because moisture was not completely excluded by the tar paper. It is believed that the atmospheric humidities were maintained nearly enough to the desired amounts, especially in the second series of experiments.

Cion wood was secured from the trees in the nursery of the Forestry Department as described before. This consisted of two year old wood as nearly as possible of diameters between 3/8 and 1/2 inch. They were grafted, tied and waxed as usual and planted in the peat and the box placed over them. The first series remained in the boxes three weeks and the second series two weeks. Twenty walnut and ten apple grafts were used in the first series and 25 walnut and 8 apple grafts in the second.

Table 17 gives the results of these experiments.

TABLE 17. INFLUENCE OF ATMOSPHERIC HUMIDITY ON CALLUS FORMATION AT TEMPERATURE OF 28° C.

Relative humidity (per cent)	0	25	50	65	75	Sat.
First series:						
Per cent callused, walnut	35	30	50	45	70	90
Per cent callused, apple	60	50	10	10	50	60
Second series:						
Per cent callused, walnut	12	52	60	-	72	95
Per cent callused, apple	89	89	78	-	100	100

From these results, it appears that the atmospheric humidity to which the walnut grafts are exposed is somewhat important. Where artificial temperatures are maintained at relatively high degree, provision should be made to keep the relative humidity comparatively high.

7. INFLUENCE OF TEMPERATURE.

In 1928, cion wood was kept in cold storage at about 5° C. Although the cion wood remained in storage several weeks, none of it formed callus. When the season's grafting was complete, there was a small amount of cion wood left over. About this time the cooling plant was shut down so that the temperature of the storage rose to 20-22° C. About ten days later, when preparing to dispose of the cion wood, nearly every piece of cion wood had formed callus, some quite profusely.

Certain grafts were made later than others this year, when the temperature was somewhat higher. These later set grafts did somewhat better than those set earlier. These observations led to an elaboration of the problem to include the influence of temperature upon grafting.

Apparatus.

To study the influence of temperature, it was necessary to construct a series of boxes by means of which the grafts could be exposed to controlled temperatures. It is often said that the cion begins growing before callus formation and union can take place, thus depleting the supply of reserve food to a point such that union cannot be formed. To avoid this, boxes were constructed in which a space 24 x 24 x 3 inches was heated. Grafts were made and inserted through holes in the 24 x 24 inch sides so that the top protruded from one side and the root from the other with the union within the heated space. This arrangement provided for the heating of about an inch and a half of the top portion of the stock and a similar amount of the basal portion of the cion.

The union of the graft was waxed before insertion in the box and the holes through which the cion and stock projected were plugged with corks and waxed. Moist peat was packed around the roots.

An ordinary 50-watt lamp bulb was used as a source of heat for the first series but was found unsatisfactory. Thereafter, a resistance coil of nichrome wire was used, being so adjusted that when full current was on the wire was just below the point of glow heat. In complete darkness, a faint red glow was produced but no glow was noticeable in diffused light. In this manner, the injurious effects of intense radiant heat were avoided.

Temperature was controlled with a de Khotinsky thermoregulator wired in series with the heating element so that a rise in temperature above the point at which the regulator was set caused the platinum points of the regulator to separate and break the current passing through the heater. The points then remained open until the temperature of the box dropped below the desired point.

After two series of experiments were conducted, these boxes were discarded, and boxes similar to those described for the atmospheric humidity studies were used for several series, when they were discarded in favor of the final form.

The final form of the constant temperature box was one 18 x 24 x 24 inches, in which the entire stock and cion was placed

(Fig. 19), the roots being packed in moist spaghnum or peat moss in a wire basket inside the box.

The box is heated by electricity, using a 110 volt current. The heating element is a conical porcelain core wound with nichrome resistance wire, such as are used in portable radiant electric heaters. This element is wired in series with a thermoregulator.

A wire basket is placed in the box, supported free from the floor so as to leave a two inch space for air circulation. An air space is provided above and on two sides of the basket, while the other two sides are placed close against the side wall of the box. This arrangement provides for circulation of air by convection, rising from the heater, passing over the basket and down on the opposite side, under the basket and back to the heater.

The basket is provided with a solid sheet iron side next to the heater to protect the grafts from direct radiant heat.

The walls of the box are made double, the outer one being of matched inch lumber and the inner of 3/8 inch wall plaster board; the space between the two walls is filled with an insulating material.

Cion Wood.

Cion wood for the first three series was obtained from the same trees in the nursery of the Forestry Department as described earlier. Cion wood for the fourth series was obtained from the orchard of Mr. Henry M. Wallace, Highland, Michigan. These trees had been neglected up to two years previous to the time when the cion wood was cut. The trees had made good growth for the past two seasons and some good wood was obtained.

For the fifth series, cion wood was obtained from a single large seedling tree on the Pecan Field Station of the United States Department of Agriculture, Shreveport, Louisiana.

Root Stock.

For the first three series, the root stock was obtained from the block of seedlings used as root stocks in the previous field work. For the other series, vigorous two year old seedlings, most of which were about 3/8 inch in diameter at the ground line, were used. Seedlings were dug and the tap root shortened to 8 to 12 inches. The top was cut back and the graft was usually placed just above the junction of the root and stem.

Procedure.

Grafts were made by the usual whip graft method, waxed and exposed to the controlled temperatures as soon as possible. Grafts were made for the first series January 10 to 21, and for the second series, March 11 and 12, and for the third series, April 28, 1929. Sixteen, 20 and 25 grafts, respectively, were used for each temperature.

Since only five constant temperature boxes were available, the temperatures to which the grafts were exposed were varied with each series. These temperatures, with the per cent of grafts forming callus, are given in Table 18.

TABLE 18. INFLUENCE OF TEMPERATURE ON CALLUS FORMATION IN WALNUT GRAFTING. FIRST THREE SERIES COMBINED.

Temperature ° C.	5	15	20	24	25	26	28	30	32	35	40
Series 1 Per cent callused	0		0		44			31		0	0
Series 2 Per cent callused		15	30		40			40		25	
Series 3 Per cent callused				32		50	54	40	20		

After the grafts were removed from the temperature boxes, those showing callus were planted in a greenhouse bench in sand and allowed to grow. The per cent of grafts which grew correlated very closely with the per cent that callused, indicating that union usually is accomplished on grafts which form callus.

The data show the influence of temperature on callus formation quite clearly. There was little or no callus formed below 20° C. in most cases, and that formed at 20° was very weak. At 25° to 30° C., callus formed more readily, especially at 26° and 28°C. Above 30° C the callus formation was weak and none occurred at 40° C.

In general where callus formation took place, it was more abundant on those grafts exposed to temperatures of 25° to 30° C. When the grafts were allowed to grow, the growth was more vigorous and the foliage a darker green, on grafts exposed at these temperatures (Fig. 20).

At 32° C. and above, many of the cions were injured apparently from dying of excessive heat. This seems to be true especially of cions having comparatively thin bark with little cork formation.

Two additional experiments on the influence of temperature were conducted. In the first of these, in addition to temperature, the effect of waxing the entire cion upon the results also received attention. In the second, the influence of various factors of gross anatomy were considered.

Grafts were made May 12, 13 and 14, 1929, and placed in boxes with temperatures maintained at 20°, 23°, 26°, 29° and 32° C., respectively. In each box were placed 23 grafts with cions of the Ohio variety, waxed in the usual way, 6 grafts of Ohio having entire cion and union waxed with paraffin, 7 grafts with cions of Ten Eyck waxed in the usual way and 4 Ten Eyck having entire cion waxed.

These grafts were allowed to remain in the boxes 14 days and were then removed and planted in the nursery. On August 9, they were examined and the number growing recorded; results are presented in Table 19.

TABLE 19. INFLUENCE OF TEMPERATURE ON THE UNION IN WALNUT GRAFTING. FOURTH SERIES.

Temperature ° C.	20	23	26	29	32	Totals
Per cent growing, all grafts	16	31	45	26	35	
Per cent growing, regular waxing	14	26	32	32	32	27
Per cent growing, waxed entire	20	44	78	16	44	42

These results agree in general with those of the preceding experiments in that the temperatures 26° to 30° C. again give best results. In this series, the cion wood was not so uniform as the preceding ones, and having such variations complicates the effect of the temperatures.

Morris (36), Nielson (40), and others have advocated strongly the coating of the entire cion with paraffin, or parapin, but have not given data on comparative tests. The results of the waxing treatments in this series indicate that some advantage is derived from such treatment but the differences are not very significant. Further investigations of waxing the entire cion are needed.

A very serious objection to the use of paraffin alone, or mixed with some other substance to raise the melting point, was observed in these trials. When this material is melted, it becomes very thin and has low viscosity, making it difficult to secure a good seal around the union.

Cion wood for the last temperature experiment was obtained from a single, large seedling tree located on the Pecan Field Station of the United States Department of Agriculture, Shreveport, Louisiana.

In this series, cion wood of one, two and three year old wood was used. There was much variation in the size of the wood; this variation was used to obtain data on the influence of gross anatomy as previously discussed. A total of 120 grafts was placed in each of the following temperatures: 20°, 24°, 28°, 32° and 36° C. The final form of constant temperature boxes was used for this series of experiments. The grafts remained in the boxes four weeks and then were removed and examined for callus formation. Results are presented in Table 20.

TABLE 20. INFLUENCE OF TEMPERATURE ON CALLUS FORMATION. FIFTH SERIES.

Temperature, ° C.	20	24	28	32	36
Per cent callus formation	13.5	72.8	86.4	31.4	24.3

Again the data indicate that temperatures of 24° to 30° C. are most favorable for callus formation in the walnut, with the optimum at or near 28° C. The degree of callus formation at 28° C. is high enough to be considered commercially satisfactory.

These data seem especially significant because of the wide variation in the cion wood used in these experiments. Much of it could not be considered suitable, and some was very small one year wood which has consistently given poor results.

DISCUSSION

It is quite clear from these experiments that, by exposure of the grafts to the optimum temperature, which apparently lies in the neighborhood of 28° C., it is possible to increase the rate at which wound parenchyma is produced. In this manner it is possible to increase the percentage of grafts forming callus and thus increase the per cent of successful unions. It must be realized that union does not always follow callus formation, but union cannot be accomplished unless callus or wound parenchyma is produced.

That other factors besides temperature are operative is indicated by the studies in anatomy, gross anatomy and chemical relations. However, if the temperature varies much from the optimum, the callus formation, even in the best types of cion wood, is very unsatisfactory. It would, therefore, appear that, of all the factors investigated affecting grafting in the black walnut, temperature is of primary importance.

It is probable that temperature acts indirectly through increased chemical activity, especially as the chemical activity is catalyzed by enzymes. If the data on the influence of temperature be plotted (Fig. 21) a curve is obtained which closely resembles the type of curve obtained for the relation between temperature and enzyme activity (18, 19 and 32). The optimum and all points on this curve occur at lower temperatures than for the typical curve as represented by the hydrolysis of maltose by maltase (19). In the latter case the optimum varies with the hydrogen ion conecntration of the medium from 35° C. to 47° C. Just why growth in the walnut stem responds to lower temperature and is prohibited at temperatures optimum for enzyme activity,as frequently reported, is not entirely clear, but the time factor probably is of great importance.

In any biological system time is a factor equal in importance to any other variable. Most of the studies on enzymatic activity have been conducted for relatively short periods, measured in hours. Very frequently statements are made that 37° C. is the optimum for enzymatic activity, which is probably true for enzymes obtained from warm-blooded animals, if the time of the reaction is short. With enzymes derived from plants and with the reaction proceeding over a longer period of time the optimum temperature will approach room temperature. Every enzymatic reaction taking place consists of at least two phases, enzyme action and enzyme destruction, and the rate of increase of enzyme activity and the rate of increase of enzyme destruction probably bear no constant relationship. For short periods of time, higher temperatures would produce greater enzyme activity but this might be accompanied by greater enzyme inactivation. With still higher temperatures the

the inactivation may be progressing at a much greater speed than the activation, so that the enzyme may be inactivated before there are any appreciable products of the reaction. It is entirely possible, and very probable, that had the reaction been continued for a longer time the total products would have been greater at a lower temperature than that which appeared to be the optimum early in the progress of the reaction.

In plant tissue the response to temperature is much slower than that of enzymes from warm-blooded animals in artificial media. In the experiments herein reported, the time was long and the results apparently show the influence of enzyme activity at temperatures between 25° and 30° C. and anzyme inactivation at temperatures above 30° C.

Among the factors which may be associated with the depression of growth at the higher temperatures is the deficiency of moisture within the stem available for chemical action. At the higher temperature the moisture may be evaporated or otherwise removed. The higher temperatures may cause a certain amount of coagulation of the colloidal material within the cell and thus retard growth.

The curve for callus formation agrees with growth curves in general except that the rise in the curve is more abrupt than the usual growth curve but, had more determinations been made, it is possible that a smoother curve would have been obtained. In certain of the experiments, apple grafts were exposed to the same temperatures as the walnut grafts. These produced more callus at both lower and higher temperatures than did walnut grafts and, if these data were plotted, a flatter curve would be obtained. It is evident that part of the response of the walnut to temperature is general and part specific for the specie.

Although the writer has been unable to secure any material and consistent improvement in grafting of the black walnut through girdling or defoliating the shoots or through nitrogenous fertilizers, it is quite evident that the condition of the shoots used for cions and also the condition of seedlings used for stock is important. Evidence of this is had in the data presented upon the influence of the gross anatomy of the cion. Reference repeatedly occurs in literature relating to propagation by budding and grafting, both in respect to the walnut and to other fruits, to the effect that well matured shoots give more satisfactory results than poorly matured shoots. The term "well matured" evidently means well nourished, that is, shoots which grew moderately vigorously and had sufficient foliage properly exposed to sunlight to supply abundant materials, both carbohydrate and protein, for storage. One prerequisite for vigorous growth is a supply of soil nutrients, especially nitrogen.

Direct evidence was not secured in this regard, but the consistently poor results with defoliated shoots indicate that stored nutrients are important. The shoots used in the last series of experiments were secured from a row of trees bordering a cultivated field. These trees were well spaced so that they received abundant sunlight. Grafts made from these shoots were exposed to the temperature which is approximately optimum and results of from 76% to 90% "take" were secured. In this case, with two and three year old wood, materials and conditions as nearly ideal as possible were secured.

There are apparently two primary requisites for successful grafting of the black walnut: suitable cion wood and optimum temperature. Probably of only slightly less importance is a stock in the proper condition of vigor.

Shoots which will make suitable cion wood usually can be obtained from trees 8 to 12 years old in a moderate state of vigor, retaining their foliage in a healthy condition until normal time for leaf fall, and well exposed to sunlight and circulation of air.

The most satisfactory method for securing the proper temperature must be adapted by the individual to suit his particular needs. A very satisfactory chamber in which the temperature can be controlled and in which a large number of grafts can be callused, can be constructed at a reasonable cost. The cost of operating such a chamber would also be reasonable. However, the walnut is somewhat difficult to transplant and it has not been determined how many of the callused grafts can be grown into good trees. Before this method of bench grafting and callusing in an incubator can be recommended generally, further experiments are needed to determine the best method of handling the grafts subsequent to callusing, also the effect of the temperature on the root and its subsequent behavior should receive attention.

Where artificial temperatures cannot be utilized, it is suggested that the grafting operations be delayed until the temperature remains above 70° F. for a considerable portion of the day. This will probably mean that the stock will have started into growth and that care must be exercised to make the grafts without tearing the bark away. After the graft is made, some provision for maintaining the temperature might be made, such as covering the union and at least a portion of the cion and stock with a black paper. The black paper would absorb heat from the sunlight and the temperature would be increased.

Possibly grafting in autumn may prove advantageous. The graft might be set in the collar, below the surface of the ground and the soil banked around the cion, leaving one bud uncovered. It is possible that the soil would be warm enough to induce uniting. The proper time for this would probably be during August in Michigan, or in September in the south. These grafts would then be allowed to pass through the winter in place and during the following spring would force into growth, giving a fair sized tree by fall, one year after grafting.

There is uncertainty as to whether the graft will form callus at that time of the year. An indication that callus will form in fall is had on cions which were ringed to prepare them for the stored nutrient studies. Some of this was done in August in Michigan and some in September in Louisiana. In both cases, callus formation took place above the wound.

Additional work might well be done with the patch bud method of propagation. The preliminary work reported here and the success of this method with pecan propagation indicate that the method could be developed for the walnut. The primary problem seems to be to find what constitutes suitable cion wood and to find a satisfactory method of storing the wood until needed. Slight modifications in the technique might well be made in order to speed the work.

On the whole, there is probably more hope for the commercial propagation of the black walnut by budding than by grafting.

SUMMARY.

1. The black walnut is very difficult to propagate asexually, as reported by most authors dealing with the subject.

2. Apparently, applications of nitrogenous fertilizers or girdling of the shoots does not have sufficient influence to materially alter the behavior of walnut cions upon grafting. Partial defoliation seems to depress the per cent of callus formation.

3. Treatment of the cion wood by chemicals did not increase the percentage of grafts uniting.

4. Propagation by the patch bud method offers promise but there are details of this method which still need attention.

5. Anatomical studies indicate that the walnut is more sensitive to wounds than is the apple. Protection of the wood from desiccation by plugging of the xylem tissues proceeds much more slowly in the walnut than in the apple. New tissue in the walnut is capable of changing direction to meet changed conditions of sap flow.

6. Study of cion wood from the standpoint of gross anatomy indicates: (a) cion wood two and three years old gave better results than older or younger cions; in general, two year wood is preferred; (b) cions having a diameter between 3/8 and 5/8 inch, inclusive, give better results than those either larger or smaller; (c) the percentage of cions forming callus is independent of the size of the pith; (d) the per cent of cions forming callus increases with the total width of the vascular tissue (xylem and phloem) from 2 mm. to 5.6 mm.

7. Grafts were exposed to atmospheres having relative humidities of 0, 25%, 50%, 65%, 75% and saturated, at controlled temperature of 28° C. Relative humidities of 75% or greater seem to give satisfactory results.

8. Grafts were made by the whip method and exposed to temperatures of 0°, 15°, 20°, 22°, 24°, 25°, 26°, 28°, 29°, 30°, 35° and 40° C. A larger per cent of grafts exposed to temperatures be-

tween 25° and 30° C. formed callus than at temperatures either higher or lower. The optimum temperature seems to be in the neighborhood of 28° C or 82° F.

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Fig. 1- Stabler on Black Walnut stock No. 1. Buds set June 1, 1928. Photographed August 8, 1928.

Fig. 2- Kinder on Black Walnut stock No. 2. Buds set June 1, 1928. Photographed August 8, 1929. This vigor is typical of all buds set on this stock.

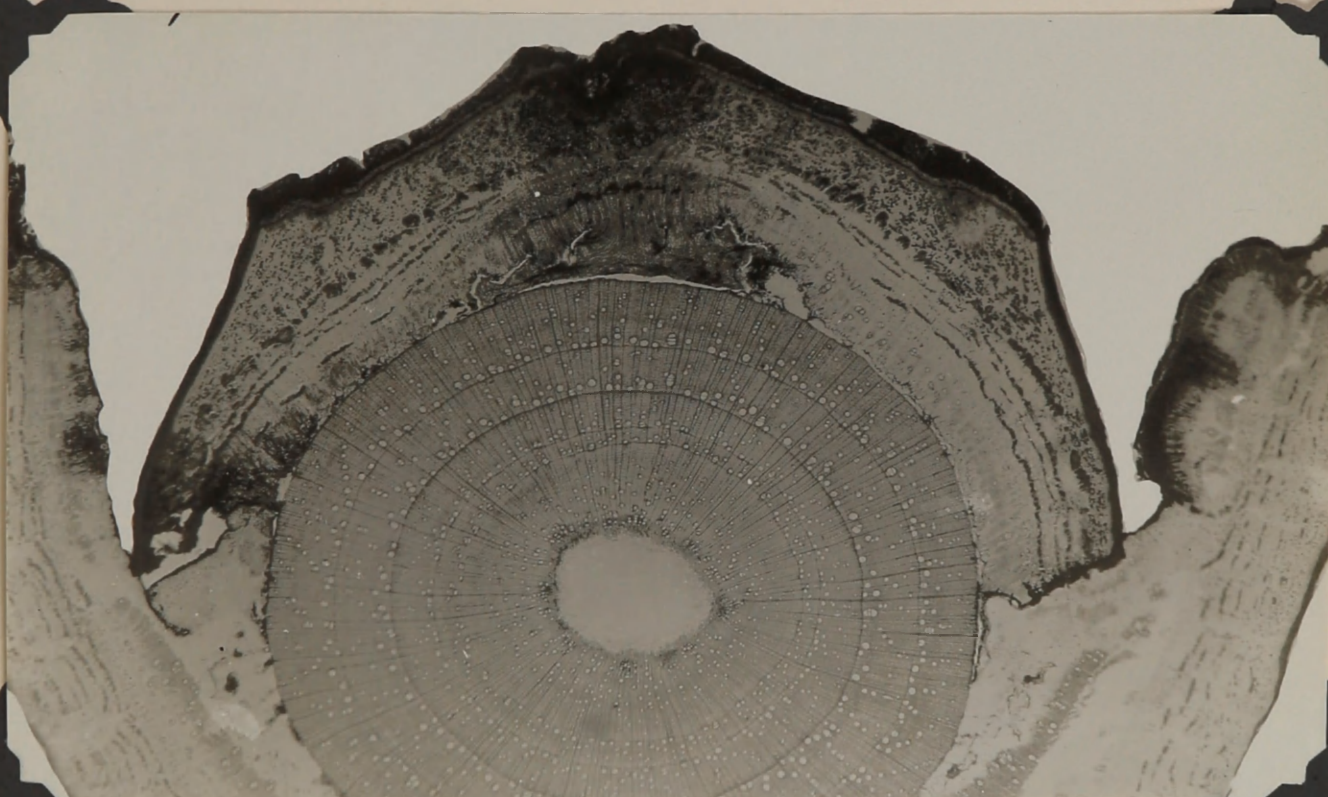

Fig. 3- Cross section of a patch bud on walnut set by the Kraus method. Bud failed to grow because union was not established directly under growing point. This may happen in any type of budding.

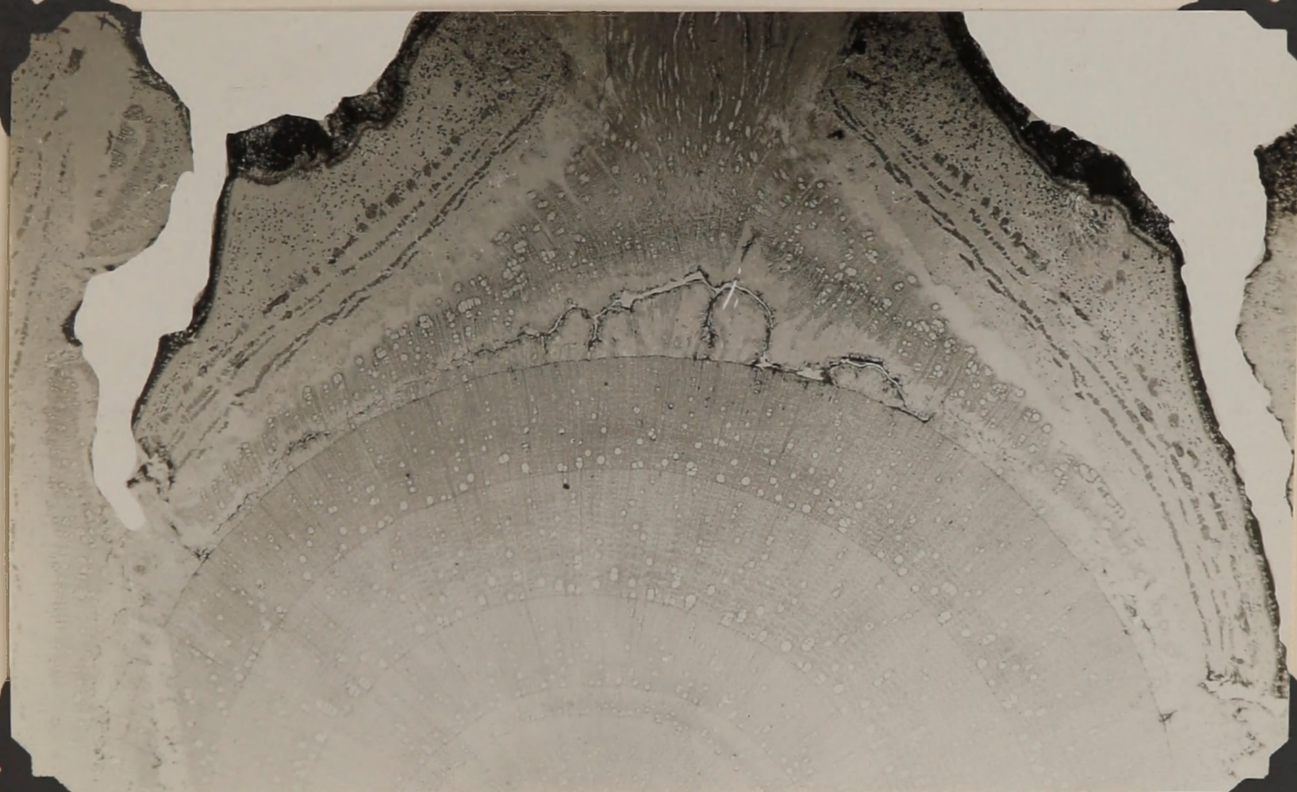

Fig. 4- Patch bud set by Kraus method which grew in spite of delay of union directly under bud.

Fig. 5- Cross section of a patch bud set June 12 and sectioned August 8, 1928. Bud had made growth of about one inch.

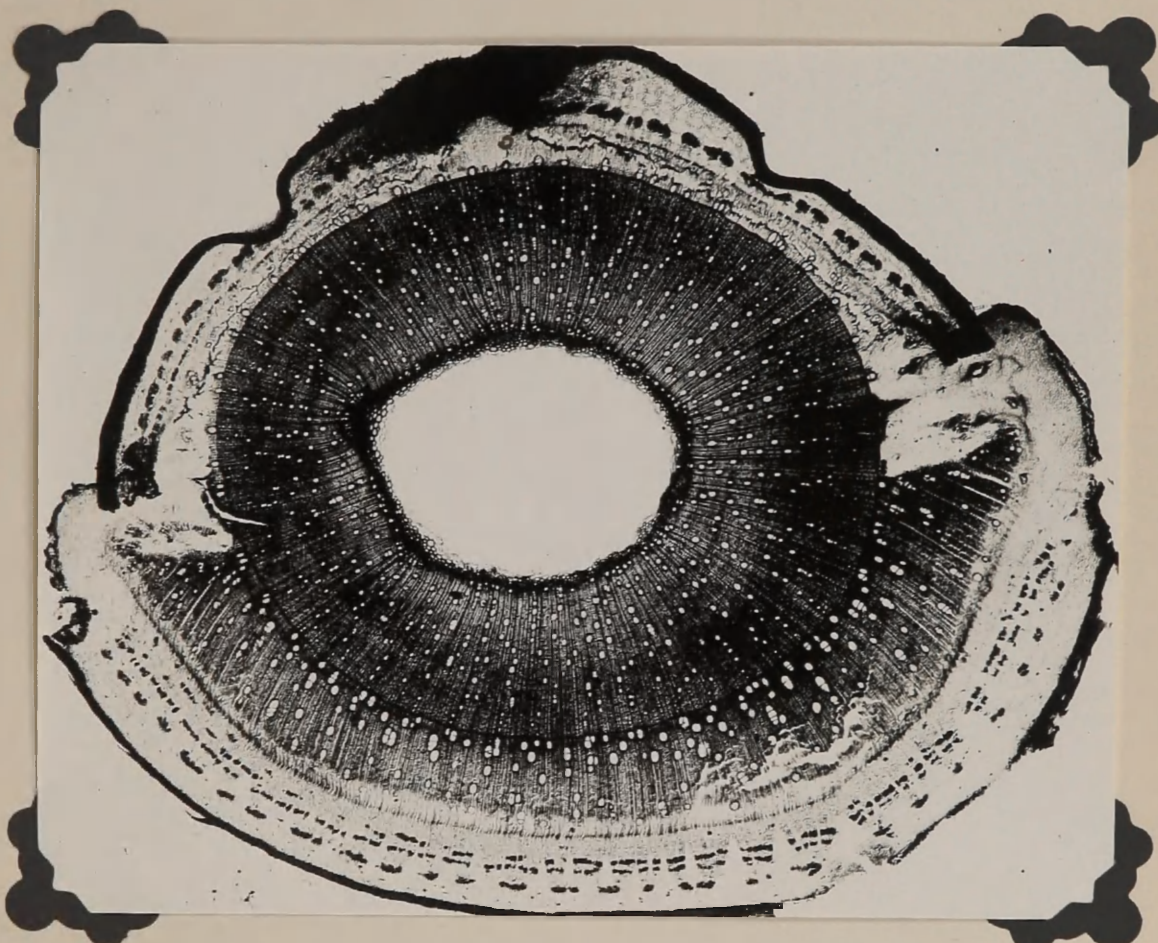

Fig. 6- Cross section of a patch bud in which the bark united but the bud failed to grow.

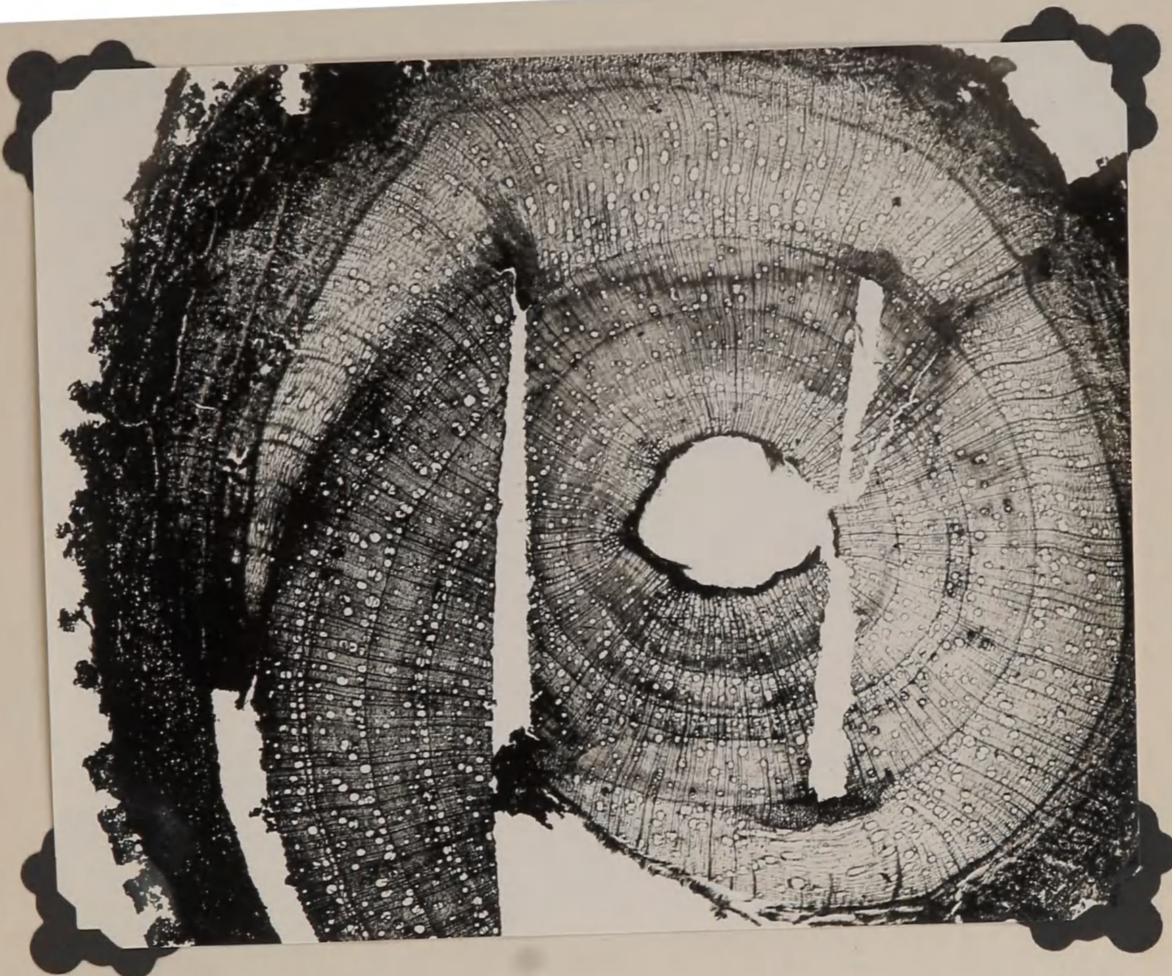

Fig. 7- Cross section of a whip graft of walnut. Most of old xylem in both cion and stock is dead. The vessels are large and not plugged. Over-walling on left and in center. Disparity in size of cion and stock.

Fig. 8- Cross section of whip graft of walnut. There is very little callus tissue on cion and the regeneration in the tongue of the stock was very weak until union became established.

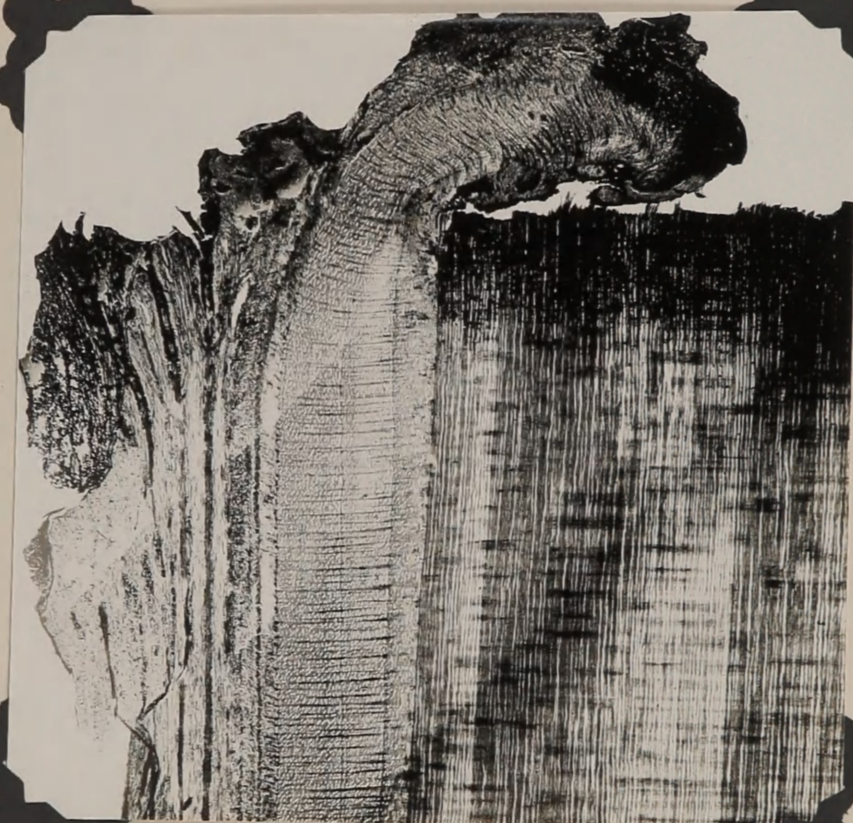

Fig. 9- Longitudinal section of amputation wound on pear showing small amount of dead tissue and completeness of the plugging of cells just under the wound.

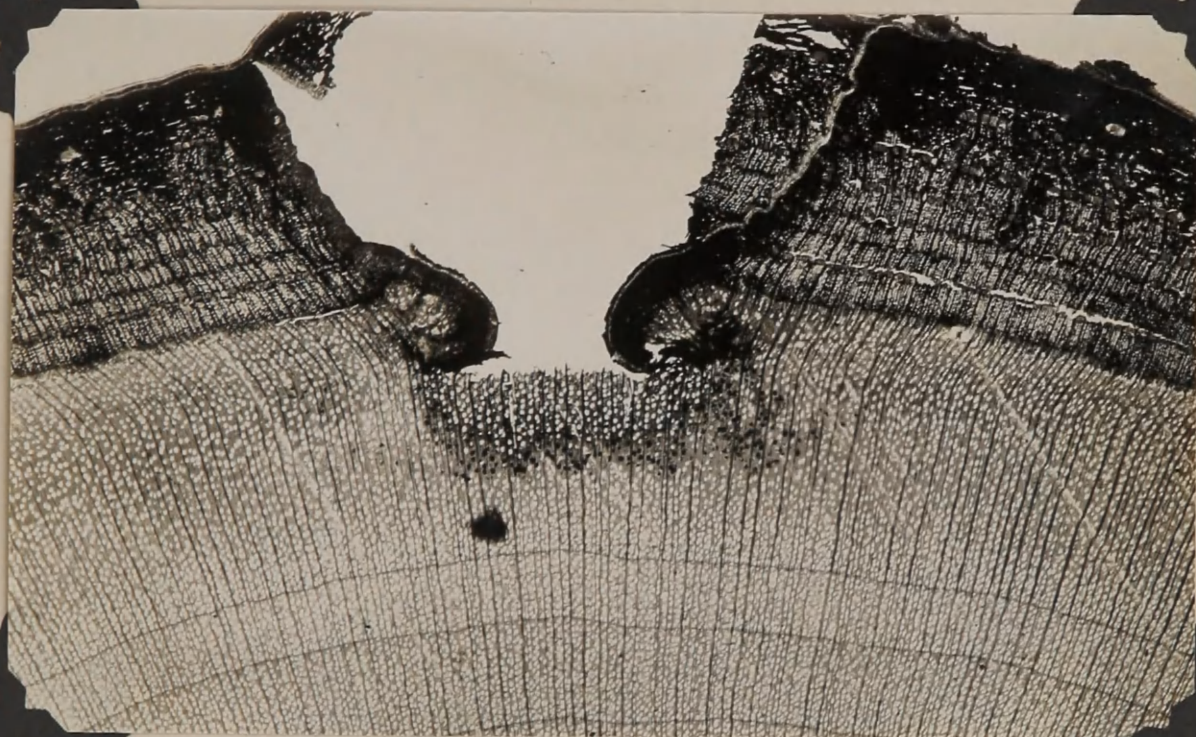

Fig. 10- Cross section of wound on apple made by removing the bark and scraping the surface of the wood. There is a small amount of dead tissue and very thorough plugging of vessels, but very little wound gum near the surface, which died quickly.

Fig. 11. Longitudinal section of an amputation wound on the walnut. The dead tissue extends in cone shape for considerable distance. The pith and wood immediately surrounding it were dead for a space of about three inches below the wound. Wound made June 6, 1928; section cut August 9, 1928.

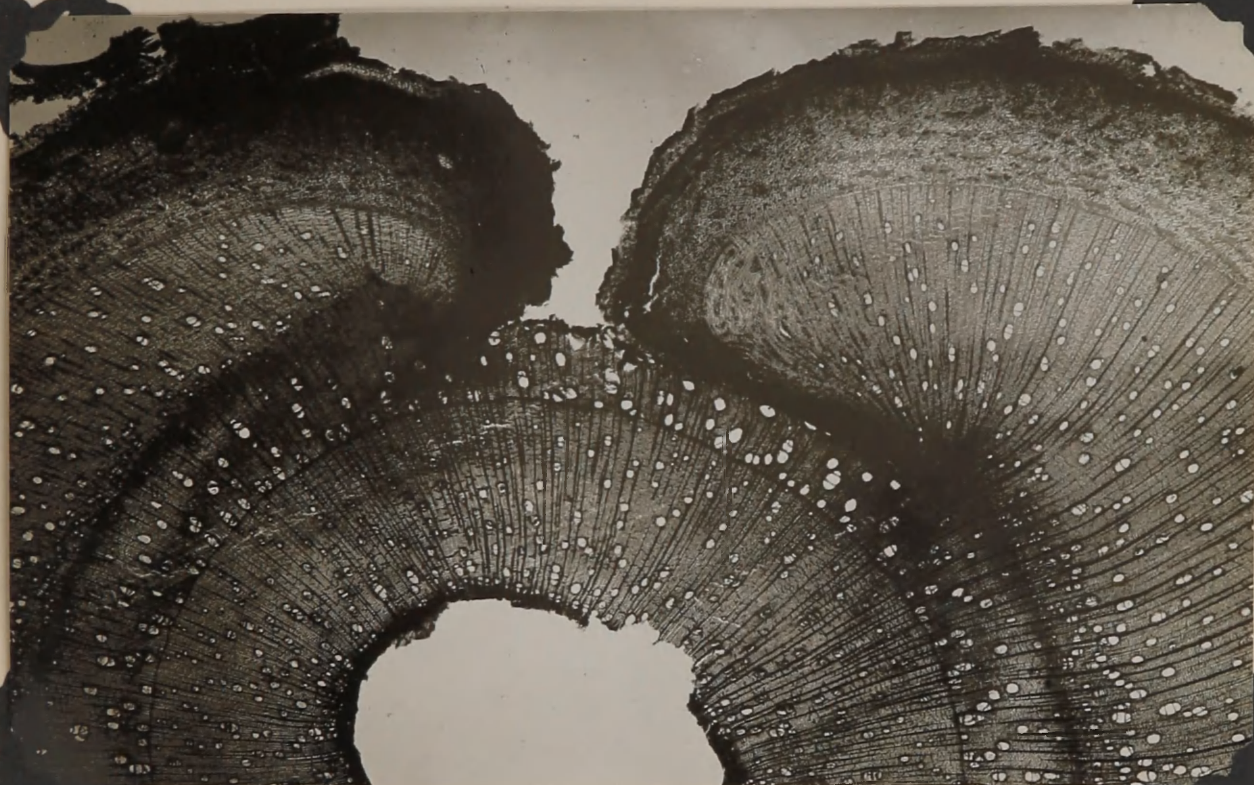

Fig. 12- Cross section of wound made by hoe in cultivation. Dead area extends to the pith over an area greater than the width of the surface wound. Very few vessels are plugged. Ray and tracheid cells at the limits of the dead area are plugged.

Fig. 13. High magnification of the margin of the dead area of wound shown in Fig. 11. Xylem rays and tracheids plugged.

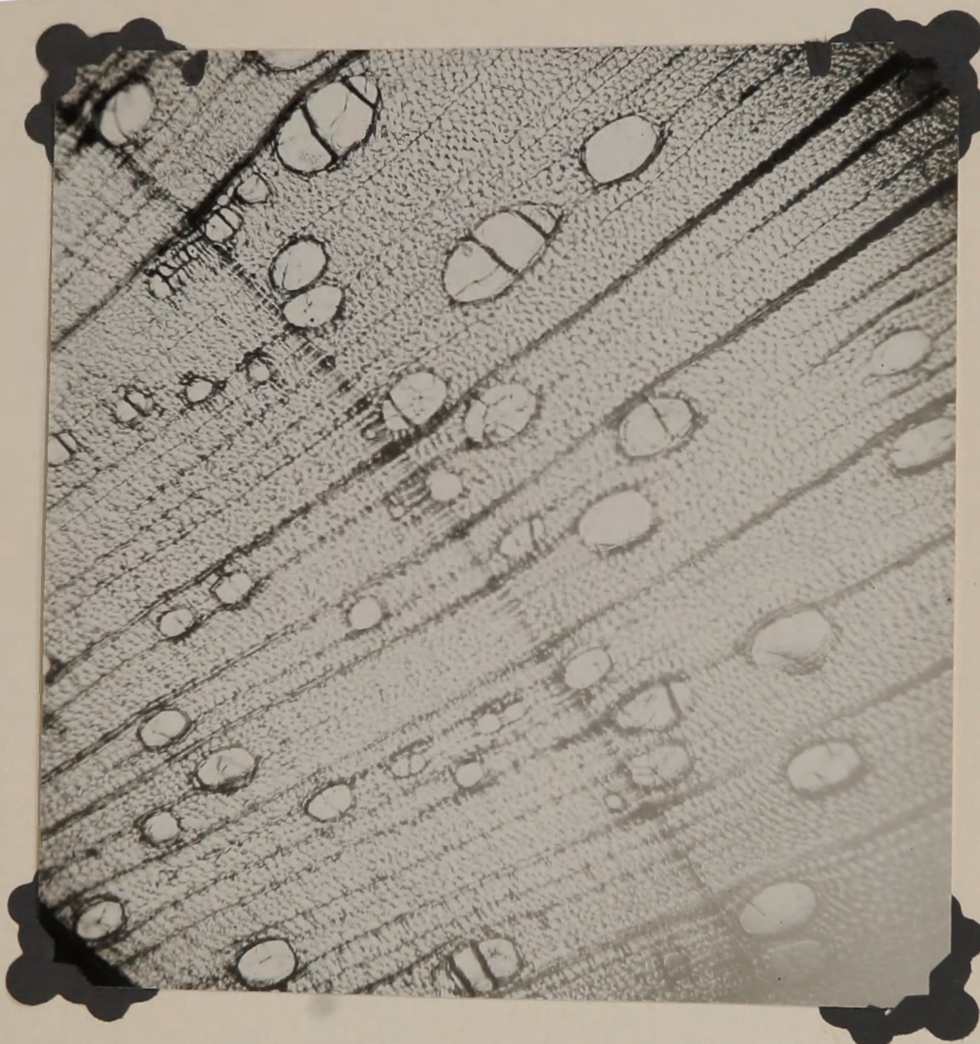

Fig. 14- High magnification of section shown in Fig. 12 showing tyloses in a few vessels. Tyloses are found in as many vessels of the wood formed after the wound as in the older wood.

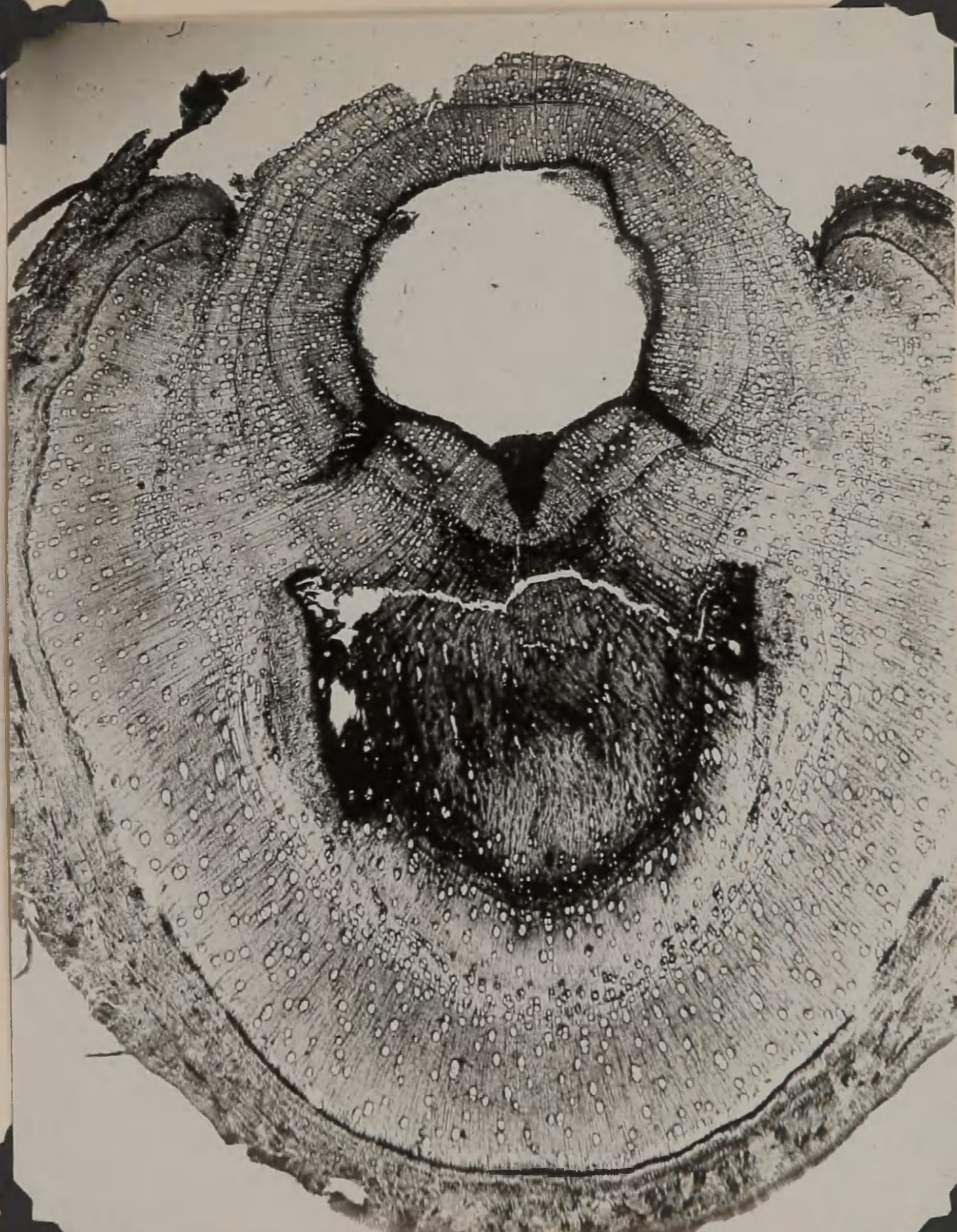

Fig. 15- Cross section of stem below an amputation wound made June 6, 1928, and sectioned August 1, showing large amount of dead tissue.

Fig. 16- Cross section of apple wound showing healing by regeneration in middle of wound and walling over at sides.

Fig. 17- Cross section of walnut wound showing healing by regeneration in center and walling over on both sides.

Fig. 18- Poor, fair and good cion wood. Group on right is two and three years old, about 3/8 inch in diameter; this is a good type of cion wood.

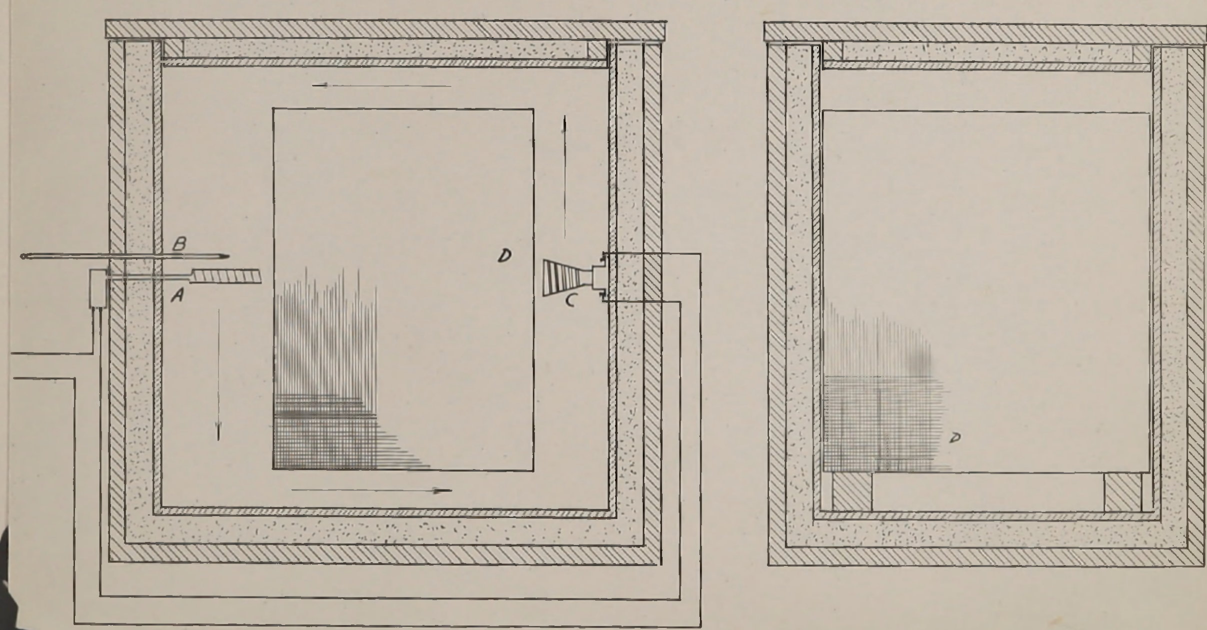

Fig. 19- Final form of constant temperature box; A, thermoregulator; B. thermometer; C, heater coil; D, basket to hold grafts and packing material.

Fig. 20- The two grafts at left were exposed to temperature of 27° C. and the two at right were exposed to 30° C. for two weeks. Both lets were grown in greenhouse bench for two weeks longer and then photographed. Those exposed to the higher temperature had more foliage of a darker green color.

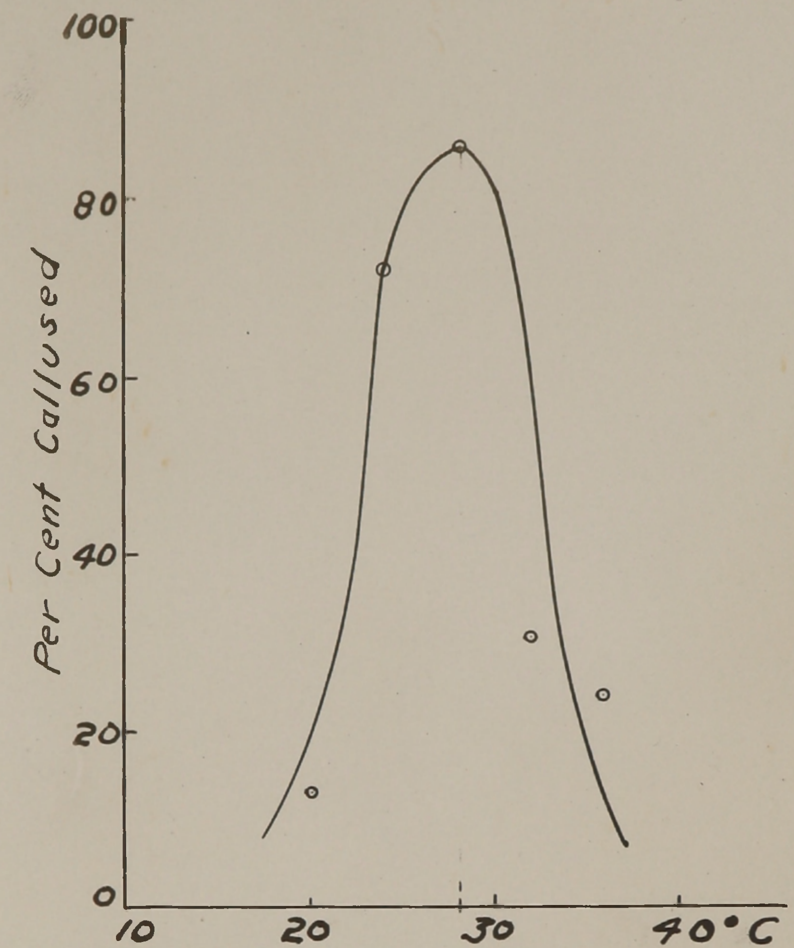

Fig. 21- Influence of temperature on callus formation, plotted from data of Table 20.