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A STUDY OF
THE PROPAGATION OF THE WALNUT

Thesis for the Degree of M. S.
Benjamin Gaillard Sitton
1928

THESIS

Walnut

Horticulture - Fruit culture.

A STUDY OF THE PROPAGATION OF THE WALNUT

With Special Consideration of the
Role of Carbohydrates and
Nitrogen and Their Re-
lation to Grafting.

A Preliminary Report

Thesis

Submitted to the Faculty of the Michigan State
College of Agriculture and Applied Sci-
ence in partial fulfillment of the
requirements for the degree
of Master of Science.

by

Benjamin Gaillard Sitton

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V. R. Gardner*

THESIS

INTRODUCTION

The native black walnut (Juglans nigra) is assuming increasing importance in recent years as a producer of edible nuts. The meats of the black walnut are in special demand for candy manufacture and confectioners are willing to pay 25 per cent more for them than for those of the English walnut. However, nuts from most seedling trees are so hard to crack that the meats are broken into small pieces. Occasionally a seedling tree which bears nuts that crack so that the meats come out in halves has been located and some are being propagated as named varieties.

It is true with the black walnut, as with most other fruits, that sexual reproduction usually does not produce fruit like that of the parent tree, therefore asexual propagation must be invoked for multiplication of desirable trees. Vegetative propagation of the walnut is very difficult and uncertain. Apparently this difficulty of vegetative propagation is at present the chief obstacle to the development of a considerable industry.

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

Asexual propagation of the Persian or English walnut (Juglans regia) seems to have received attention some time ago. In the early writings of Thomas Andrew Knight a discussion of the budding of the walnut appears. He was entirely unsuccessful in his first trials and discouraged the attempt to propagate the walnut by methods other than by seed. Later and more successful efforts were reported before the London Horticultural Society in 1818 (6). He said that the walnut bud, when inserted into the shoot of the same season's growth, fails, possibly because the branch into which the buds are inserted had stopped growth long before any of the buds were mature enough for use. To obviate this difficulty he retarded the growth of the stock (probably by placing it in a pot in a cool place) and when the bud was ready the stock was allowed to vegetate. In this manner he secured fair success. In another experiment he inserted one of the minute buds from near the base and one of the larger ones from the middle portion of one year wood into the same stock. The small buds took freely while the larger buds failed entirely.

Later (1832) Knight (7) stated that the following mode of grafting succeeded under many unfavorable

circumstances: Allow the primary buds to unfold on both stock and cion, then remove all the open buds. After the small buds, which have previously been almost invisible, begin to swell, take off the cion and immediately insert upon the stock which should be wood of the previous year. He reported saddle or cleft graft equally successful.

Baltet in 1869 (1) recommended crown grafting and flute budding. He said the cion should be cut obliquely, exposing the pith on one side only. He advised the use of cions whose base consists of two year old wood.

Oliver (1902) (12) recommended the patch bud method of propagating pecans. He advised the use of buds from near the base of the past season's growth, from shoots of large diameter with short plump buds. He gives the following directions: A rectangular piece of bark, with the bud near the center, is removed from the bud stick. The outer bark at the sides of the bud is shaved off so that the edges will make a perfect fit. Make an I cut in the stock, the distance between the horizontal cuts being the same as the length of the bud piece, raise the top portion of the flaps slightly and insert the bud. The flaps are pressed down snugly, tied and the whole covered with a wax.

Kraus (1911) (8) reports good results from using a similar method in walnut propagation.

Lake (1913) (9) assembled all the ideas on walnut propagation to date and gave a description of desirable cion wood: from suitable trees take normal one year old wood from the middle more or less erect portion of the tree. Under no circumstance use suckers. Cion wood should be one-fourth to three-eighths of an inch in diameter, with small pith, round, not angular, thrifty, plump about the buds, with short internodes, straight, preferably not over a foot long. The terminal portion is unfit, because its buds are immature. Cions should be cut during the period of dormancy, probably two or three weeks before vegetation is due to begin. The cut ends should be dipped promptly in melted paraffin so that evaporation from the cut ends may not reduce the vitality of the cion. Batchelor (2), Fuller (5), Lewis (10), Reed (13), Stuckey and Kyle (14), Woolsey (15) and others give much the same general directions; apparently all draw their information from the same original source.

Cooper quotes H.E.Dosch who in turn quotes Professor Lechemby as saying that 90% of walnut grafts will grow if these directions are followed: use one gallon of water with four teaspoons of sulphate of

quinine. The cions should be cut submerged in this solution and the cut tree should be washed at once to prevent it from turning black; then the cion is to be inserted as on other fruit trees.

There seems to be a dearth of original thought on the subject of vegetative propagation of the walnut and indeed of the nuts in general. Writers have passed the opinions of others on without actually trying out the methods advocated. With a few exceptions, careful investigation of the propagation of the walnut seems to have been neglected.

EXPERIMENTAL METHOD

This problem has been approached from three angles: first, field or nursery trials; second, chemical analyses; and third, anatomical examinations.

1. Field Work

Cion wood was obtained from a block of seedling trees in the nursery of the Forestry Department of this college, which were probably twelve years old and 10 to 15 feet high. They were moderately vigorous, making a growth of about 12 inches a year, although they were not uniform. Cion wood was selected from those in the best state of vigor and was usually

obtained from the lower portion of the trees. A small lot of cion wood was obtained from a block of seedling trees at the Graham Horticultural Experiment Station, near Grand Rapids. These seedlings were grown from nuts from a single tree, were very uniform, and were making from 18 to 24 inches growth per year. Some of these had been ringed, some girdled, and others left in the natural condition.

The stock into which grafts were set consisted of a block of seedling trees in the Forestry nursery, about five years old, four feet high, low in vigor and not uniform.

In the hope of securing varying chemical conditions of wood to be used as cion and stock, and in that manner, to secure various degrees of success in grafting, both cion and stock were treated in five ways.

One lot was left in the natural condition without treatment; a portion of this lot was collected in the fall and a portion in the spring. These are designated "Natural-fall" and "Natural-spring" respectively. Each of these portions was divided into five lots:- 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. Lot 4 corresponds

to the portion above the girdle and Lot 5 to the portion below the girdle as mentioned below.

In the latter part of August 1926 a wire was twisted tightly around the stem at the base of the two year wood on another lot of cions, which are referred to as "Girdled".

A fourth lot was partly defoliated by cutting the mid-rib of the compound leaves so that approximately half of the leaf area was removed. This lot is called "Defoliated."

A fifth lot received an application of sulphate of ammonia about the middle of September, after growth had ceased. This did not cause a resumption of growth but caused the trees to retain their leaves later than those not receiving the treatment, and to begin growth earlier in the spring. This treatment is designated by the word "Nitrate."

Seedling trees to be used as stocks were given the same treatment except that the girdle was placed about 18 inches from the ground.

Cion wood from the "nitrated" class was gathered December 11, 1926 and buried about 18 inches deep in a well drained place. Cion wood from the "nitrated" and other classes was gathered April 6, 1927, packed in moist sawdust, and placed in cold storage until needed. Samples for chemical analysis were taken at

the time of collecting cion wood.

Grafting began on April 25 and was completed May 4. Various combinations of the treated and the untreated cion with those of the stock were made. These combinations, together with the number of grafts and the percentages of successful unions are presented in Table 1. The whip or tongue graft, made in the usual way, was used. The cion was fitted to the stock, tied with cotton string and the entire union and the tip of the cion was covered with a brush wax, the formula for which is found in Michigan Experiment Station Special Bulletin 142 (3). The entire cion and union was covered with a kraft paper bag.

Budding

A limited amount of budding was done and, though no detailed study was given this method of propagation, it seems very promising. The patch bud method, as described by Stuckey and Kyle (14) was followed fairly closely. The budding knife described by them under the name of "Texas Agie" was used in this budding. Buds were obtained from one year wood cut in spring before growth began.

Root Grafting

On February 6 to 8, natural cions, nitrate cions, girdled cions and some which had special treatment were whip grafted on pieces of walnut root. Special treatment was given some of the natural cions as follows: the cions were placed into three suction flasks, containing respectively a 3% solution of sucrose, a 3% solution of dextrose and a 2% solution of potassium nitrate. The flasks were then evacuated for 20 minutes and the cions allowed to remain in the solution for two hours. A fourth lot was placed in a Mason jar and enough ether added to saturate the air of the jar. The jar was then sealed and allowed to stand for 16 hours.

Natural walnut cions were placed on apple roots, apple cions on walnut roots, apple cions on apple roots and apple cuttings similar to the cions were made. All grafts were packed in moist sawdust and placed in storage at about 40°F.

CHEMICAL

Carbohydrate Analysis

Samples for chemical analysis were cut into half inch pieces, weighed in a tared sample bottle and heated to 95°C. for one hour. Later they were

dried at 90°C., ground to pass a 60-mesh sieve and an aliquot dried to a constant weight at 95°C. Two grams of the dry ground material were placed in a 250 ml. Erlenmeyer flask and extracted with five 80 ml. portions of 80% alcohol. The alcohol was brought to boiling and boiled for five minutes, cooled and filtered. 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 delead with dibasic sodium phosphate. The reducing power of an aliquot was determined and expressed as dextrose.

The Shaffer and Hartman iodine titration method was used to determine the reducing power. Twenty-five ml. each of copper sulphate and Rochelle salts solution were placed in a 300 ml. Erlenmeyer flask and 50 ml. sugar solution added from a pipette. This was brought to boil in four minutes and boiled two minutes, cooled to 40°C. Twenty-five ml. iodine-iodate solution (60 gms. potassium iodide and 5.4 gms. potassium iodate to one liter) added and mixed, 17 ml. H_2SO_4 (175 ml. concentrated acid to one liter) added and then 20 ml. saturated potassium oxalate solution. The excess iodine was

titrated with 0.1N sodium thio-sulphate. The difference between the amount of thio-sulphate used and that used in a blank determination multiplied by 6.36 equals milligrams copper reduced by the sugar solution. Duplicate determinations were made which checked to 0.1 ml. thio-sulphate.

One hundred ml. of the clarified extract was hydrolyzed by adding 10 ml. concentrated HCl and holding at 70°C. for 10 minutes. This was cooled, neutralized, and the reducing power of an aliquot determined and expressed as total sugars.

The residue from the alcohol extraction was washed into the Erlenmeyer, heated for 30 minutes over a water bath, cooled and a 1% solution of taka-diastrase added. This was digested for 36 hours at 38°C., filtered, washed with enough water to make 150 ml., then 8 ml. concentrated HCl added and refluxed over a water bath for two and one half hours. It was cooled, neutralized, made up to 250 ml. and the reducing power determined without previous clarification. From this the starch content was calculated.

The residue from the taka-diastrase digestion was washed into the Erlenmeyer with dilute HCl (8 ml. to 150 ml. water) and hydrolyzed for two and one half hours, cooled, neutralized, filtered, and made up to

250 ml. and the reducing power determined. From this, calculations were made for acid hydrolyzable 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.

Histological

Sections were made from girdled and natural stems, of successful and unsuccessful grafts, and also of 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 100% alcohol, standing in each only a few minutes. They were then passed through 75% xylol, 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.

PRESENTATION AND DISCUSSION OF DATA

Counts of grafts growing, live and dead were made on June 28 and August 9. On the earlier date many cions were still alive but it was impossible to tell whether or not union was established. On the later date, however, all cions were either growing or dead. Table 1 gives the results of the August count.

When the count was made, several cases of defective waxing were discovered; these were discarded from the record.

It will be observed from this table that the stock apparently is not the controlling factor in the process of uniting of the graft. The degree of success on the natural stock varied from 0 to 52.7 per cent. The stock above the girdle showed varying success from 12.5 to 58.8 per cent and the stock below the girdle varied from 13.0 to 61.6 per cent living. The nitrate stock gave more uniform results but in this case the cions used were the types which gave approximately the same percentages on other types of stock. These data substantiate field observations. All grafts which failed to unite were examined and in practically every case the stock formed callus quite freely but the cion did not; apparently it was the failure of the cion to form callus which was responsible for the failure to establish union.

Table 1.- Results of field work 1927

Cion	Stock	Number of grafts	Percent success
Natural-spring apical 1 year	Natural	25	0.0
" " basal 1 "	"	23	21.7
" " apical 2 "	"	18	33.3
" " basal 2 "	"	19	31.6
" " apical 3 "	"	19	52.7
" " " 3 "	Nitrate	19	36.8
Natural-fall apical 1 "	Natural	14	0.0
" " basal 1 "	"	13	0.0
" " apical 2 "	"	10	20.0
" " basal 2 "	"	11	9.1
" " apical 3 "	"	13	30.8
Nitrate	Nitrate	18	33.3
" " " "	Natural	24	37.5
" " " "	Above girdle	17	58.8
Above Girdle	Natural	15	26.7
" " " "	Above girdle	15	40.0
" " " "	Below "	13	61.6
" " " "	Nitrate	25	40.0
Below Girdle	Natural	16	37.5
" " " "	Above girdle	13	15.4
" " " "	Below "	20	20.0
Defoliated	Natural	18	33.3
" " " "	Above girdle	24	12.5
" " " "	Below "	23	13.0
" " " "	Defoliated	25	20.0

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There does seem to be some influence of the condition of the cion upon the degree to which grafting is successful. With the natural cions, there was an increase in the percentage of success with increased age of cion wood. Spring cut cion wood gave considerably better results than fall cut cion wood. This difference might appear to be due to the size of the buds and the readiness with which they begin growth. Supposedly the early growth would cause excessive drain on the cion before union was formed and thus prevent the union. This was not the case, however, for cions of the one year old wood where the buds were the largest did not start to grow and die later, the cion apparently died before the buds could begin to grow.

Cions treated by girdling, defoliation, and nitrogen application gave inconsistent results when grafted on various stocks.

Carbohydrate and nitrogen analyses of the cion wood, with the percentages of success in grafting are presented in Table 2. In this table the results of the nitrate cion upon various stocks are averaged, which is true of those of above the girdle, below the girdle and defoliated cions. It will be noted that the C/N ratio in the "Natural-fall" cion increases with the age of the wood and that the degree of success also increases in

Table 2.- Carbohydrate and nitrogen analyses of cion wood and field results

	Percent Dry Matter	Percent Reducing Sugars	Percent Total Sugars	Percent Starch	Percent Acid Hydrolyzable	Percent Total Carbohydrates	Percent Insoluble Nitrogen	Percent Soluble Nitrogen	Percent Total Nitrogen	C/N	Number of Grafts	Percent Success
Natural-Fall												
Apical -1 yr.	49.6	2.38	4.92	1.95	13.05	19.92	1.12	.17	1.29	15.46	14	0
Basal -1 yr.	51.8	2.38	4.69	1.46	11.93	18.08	.74	.06	.80	22.60	13	0
Apical -2 yr.	54.0	3.87	4.45	1.40	11.79	17.64	.57	.05	.62	28.45	10	20.0
Basal -2 yr.	54.8	2.81	3.90	2.48	9.83	16.22	.63	.11	.74	21.92	11	9.1
Apical -3 yr.	----	2.63	6.95	1.48	14.41	22.84	.62	.08	.70	32.63	13	30.8
Natural-Spring												
Apical -1 yr.	48.2	----	2.91	1.18	13.72	17.81	1.01	.33	1.34	13.29	25	0
Basal -1 yr.	54.1	----	2.50	.38	11.81	14.69	.61	.16	.77	19.08	23	21.7
Apical -2 yr.	57.4	----	4.30	1.91	13.05	19.26	.61	.12	.73	26.38	18	33.3
Basal -2 yr.	54.4	----	3.67	.97	12.04	16.68	.66	.09	.75	22.24	19	34.2
Apical -3 yr.	52.7	----	2.79	1.15	11.25	15.19	.54	.10	.64	23.73	19	52.7
Above Girdle	51.9	----	3.35	2.75	12.49	18.59	.58	.14	.72	25.82	68	42.1
Below Girdle	52.9	----	2.57	.91	11.81	15.29	.55	.08	.63	24.27	49	34.4
Defoliated	54.8	2.06	3.20	.98	12.38	15.56	.47	.05	.52	31.84	90	37.5
Nitrate	54.1	1.93	2.97	.98	11.74	15.74	.82	.05	.87	18.09	59	43.2

the same direction. The same holds true also in the "Natural-spring" cion, but comparison of the fall and spring ratios show that the fall ratio is larger than the spring ratio for the same type of wood in every case except one. If increased success in grafting is associated with increased C/N ratio, the fall cut cion should be superior to the spring cut cion, but this is not the case. This ratio is higher also in the girdled and defoliated cion but the percentage of success is smaller. In the nitrate cion, the ratio is smaller while the percentage successful grafts is not materially changed. In the same manner, no single class of carbohydrates or nitrogen varies consistently with or inversely to the degree of success in grafting.

Carbohydrate and nitrogen analyses of the stock appear in Table 3 and here again there is no consistent nor significant variation.

Chemical analyses and field results of the cion wood from the Graham Station are given in Table 4. These cions were treated about a month before those from the Forestry nursery and showed considerable swelling above the girdle and ring, Figure 1. They were grafted on natural stock in the Forestry nursery in the same manner as the other cions. It was expected that the results from this lot of cions would show marked differences.

Table 3.- Carbohydrate and nitrogen analyses of stock

	Percent Dry Matter	Percent Reducing Sugars	Percent Total Sugars	Percent Starch	Percent Acid Hydrolyzable	Percent Total Carbohydrates	Percent Insoluble Nitrogen	Percent Soluble Nitrogen	Percent Total Nitrogen	C/N
Natural	57.6	2.81	5.08	1.56	12.38	19.02	.58	.06	.64	29.72
Nitrate	53.1	1.50	3.52	1.91	13.61	19.04	.58	.06	.64	29.75
Above Girdle	58.1	3.25	6.25	1.65	14.29	22.19	.51	.09	.60	36.98
Below Girdle	57.1	2.63	4.53	1.56	14.41	20.50	.48	.08	.56	36.61
Defoliated	56.2	4.20	5.41	1.12	12.04	18.57	.47	.06	.53	35.02

Table 4.- Carbohydrate and nitrogen analyses and field results of cion from Graham Station, Grand Rapids

	Percent Dry Matter	Percent Reducing Sugars	Percent Total Sugars	Percent Starch	Percent Acid Hydrolyzable	Percent Total Carbohydrates	Percent Insoluble Nitrogen	Percent Soluble Nitrogen	Percent Total Nitrogen	C/N	Number of Grafts	Percent Success
Natural	58.2	5.25	8.82	1.21	13.72	23.75	.70	None	.70	33.93	25	44.0
Above Girdle	57.7	4.77	11.11	2.07	13.52	26.70	.70	.03	.73	36.57	14	16.7
Below Girdle	58.6	6.06	8.75	2.14	12.26	23.15	.68	None	.66	34.04	13	30.8
Above Ring	54.3	13.56	13.91	2.14	12.15	28.20	1.24	.12	1.36	20.73	6	14.3
Below Ring	57.2	3.93	5.94	.53	13.28	19.75	.67	.03	.70	28.21	6	0.0

There are some differences both in field results and in carbohydrate content but they do not tend in the same direction in the girdled and ringed cion nor are the results of grafting consistant with the analyses.

Histological

The most striking aspect in the sections of walnut grafts (Figures 2 and 3) is the large size and number of the 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 tissues of the apple (Figures 4 and 5).

Sections of pruning wounds on walnut stems (Figures 6 and 7) show some degree of closure of the vessels due to the formation of tyloses (Figure 8) but little or no gum formation. The degree to which the vessels are closed is considerably less than in apple wounds. The tracheids and vascular rays seem to be plugged. These observations may be significant in partially explaining the difficulty in propagating the walnut by grafting. If the vessels remain open so that rapid loss of water takes place and the tracheids and rays become plugged, making translocation of stored food materials difficult, active division of the cambium cells would be limited or prevented, thus reducing the

amount of wound meristem formed. Since union of stock and cion results from the meeting and fusion of the wound meristem arising from each, no union could take place if the meristem were not formed by the cion. Since the cion is 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.

Root Grafting

A few rather interesting observations were made regarding the root grafts. Cions treated with sucrose, dextrose, and those from above the girdle remained alive longer than other cions, although no successful unions were obtained; treatment with sugar solution seems promising for further study. Treatments with potassium nitrate and ether were very injurious; the cions turned black and died in a very short time.

Walnut cions depressed callus formation by the apple roots and walnut roots depressed callus formation in the apple cions placed on them (Figures 9 and 10). This suggests the possibility that the walnut contains some substance inhibitory to callus formation.

CONCLUSIONS

At the present stage of the experiment, the condition of the cion wood seems to be the limiting factor in successful propagation of the walnut by grafting. The carbohydrate and nitrogen content of the cion does not seem to have the controlling influence. It may be possible that some other substance, not taken into consideration in the present work does exert more influence.

The anatomical and morphological relations seem to be very important and deserve more study. Treatments of the cion with sucrose and other solutions should receive further attention.

SUMMARY

1. The stock of the walnut seems to be the least variable of the two symbionts in behavior in grafting.
2. The percentage of success in grafting increases with age of wood, up to three years old.
3. Cion wood cut in spring seems to be superior to that cut in fall.
4. Girdling of the stem and application of a nitrate fertilizer do not seem to influence greatly the degree of success in grafting.
5. Plugging of tracheids and vascular rays, associated with the failure of vessels to become plugged, seems to limit callus formation and success in grafting.

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Fig. 1.- Walnut branches from the Graham Horticultural Experiment Station, near Grand Rapids showing girdle and ring.

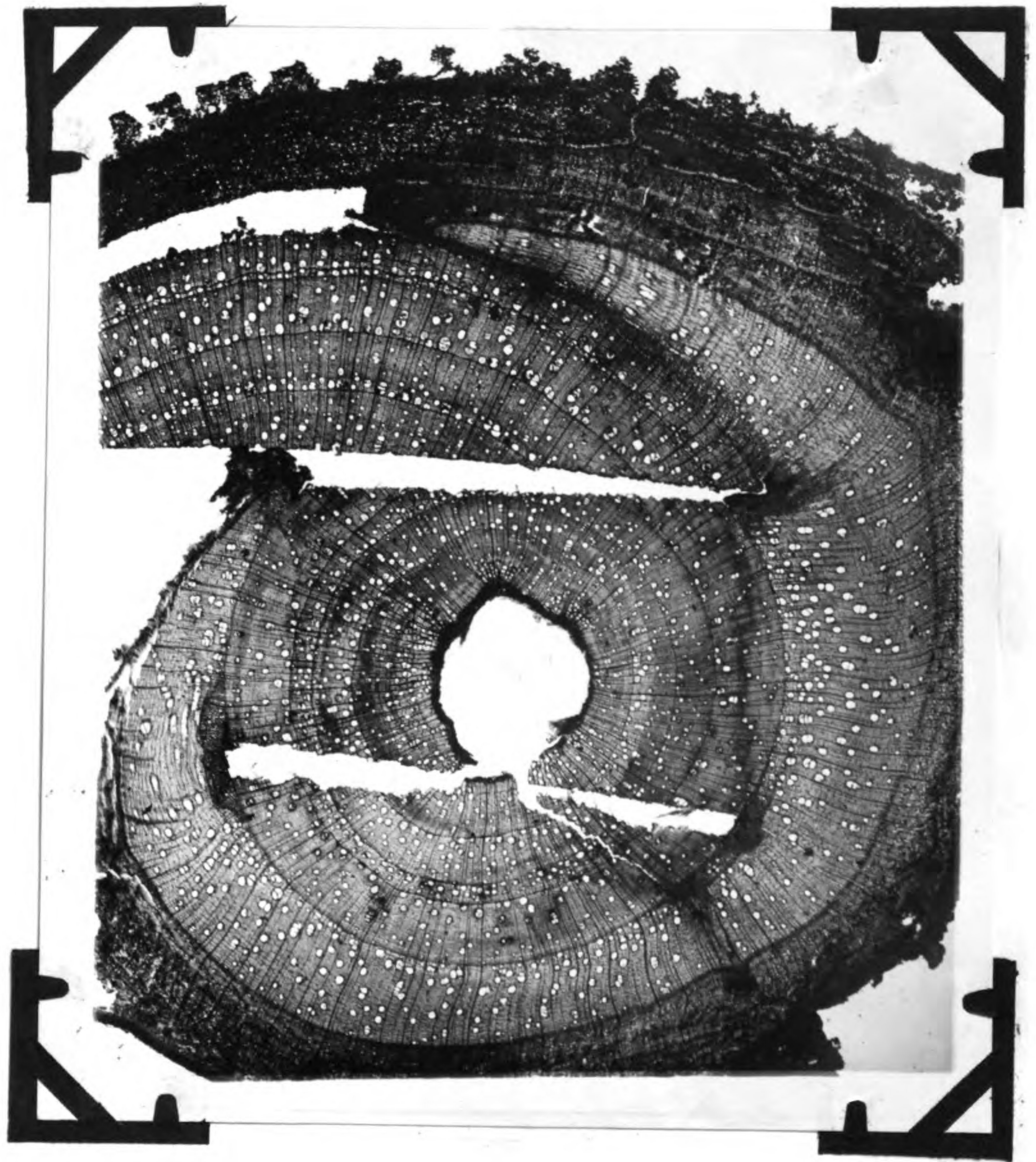


Fig. 2.-Cross section of whip graft of walnut. Most of old xylem in both cion and stock is dead. The vessels are large and not plugged.



Fig. 3.-, 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.

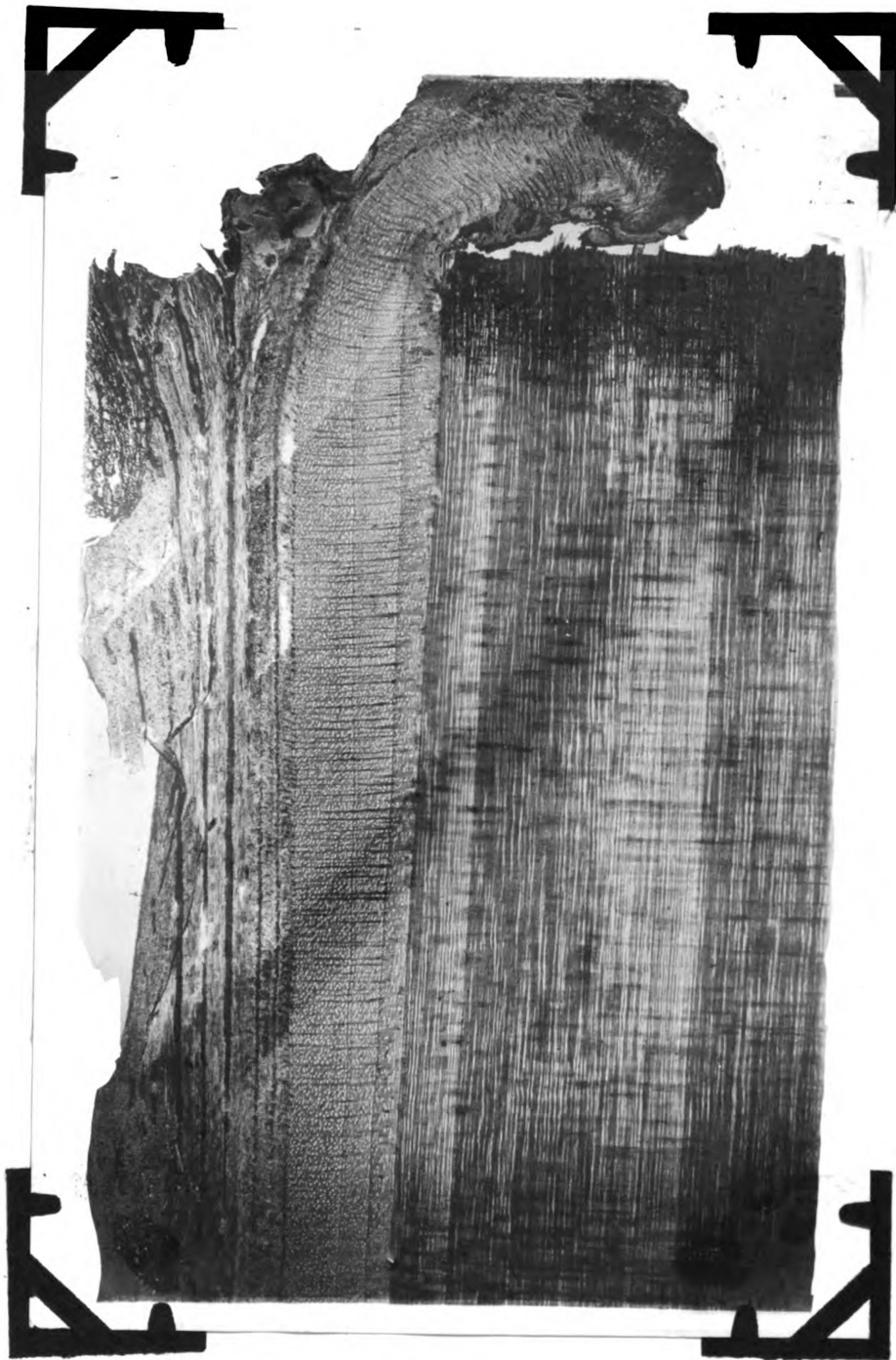


Fig. 4.- Longitudinal section of amputation wound on apple stem. Vessels are completely plugged near cut surface and walling over callus plentiful.

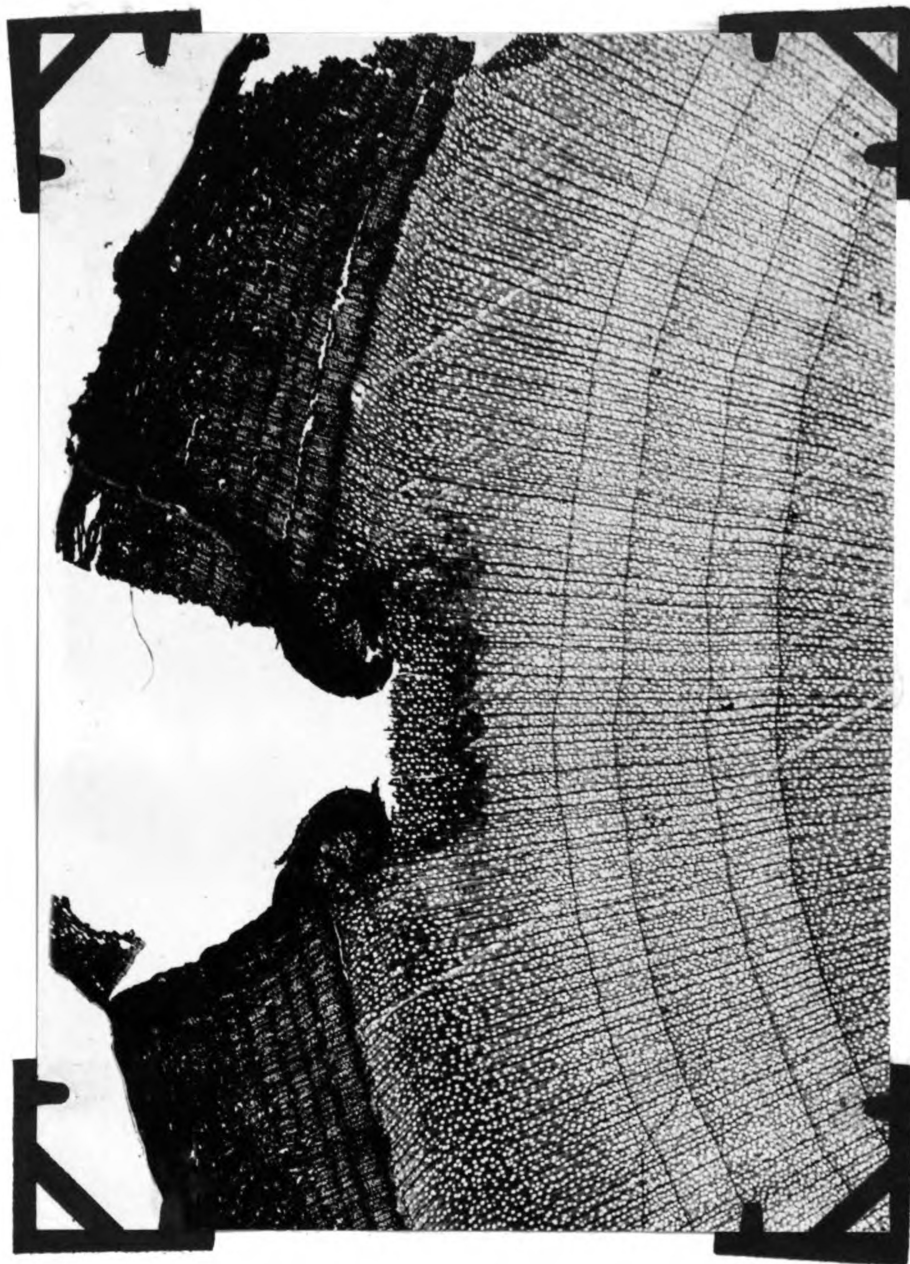


Fig. 5.- Wound on apple stem made by removing the bark and scraping away the young xylem tissue. Dead xylem is very limited and plugging of vessels protects underlying tissues.

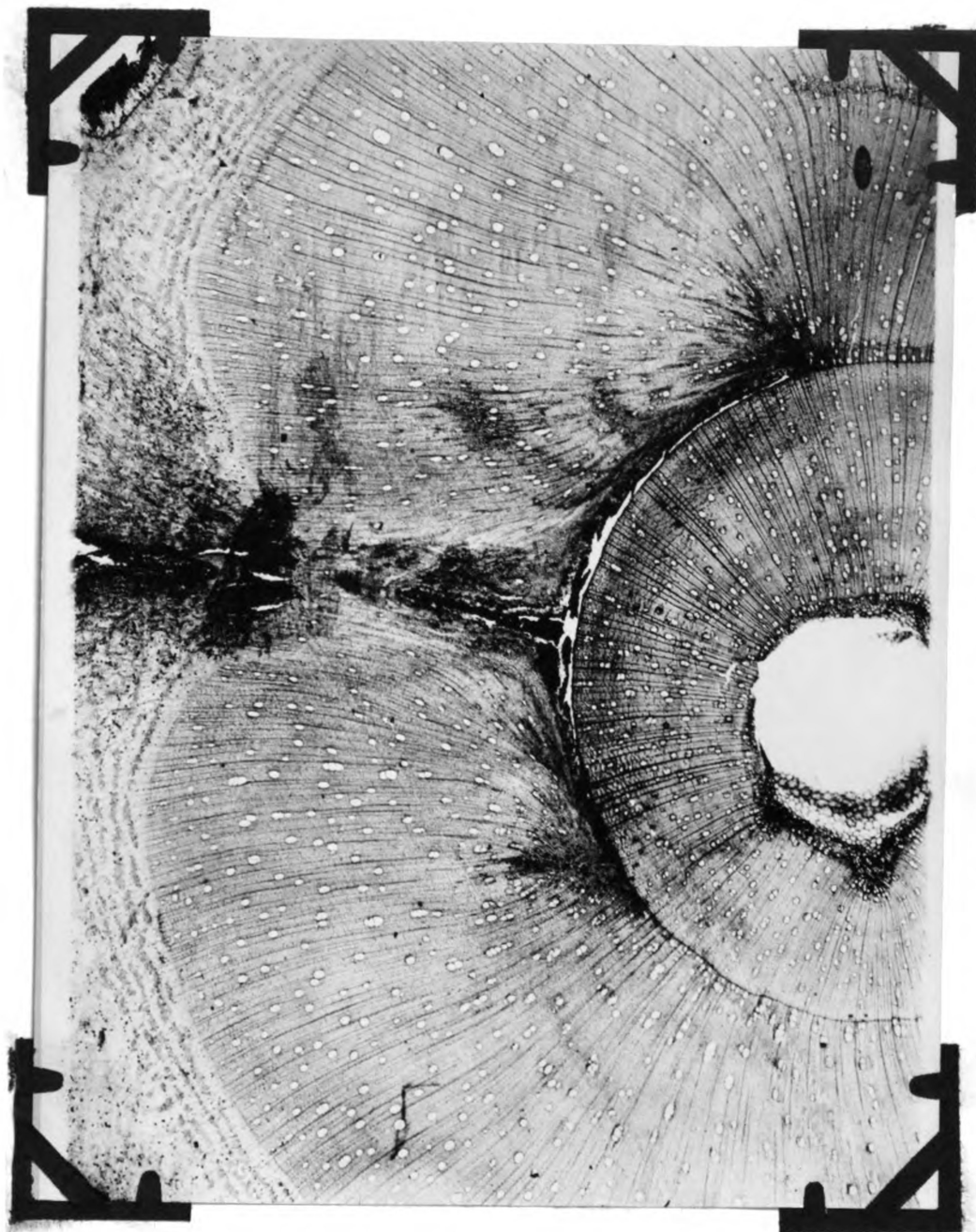


Fig. 6.- Amputation wound on walnut stem, slightly below the injury. Xylem is dead for nearly $\frac{1}{3}$ the circumference. Tracheids are plugged, vessels are open except for occasional ones having tyloses.

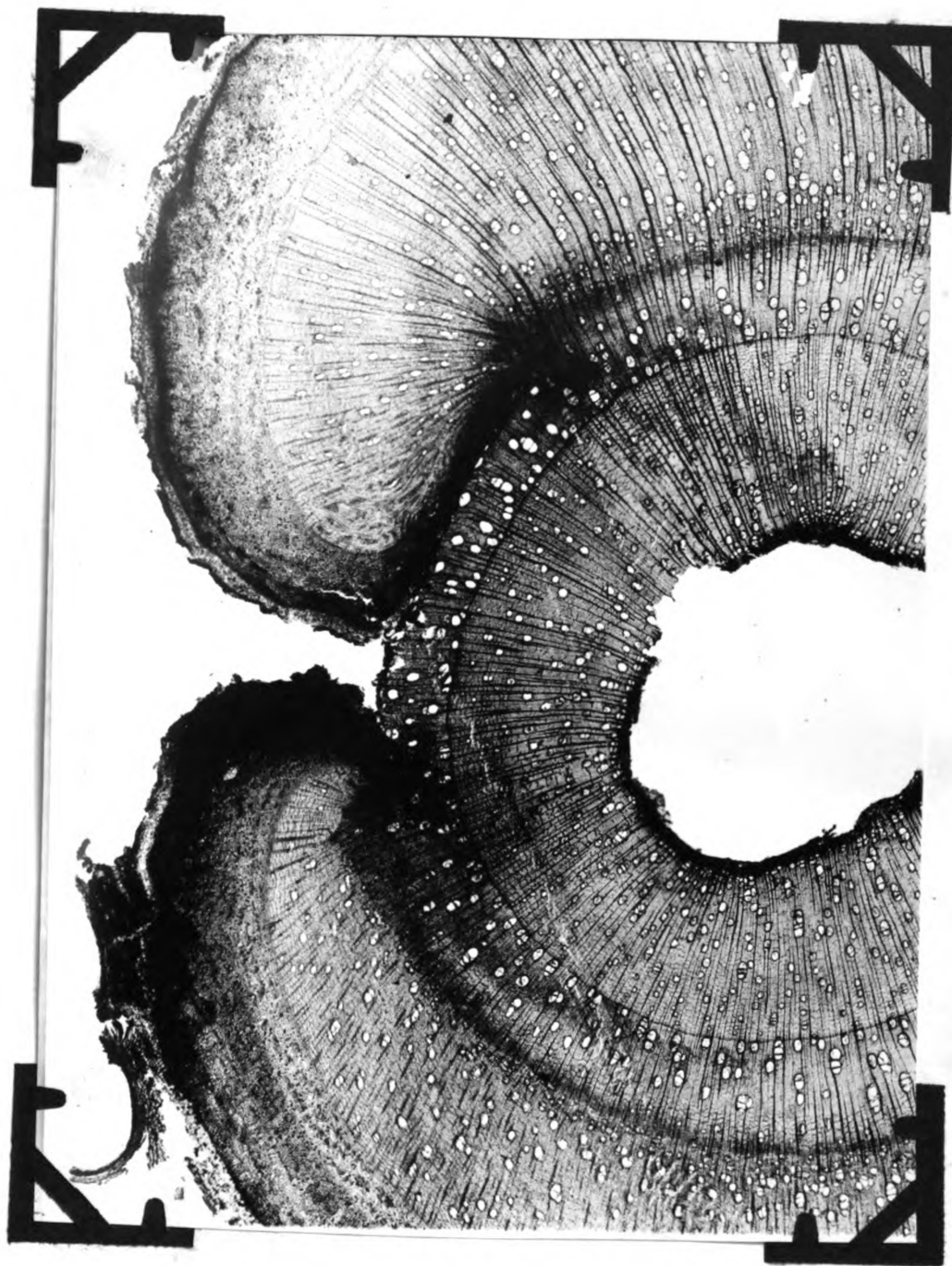


Fig. 7.- Wound on walnut stem made by removal of bark when cultivating. Compare with Figure 5. The xylem is dead the entire extent of wound and to the pith. Very few vessels are closed but many tracheids and ray cells are.

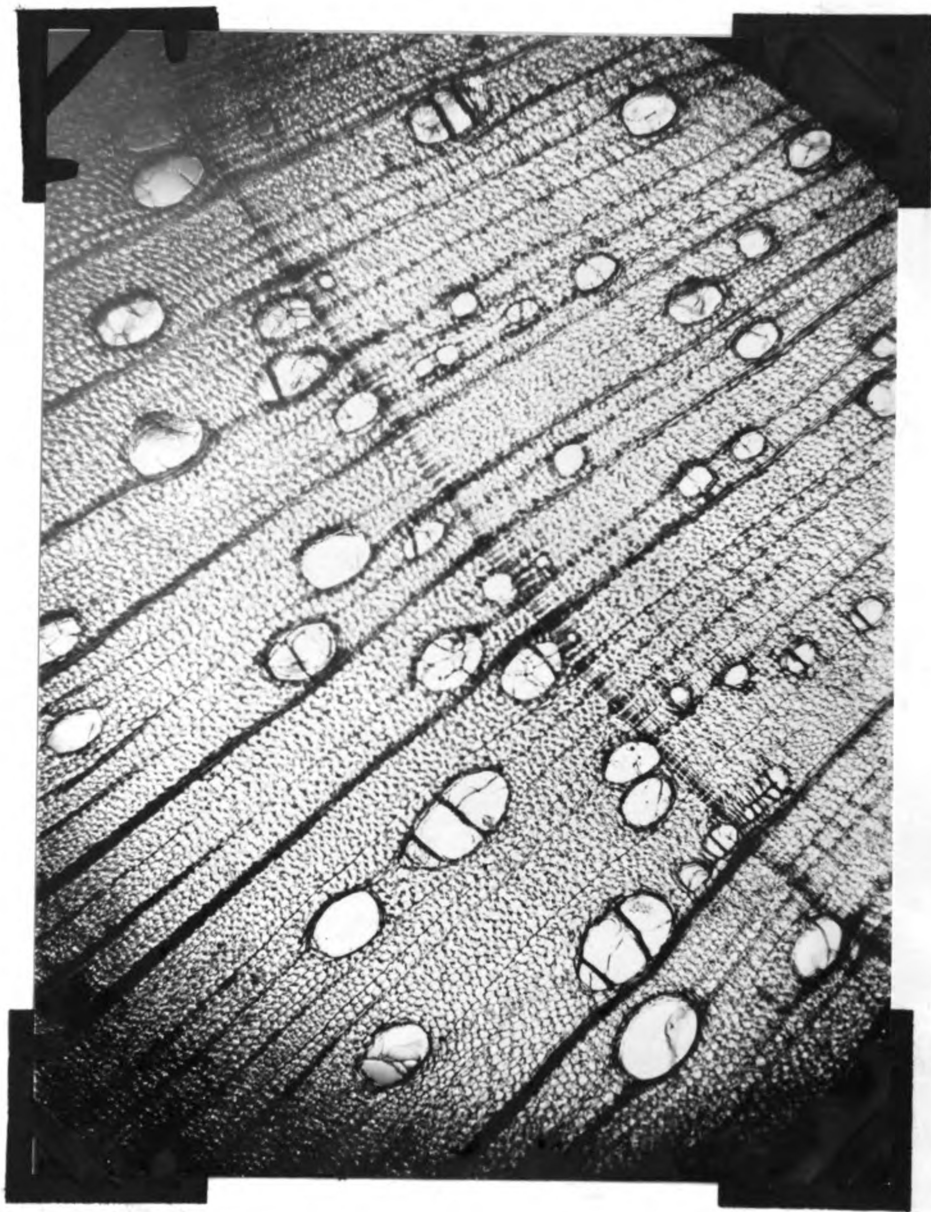


Fig. 8.- Higher magnification of a portion of Figure 6 showing tyloses in a few vessels.



Fig. 9.- Normal apple cuttings showing callus.

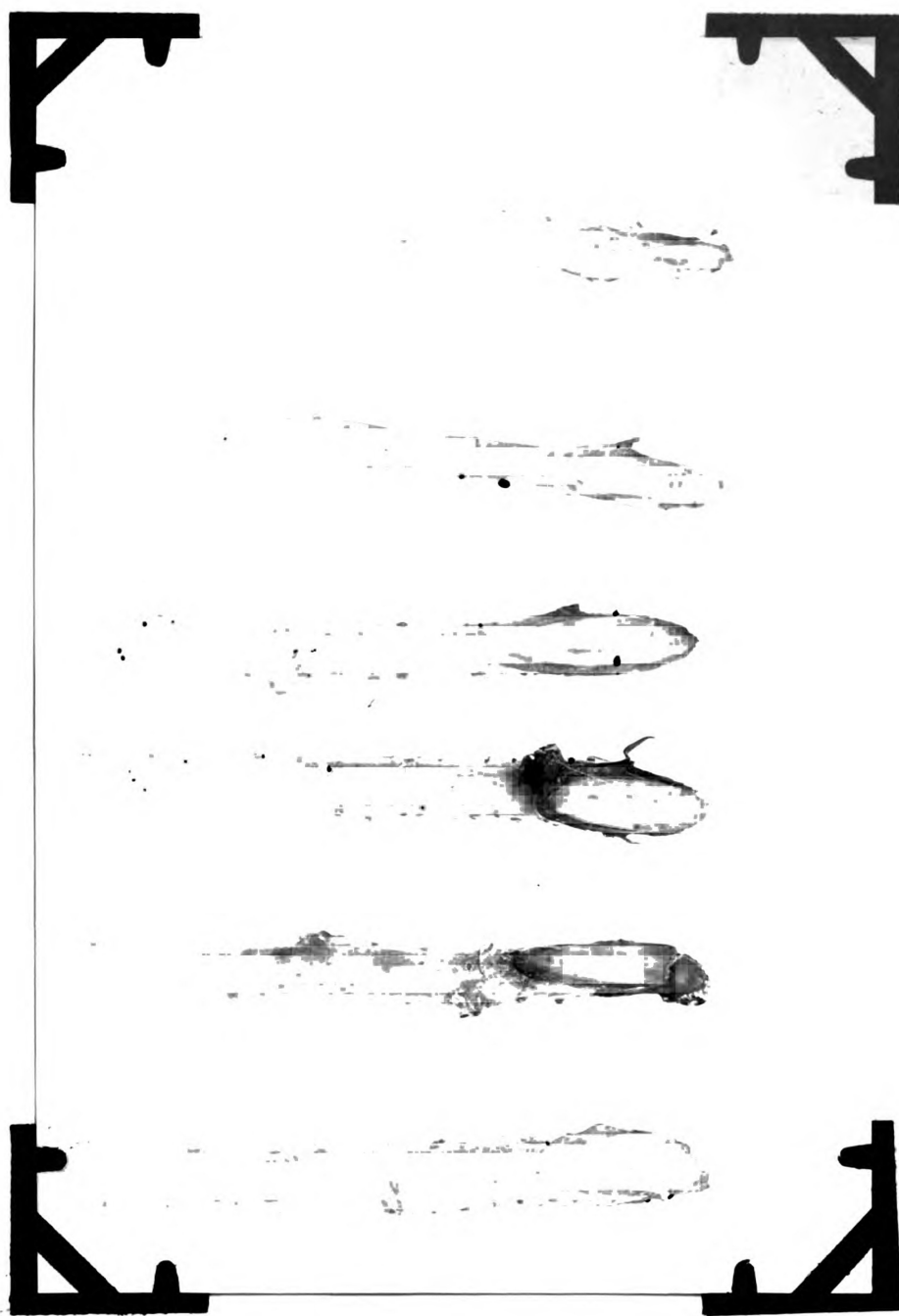


Fig. 10.- Apple cions which had been placed on walnut roots.
Callus formation very weak or absent.

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