THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Purley Lorenzo Keene for the degree of Master of Science. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

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Date May 31, 1922

10-20 5M

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Purley Loranzo Keene final oral examination for the degree of

Master of Science

We recommend that the degree of

Master of Science

be conferred upon the candidate.

W H Alderman
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Date May 31 1922

10-20 5M

A STUDY
OF THE FACTORS AFFECTING
THE
CALLUSING AND ROOTING
OF
HARDWOOD APPLE CUTTINGS

A THESIS

SUBMITTED TO THE FACULTY

OF THE

GRADUATE SCHOOL

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BY

PURLEY L. KEENE

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FOR THE DEGREE OF

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INTRODUCTION

The apple is propagated commercially by budding and grafting which are rather cumbersome and expensive methods. Common apple stocks are usually secured from seed. Seedlings of a single variety, even if from self-fertilized seed, show great variation in characters owing to the heterozygous condition of our cultivated varieties of apples. Vegetative propagation of plants is most readily and most economically accomplished by cuttings; and since the apple must be propagated vegetatively, the value of further definite knowledge upon this subject is quite apparent. If apple stocks could be secured by hardwood cuttings, we would at least have a method of securing a uniform root system in our trees for experimental purposes, even though the method might not be adapted for commercial propagation.

Bearing in mind the scientific as well as economic importance of increasing our knowledge of propagation this investigation was undertaken. The object was to analyze more clearly the various factors affecting and governing the callusing and rooting of hardwood apple cuttings.

ACKNOWLEDGEMENTS

A considerable amount of valuable material and suggestions were received through personal correspondence with horticulturists and nurserymen of various parts of

the country. The work was done under the direction of Prof. W. A. Alderman, to whom the writer is very grateful for the many helpful suggestions which he gave upon the various phases of the problem.

LITERATURE REVIEW

While the apple is propagated commercially by budding and grafting, various forms of cuttage and layerage are used in closely related species of plants. Du Breuil (8) states that, "Quince, Doucin and Paradise stocks are propagated by mound and simple layering and by cuttings." And according to Sorauer (29) Paradise stock cuttings can readily be struck. Hatton (13) states that Paradise stocks of both free growing and dwarfing habits can be raised quickly by layers, wood and root cuttings. Kains (17) states that, "French Paradise and Doucin trees are grown mainly by mound layering." Welsh (31) of the Mound Arbor Nurseries states that some years ago a nursery firm in the South grew the Le Conte pear from stem cuttings. The practice was discontinued on account of the poor root formation. Scott (26) of the United States Department of Agriculture recently stated that the Keiffer and other varieties of pears were formerly propagated by hardwood cuttings in the Southern states, and that the quince is commonly propagated by this method at the present time. Luke (20) was able to root a small percentage of his hardwood pear cuttings.

In the apple layerage and cuttage have been tried with varying degrees of success. Nordine (22) writes that while in Denmark he propagated two year old apple trees by simple layerage. A slight incision was made under a bud which was covered with earth. He states that good results were secured by this method. Shaw (27) gives an account of an experimental attempt to propagate the apple by mound layering new sucker shoots of four or five inches in height. The following four varieties were used: Ben Davis, Bough, Rhode Island Greening and Transcendent. At the end of two years it was found that most shoots of all varieties bore small roots which appeared near the junction with the cut off stump. However, up to the time this report was made, none of these shoots had been separated in an attempt to establish them as independent trees. To get a stock, which is resistant to the attacks of the woolly aphis, the nurserymen of Australia are very largely using the Northern Spy for stock purposes. This variety may be propagated, according to Watson (30), by mound layering similarly to the Doucin and Paradise. Watson (30) states, however, that the usual practice is to propagate it by root cuttings, two and a half inches long, and planted one inch below the surface of the soil. Shaw (27) tried root cuttings and secured a good stand, although the growth was very slow the first season. Luke (20) also was able to induce root cuttings to grow with fairly good success.

The nurse-root method has been used to secure varieties upon their own roots. This method consists of making

ordinary root grafts, using a long cion and a short root, and planting them deep to encourage rooting from the cion. After two or three years of growth the graft is dug and the stock portion cut away leaving the new plant to exist on its own cion roots. Shaw (27) worked extensively with this method and found that varieties vary greatly in the readiness with which they send out adventitious roots from the cion, the proportion varying from zero to practically one hundred with different varieties; that varietal differences may be loosely correlated with density of wood, the softer the wood the higher the proportion rooting from the cion. He found little or no correlation between the ability to form cion roots by this method and the ability to form a callus on a cutting. Moore (21) found that with most varieties, even after three years in the nursery, the number of trees having sufficient cion roots to support the tree was very small. In no case was a two year old tree found to have sufficient cion roots to support itself. Experiments with this method at the Iowa Agricultural Experiment Station gave only partial success. It is quite evident that the apple does not readily form adventitious roots. Howard (15) gives a detailed description of the propagation of plants by the nurse-root method.

A few attempts have been made to root green wood cuttings of the apple. Shaw (27) made cuttings three or

four inches in length in August and September and placed them in sand and other mediums in the greenhouse. Bottom heat in varying degrees was used in some cases, and also an enclosed propagating frame. Something over a thousand cuttings of several varieties were made. The cuttings formed a callus varying somewhat with the variety and the buds started out until the leaves were about one-fourth inch in length. This occupied about two weeks after which growth ceased. The final result was the same in practically all cases, and of the thousand or more cuttings only a single one of the Fall Pippin variety rooted and that only a single short root which was broken off in removing from the sand so that it failed to grow. Luke (20) also failed to root green wood cuttings of the apple.

Up to the present time attempts to propagate the apple by stem cuttings have met with little success. In spite of this fact, however, many commerical propagators and professional horticulturists believe that the apple may be increased by hardwood cuttings, if the proper conditions are given. They admit, however, that such cuttings root with difficulty. Klehm (18), a nurseryman of Arlington Heights, Illinois, states, "I know from my limited experience that you will find some difficulty in rooting apple cuttings, yet I know it can be done." Balfour (2) states, "I believe all plants can be propagated vegetatively by cuttings, some easily, some with more or less difficulty." Shaw (27) says

that in spite of his failures, he is of the opinion that it is possible to grow apple trees from cuttings. And it is quite evident that Bailey (1) had the same opinion when he wrote, "Dwarf stocks are mostly obtained from mound-layering.....The Dwarf stocks, in common with all apple stocks, may be sparingly propagated by root cuttings and by hardwood cuttings."

No doubt many attempts to root hardwood cuttings of the apple have been made in the past. One attempt was made at the Maryland Agricultural Experiment Station (3) a few years ago. In regard to this attempt Ballard (4) writes that the work consisted mainly of making hardwood cuttings of a number of varieties, heeling them in for the winter and setting into nursery rows in the spring. Most of these cuttings callused and a number of them put out foliage, but in no case were roots formed. The most extensive work bearing directly upon this problem was done by Luke in 1896-7 (20), when he made more than seven thousand hardwood cuttings of the apple, plum, pear and grape. However, he used three year old wood making the cuttings one, three and six inches in length. The first group of cuttings was set directly in beds in the greenhouse at 64° and 77° F. After three months they were discarded with a hundred per cent failure to secure roots. The second group was stored in sand for three months and then placed in the greenhouse beds at similar temperatures. The same results were secured as with

the first group. The rooting ends of some of the cuttings were placed in contact with brick, the edge of the pot and other obstacles with no results. In no case did he succeed in getting stem cuttings of the apple to grow.

Nevertheless, there are clearly determined records in which hardwood cuttings of the apple have developed roots. Pickering (9) states that out of twenty apple cuttings, planted in earth, three formed roots. Fagan (10) succeeded in growing one apple tree from a cutting but was unable to give any reasons for one growing when all others failed to grow. Gould (12) gives an account in which he states that prunings which had been stuck three or four inches in the ground under the trees for cion use later on developed roots. An unsuccessful attempt to transplant them was made. Shaw (27) quotes the late T. V. Munson as stating that he had often placed apple shoots which were cut from the trees in February into the ground for marking rows and had them take root. Curtis (7) writes, "Among the apple cuttings I tried in my earlier experiments, a few twigs rooted."

STATEMENT OF THE PROBLEM

Since hardwood cuttings of the apple will develop roots under certain conditions, and since so little is known about the conditions under which roots will be formed it was thought highly desirable to take up a study of the factors affecting their callusing and rooting. The work

upon this problem was started during the summer of 1920 as a station project. The writer took up the work in October of the same year and carried it on until July 1921. Such factors as the season, the storage medium, the storage temperature, the position of the cutting, the variety and the potassium permanganate treatment were given careful study.

MATERIALS AND METHODS OF THE PRESENT INVESTIGATION

Materials and methods

With the exception of some preliminary work with green wood cuttings this investigation was confined entirely to hardwood cuttings of the apple. During the progress of the investigation data were taken on sixteen thousand six hundred fifty (16,650) individual cuttings undergoing various treatments. All of the cuttings with the exception of those in one experiment were made of a uniform length, eight to nine inches, the lower end being cut slanting just below a bud. Only wood of the preceding year's growth was used. The coarser, harder, lower portions of long water shoots were discarded, as were also the tender, unripened, upper portions of long growths. The cutting wood was gathered from the field at various intervals and stored with the lower ends of the shoots buried two or three inches deep in moist sand in the cellar until they were made into cuttings and stored in their respective places. Ten cuttings were taken as a unit number. Each bundle of ten cuttings was held together with a wire tree label upon which the key numbers were placed.

The various mediums in which the cuttings were stored were carefully guarded against becoming too dry as well as too damp. The thermometer used in the experiments was tested both at the beginning and close of the work.

The major experiment consisted of a study of the callusing and rooting of the cuttings, when made at different seasons of the year and when stored in different mediums, positions and temperatures. Eight varieties were used and a set of cuttings were made the first of each month beginning with November first and continuing to April first. They were stored in the greenhouse, cellar and outside, where different temperatures were maintained. Sand, soil and sawdust were used as storage mediums in each location. Two bundles of ten cuttings each were used in each instance, one being stored in a normal upright condition and the other inverted with the butts of the cuttings up. This was done to test the effect of the difference in temperature between the lower and upper part of the mediums where such existed. Ten cuttings of each of the eight varieties which were used in this experiment were planted the first of each month both in sand and in soil in a propagating bench in the greenhouse.

In addition to the main line of work a considerable number of cuttings of available varieties were made during the winter months to further test varietal variability. Some of these cuttings were also used in other minor experiments to test certain recommended practices and suggested treatments

of cuttings for the stimulation of root formation. The cuttings of the variety experiment were stored in sand in the cellar with their butt ends up. Other experiments consisted of storing them in soil over a heating tunnel where the temperature was from 22° to 28° C. One group of cuttings was treated with potassium permanganate in strengths of two, one, and one-half per cent for twenty-four hours and planted directly into the field in soil. A check was made by treating one group of cuttings for twenty-four hours in tap water. Other groups were placed over a heating tunnel for three weeks after being treated with potassium permanganate for eighteen, twenty-four and thirty hours using the same strengths as in the preceding experiment. A check was run with this experiment also. Still another group was made into various lengths and planted directly into the field. The lower ends of a number of cuttings were split, others split twice, and in others a small kernel of oats was placed in the slit. These were also planted directly into the field.

Methods of recording data

Considerable data were kept on each bundle of ten cuttings in order to be able to make intensive and accurate comparisons. A large outline was prepared and data added from time to time as it was taken. The records of the date gathered from the field, the variety, the average size of the cuttings, the date made and stored, the storage medium and temperature, and the position of the cuttings in the storage mediums were

taken at the beginning of the work with each group of ten cuttings. At the time of planting the following records were taken; the date and place of planting, the medium in which planted, the condition of the cutting, the number showing calluses and their size. Upon removal from the bed the date, the condition of the cutting and the degree of callus formation were recorded. At any time that root development was noted record was made of the character of the individual cutting, the length, thickness and source of the roots.

Three grades of callus formation were made depending upon the extent of the formation. These were designated as poor, medium and good. Figure 1 shows calluses of the grade "good". The grading of the calluses was performed arbitrarily without taking exact measurements of callus growth in each case. This was necessary due to the fact that over sixteen thousand cuttings were made. It is evident, however, that a reasonably accurate comparison was obtained by the method used. In order to compare the degree of callus formation an index number was found to be necessary. This was obtained for each bundle of ten cuttings by giving each cutting which had a callus falling in the grade 'good' ten, in the grade'medium' seven, and in the grade 'poor' four. Thus a bundle of ten cuttings all having good calluses would have an index of one hundred, one having five medium and five good eighty-five, and one having four poor and six medium fifty-eight, etc. All averaged indexes were weighted.

FACTORS AFFECTING CALLUS FORMATION

Seasonal effects

Taking up the study of callus formation under the various conditions, we will consider first the effect of making the cuttings at various seasons of the year. Table 1 shows the number of cuttings and their callus index by months for the six month period covering the major part of the work. These callus indexes were secured from data which were taken at the time of planting, the first part of May, with all cuttings which were made prior to April first. While the callus indexes of cuttings which were made April first were secured from data which were taken at the time of removal from the field during the first part of July.

Table 1. Effect of time of making cuttings upon callus formation

Date made	No. cuttings	Callus index
November 1st.	1420	30.7
December 1st.	1440	27.1
January 1st.	2880	45.1
F ebruary 1st.	2135	55.6
March 1st.	1745	64.2
April 1st.	1645	43.4

These results indicate that cuttings which were made the first of March showed the greatest tendency to callus; that cuttings which were made February first showed the next greatest tendency to callus; that cuttings which were made January first showed a slightly greater tendency to callus than those which were made April first; and that cuttings which were made earlier in the season than January first showed a decidedly lessened tendency to callus. Other minor experiments showed that cuttings which were made after the first of April gave very poor calluses.

As a whole, the work indicates that cuttings, gathered and made during the later part of the winter, gave decidedly better results than those made either during the fall or early spring. This emphasizes the fact that the cutting wood must be taken from the field before any activity starts in the spring, and also the necessity of taking into consideration the rest period of woody plants. Howard (16) concludes that, "The fact of the existence of a rest period in a large number of species of woody plants has been established and the conclusion seems justifiable that practically all woody forms rest for a longer or shorter period of time."

Comparison of storage mediums and positions

In comparing the value of sand, soil and sawdust as storage mediums, it was found that, outside where the temperature was low most of the time until the warm days of spring came, the sawdust gave the best results. But the development

of fungus diseases in the sawdust, which was used in the cellar where the temperature was around 10° C., made it nexessary to abandon this part of the experiment and to discard the data which were taken on the cuttings stored here. The cuttings which were stored in soil showed a slightly higher index of callus formation than those which were stored in sand. Of twenty-four hundred (2,400) cuttings which were stored in sand the index of callus formation was forty-four and five-tenths (44.5); and of twenty-three hundred eighty (2,380) cuttings which were stored in soil the index of callus formation was forty-six and three-tenths (46.3).

In comparing the value of storing the cuttings with their butt or top ends up, we find that of twenty-seven hundred eighty (2,780) cuttings which were stored with their top ends up the index of callus formation was forty-four (44.0); and that of twenty-eight hundred (2,800) cuttings which were stored with their butt ends up the index of callus formation was fifty and four-tenths (50.4). The greatest difference was shown in the cuttings which were stored outside in sawdust in favor of those stored with their butt ends up. This was very likely due to the quicker response of the sawdust to the sun's heat. The difference in temperature between the upper and lower parts of the sawdust was greater than the difference in temperature between the upper and lower parts of either soil or sand. The cuttings which were stored in the greenhouse showed comparatively little difference, while those which were stored in the cellar showed only a very slight difference in favor of those stored

Table 2. Effect of various storage mediums upon development of callus tissue

Storage medium	Storage place: Greenhouse No. Cuttings	Greenhouse Callus index	Cellar No. Cuttings	Cellar Callus index	Outside No. Cuttings	Outside Callus index	Average of all locations: No. Cuttings	Average of all locations: Callus index
Sand	800	70.7	800	33.7	800	29.2	2400	44.5
Soil	780	69.8	800	30.2	800	39.7	2380	46.3
Sawdust					800	57.3	800	57.3

Table 3. Effect of the position of cuttings upon the development of callus tissue

Position	Greenhouse Sand	Greenhouse Soil	Outside Sand	Outside Soil	Outside Sawdust	Cellar Sand	Cellar Soil	Average all conditions
Inverted	73.6	68.1	25.5	46.6	69.8	37.9	31.2	50.4
Upright	67.8	71.5	32.9	32.7	44.8	29.4	28.9	44.0

with their butt ends up. Here again, the difference was due to the slightly higher temperature which the upper part of the storage mediums maintained over the lower part, the difference being approximately 2° C. Data taken on fifty-five hundred eighty (5,580) cuttings bearing upon these comparisons will be found presented in tables 2 and 3.

Effect of temperature

An analysis of the effect of the different temperatures secured in the several locations brings out what is perhaps the most significant features of the work. Summarizing the results from forty-seven hundred eighty (4,780) cuttings we find that of sixteen hundred (1600) cuttings, which were stored outside in sand and soil with a temperature varying from zero or below to 20°-22° C., the callus index was thirty-four and four-tenths (34.4); of sixteen hundred (1600) cuttings, which were stored in the cellar in sand and soil at 9.5°-11.5° C., the callus index was thirty-one and nine-tenths (31.9); and of fifteen hundred eighty (1580) cuttings, which were stored in the greenhouse in sand and soil at 17°-18° C., the callus index was seventy and three-tenths (70.3).

While the temperature of the sand and soil outside is given at zero to 20°-22° C., it must be borne in mind that this maximum temperature was only attained during the day in the later part of the spring; that it existed for only a few weeks before planting; that the temperature fluctuated with the climatic conditions, low at night and high during

the day; and that during most of the period in which these cuttings were stored the mediums were in a frozen condition. The low temperature which prevailed throughout the winter merely preserved the cuttings in a healthy condition. Callus formation took place only during the last few weeks of the storage period in spring when the temperature rose to a favorable degree.

Of seven hundred fifty (750) cuttings, which were stored in soil over a heating tunnel for periods of eight and two weeks at 22° — 25° C., the callus index was eighty-four and six-tenths (84.6); and of five hundred forty-five (545) cuttings, which were stored there for three weeks at 26° — 28° C., the callus index was forty-two and seven-tenths (42.7). The apparent reason for the latter index being so much lower than the preceding one is due to the temperature, which attained too high a degree, and thus devitalized the cuttings. It would appear that the optimum temperature for callus development would not exceed 25° C.

It is readily seen that the callus index varies directly with the temperature; and that, as far as can be interpreted from the data collected, it is quite evident that temperature is one of the most important factors affecting the callusing of the cuttings, and one that should receive much more intensive and accurate study than was given it in these experiments. A brief summary of these comparisons is presented in table 4.

Table 4. Effect of temperature on callus development

Temperature	Location	No. cuttings	Callus index
9.5°-11.5° C.	Cellar	1600	31.9
0.0°-22.0° C.	Outside	1600	34.4
17° -18.0° C.	Greenhouse	1580	70.3
22° -25.0° C.	Over tunnel	750	84.6
26° -28.0° C.	" "	545	42.7

Varietal variability

Several separate tests were conducted with varieties of apples and crab apples to determine their variability in the production of callus tissue. The following eight varieties, Anisim, Florence, Hibernal, McIntosh, Oldenburg, Patten, Virginia and Wealthy were used in the major experiment and cuttings were taken of each once a month during the winter. Relatively large numbers of cuttings were used of the above eight varieties, while over one hundred cuttings were made of several other varieties which were used in minor experiments. Table 5 presents data bearing upon thirty varieties which were used in quantities of eighty cuttings or more. In studying the callus index of the various varieties used, it is found that they vary widely as may be noted by the data in table 5.

Table 5. Variability of callus index and rooting of thirty varieties which were used in quantities of eighty cuttings or more.

Variety	No. of cuttings	Callus index	No. rooted	Per cent rooted
Christmas	210	69.9	0	.00
Cross	160	67.5	0	.00
Martha	100	63.2	0	.00
Berlin	160	62.4	0	.00
Brier	220	62.0	13	5.91
Charlamoff	260	61.4	0	.00
Gen. Grant	100	58.6	0	.00
Babbitt	100	58.0	2	2.00
Antonovka	120	57.3	3	2.50
Hibernal	1240	55.0	7	.57
Shields	130	52.8	0	.00
Alexander	320	52.3	0	.00
Beach	110	51.6	0	.00
August	100	50.9	0	.00
Virginia	1080	49.5	7	.65
Veteran	110	49.1	0	.00
Bethel	50	48.8	1	2.00
Okabena	80	48.6	0	.00
Wealthy	1140	48.2	0	.00
Patten	1060	46.3	0	.00
A. Spaneholms	130	44.8	2	1.54
Oldenburg	740	43.1	0	.00
Duchess No.4	360	42.5	1	.28
McIntosh	870	40.5	1	.11
Anisim	870	40.4	0	.00
Whitney	100	39.1	1	1.00
Florence	700	38.7	6	.86
Jewell Seedling	80	35.1	1	1.25
Breskovka	175	17.7	0	.00
Bellflower	130	13.8	0	.00

Table 6 presents data on cuttings of seventy-eight varieties which were made the first of January and the first of April and which were stored in sand in the cellar at 9.5°-11.5° C. The data of table 5 were based upon the total number of cuttings which were made of the varieties given in the table. Consequently, they were stored under varied conditions in regard to medium and temperature, and made over a wider period. Table 7 presents data on cuttings of eighteen varieties which were stored in soil over a heating tunnel at 22°-25° C.

It will be noticed that while there is great variation within a variety depending upon the conditions under which it is stored, this variation is not as great as that between different varieties. For instance, in comparing the callus indexes of varieites which were stored over the heating tunnel, table 7, with those of varieties which were stored in the cellar, table 6, a marked variation is apparent. Thus the Sweet Russet, Oldenburg and Wealthy rank high among the varieties tested over the tunnel but below the average when stored in the cellar. This variation within a variety might be explained by the quicker and more sensitive response to the increased heat of some varieties over others, or to a greater retardation of callus development under low temperatures.

However, the variation between different varieties even when stored under the same conditions, table 6, is extremely wide. Six varieties have callus indexes below twenty, while

Table 6. Variability of callus index of cuttings of seventy-eight varieties which were stored in sand in the cellar at 9.5°-11.5° C.

Variety	No. of cuttings	Callus index	Variety	No. of cuttings	Callus index
Soulard	40	100.0	Sweet Russet	20	43.0
Beach	60	92.0	Christmas	20	42.0
L.Raspberry	40	82.8	Baxter	50	41.0
Babbitt	60	81.5	Gano	30	41.0
Borovinka	60	79.0	Jewell Sdlg.	60	40.8
Charlamoff	20	79.0	Ivan	60	39.0
University	40	78.8	Boiken	40	38.8
Shield	60	77.2	Gideon	20	38.5
Antonovka	60	76.8	Fanny	20	37.5
Berlin	40	76.5	Bayfield	40	37.3
Duchess #4	20	76.0	McIntosh	50	36.6
Brier	40	75.3	Red Wing	40	36.0
A.Spancholms	60	69.0	Oalfka	60	34.8
Martha	60	66.8	Florence	40	34.5
Winter	50	64.0	White Pigeon	35	34.0
P.Prolific	20	64.0	Harry Kaump	30	34.0
Veteran	60	62.8	Jefferies	20	32.5
Aitken	40	62.5	Wealthy	50	31.2
Iowa Beauty	20	62.5	Cross	40	30.0
Gen. Grant	60	62.0	Plumb Cider	60	28.7
Alexander	60	60.2	Okabena	40	28.5
August	60	60.0	Anisim	50	28.4
Wild Crab	50	57.4	Oldenburg	40	28.3
Goodhue	15	54.0	Delicious	30	28.0
Hibernal	90	53.6	Gilbert	60	27.7
Plikanoff	35	52.0	Dolgo	60	27.3
Napoleon	60	51.2	Sdlg. #7045	60	27.3
Siberian	60	50.7	Glenton	50	26.8
Bingo	50	49.2	Sdlg. #90	40	22.0
Virginia	50	49.2	McMahon	30	21.0
Bethel	50	48.8	Breskovka	60	20.7
Sdlg. #1	40	48.3	Douglas	20	20.0
Whitney	60	48.2	Bellflower	60	18.0
Pewaukee	20	47.5	Herkimer	15	16.0
Radiant	60	45.7	Ben Davis	10	16.0
Hyslop	30	45.0	Allen Choice	60	12.0
Jewell	50	43.6	Ambo	60	11.3
Perfection	60	43.5	Broome	20	10.0
Patten	50	43.2	Iowa Blush	10	8.0

Table 7. Variability of callus index of eighteen varieties which were stored in soil over a heating tunnel at 22° - 25° C.

Variety	No. cuttings	Callus index
Lowland Raspberry	40	96.3
Sweet Russet	40	96.0
Wealthy	60	93.7
Oldenburg	40	93.5
Cross	40	91.6
Anisim	20	90.0
Hibernal	100	88.3
Duchness #4	40	85.5
Berlin	40	85.0
Brier	60	82.7
Virginia	40	80.0
Patten	40	79.3
Christmas	30	78.0
Jenkins	20	75.5
University	40	73.3
Charlamoff	40	70.6
McIntosh	20	70.3
Okabena	40	68.6

four have callus indexes above eighty. Tables 8 and 9 present the callus index of varieties of crab apples and apples. The callus index of the different varieties of apples and crab apples varies widely. But the callus index of all varieties of crab apples compares very closely to that of all varieties of large apples.

Table 8. Callus index of varieties of crab apples

Variety	No. cuttings	Callus index
Soulard	40	100.0
Sweet Russet	60	78.3
P. Prolific	20	64.0
Martha	100	63.2
Brier	220	62.0
Gen. Grant	100	58.6
Wild crab	50	57.4
Shields	320	52.8
Beach	110	51.6
Siberian	60	50.7
Virginia	1080	49.5
Hyslop	30	45.0
Whitney	100	39.1
Ivan	60	39.0
Florence	700	38.7
Gideon	20	38.5
Dolgo	60	20.7
All varieties	3130	49.3

Table 9. Callus index of varieties of apples

Variety	No. of cuttings	Callus index	Variety	No. of cuttings	Callus index
L.Raspberry	40	82.8	A.Spaneholms	130	44.8
Borovinka	60	79.0	Oldenburg	740	43.1
University	40	78.8	Jewell	50	43.6
Christmas	210	69.9	Duchess #4	360	42.5
Cross	160	67.5	Gano	30	41.0
Winter	50	64.0	McIntosh	870	40.5
Aitken	40	62.5	Baxter	50	41.0
Iowa Beauty	20	62.5	Anisim	870	40.4
Berlin	160	62.4	Boiken	40	38.8
Charlamoff	260	61.4	Fanny	20	37.5
August	60	60.0	Red Wing	40	36.0
Babbitt	100	58.0	Jewell Sdlg.	80	35.1
Antononka	120	57.3	Harry Kaump	30	34.0
Hibernal	1240	55.0	White Pigeon	35	34.0
Goodhue	15	54.0	Jefferies	20	32.5
Alexander	320	52.3	Plumb Cider	60	28.7
August	100	50.9	Delicious	30	28.0
Plikanoff	35	52.0	Gilbert	60	27.7
Veteran	110	49.1	Sdlg.#7045	60	27.3
Bethel	50	48.8	Sdlg.#90	40	22.0
Okabena	80	48.6	McMahon	30	21.0
Sdlg.#1	40	48.3	Breskovka	175	17.7
Wealthy	1140	48.2	Ben Davis	10	16.0
Pewaukee	20	47.5	Bellflower	130	13.8
Patten	1060	46.3	Iowa Blush	10	8.0
			All varieties	9500	49.0

Potassium Permanganate treatment

In regard to the potassium permanganate treatment, Curtis (7) writes, "Apple cuttings callus readily, but are very slow to root and since the treatment (with potassium permanganate) serves to keep the twigs in a healthy condition for a long time, I think it ought to be of some value." In Curtis' work the time ranged from one to two days for the limited treatment. The cuttings were then rinsed and placed in flanks or in sand. One hundred cubic centimeters of the solution was used in most cases. The treatments were administered either in tall glass cylinders, in large test tubes, in flasks or in tumblers. From his work Curtis (6) concludes that treatments with potassium permanganate may result in a very marked increase in root growth of various woody cuttings. He offers five possible explanations for this stimulation. Following his suggestions and methods a considerable number of cuttings were treated similarly to his twenty-four hour treatments.

In one experiment, table 10, the cuttings were treated for periods of eighteen, twenty-four and thirty hours in two, one, and one-half per cent solutions of potassium permanganate. The cuttings used as checks were treated in tap water. The work was done in the cellar, where the temperature was approximately 10° C. The time of collecting the cutting wood in the field, the size of the cuttings, and the method of handling up to the time of the treatment was comparable

for all varieties used. The cuttings were made just before being treated and stored immediately afterwards in soil over a heating tunnel, where the temperature was 26°-28° C. They were left here for an average period of nineteen days, when they were removed, notes taken, and planted in soil in the field on May twenty-first to twenty-third. On July third after six weeks in the field they were removed with practically one hundred per cent in a decayed condition. The cuttings were treated in battery jars with enough solution to cover the lower inch of the cuttings. A solution was used only once and the cuttings rinsed in tap water upon removal.

In all cases the treatment was decidedly detrimental to callus formation and stopped all activity. The outer layers of cells to a depth of three or four millimeters were heavily discolored and apparently killed. The callus index increased inversely with the strength of the solution used and also with the duration of the treatment. It should be noted that even the treatment of the cuttings in tap water had a detrimental effect on callusing. The data presented in table 10, show clearly these results.

Five hundred seventy (570) cuttings with varying degrees of callus formation were treated for twenty-four hours in solutions of two, one, and one-half per cent and stored over the heating tunnel for one week at 26°-28° C. The result was that no new calluses were formed and all activity stopped. Sixteen hundred seventy (1670) callused cuttings were treated

for twenty-four hours with two, one, and one-half per cent solutions and planted directly into the field with decidedly negative results as far as callus formation was studied.

Table 10. Effect of potassium permanganate on callus formation.

Treatment	18 hours		24 hours		30 hours	
	No. cuttings	Callus index	No. cuttings	Callus index	No. cuttings	Callus index
2% K Mn O4	190	5.5	180	0.0	130	0.0
1% " " "	190	14.0	180	1.1	130	0.8
.5% " " "	#190	22.3	170	6.1	130	1.5
Tap water	&180	52.6	170	14.1	130	3.9
No treatment	No. cuttings 420		Callus index 53.2			

One of these cuttings developed roots
& Four of these cuttings developed roots

Special forms of cuttings

Several minor experiments were performed on various forms of cuttings. Of the one hundred (100) mallet cuttings of Wealthy and Oldenburg the callus index was twenty-two and six-tenths (22.6). The cuttings were stored in sand in the cellar at various intervals during the winter. The only cuttings which showed a callus index worthy of mentioning were ten of the Wealthy cuttings which were made March first and whose index was fifty-five (55.0). However, when compared to the callus index of all cuttings of the Wealthy (48.2) and of the Duchess (43.1), the degree of callus

formation is considerably less; and when compared to the callus index (33.7) of eight hundred (800) cuttings which were used in the major experiment and stored in sand in the cellar the degree of callus formation is noticeably less. Further comparisons may be made by referring to table 11. Thus, as a whole, the mallet cuttings gave poorer calluses than the ordinary cuttings.

Using the Wealthy, Patten, Oldenburg and Brier varieties, five hundred seventy (570) cuttings were made into the following lengths; six, nine, twelve, fifteen and eighteen inches, with some of them cut to the second year wood (Heel cuttings). Following the suggestion made by several people, they were planted directly into the field on May thirteenth. After remaining there for two months, it was found that practically no calluses had formed. Consequently no comparison of the value of the various lengths can be made. It does, however, prove the inadvisability of trying to callus and root the cuttings under ordinary field conditions and temperatures.

Using ten varieties and a total of six hundred twenty-five (625) cuttings, one group had their lower ends split for a quarter of an inch, another group had them split twice and a third group had them split for half an inch and a small kernel of oats placed in the opening to keep it from closing. These cuttings were made and planted directly into the field. After two months the results were entirely negative, thus substantiating the conclusions of the preceding

experiment. A number of cuttings were made as checks on the preceding two experiments with analogous results.

Two hundred seventy (270) green wood cuttings of several varieties were made on the fifteenth and thirteenth of September, and were planted in sand in the greenhouse. The calluses formed were very poor in the majority of the cuttings, and only negative results were obtained. A general comparison of the results of all experiments may be found in table 11, which includes a total of sixteen thousand six hundred fifty (16,650) cuttings.

Table 11. A general summary of all experiments

Exprmt	Time stored	Storag locatn	Storag medium		Time plntd	Time dis-carded	Notes callus	No. cutngs	C'lus index	No. rooted
Gr wood		Gr ho	Sand	#1	Sept.	May 30	May 30	270	1.2	0
Major		"	"	1	Nv-Mr	"	"	400	49.5	2
"		"	Soil	1	"	"	"	400	28.8	4
"	Nv-Mr	"	Sand	1	May 3	Jun 26	May 3	400	67.8	3
"	"	"	"	2	"	"	"	400	73.6	3
"	"	"	Soil	1	May 4	"	May 4	380	71.5	2
"	"	"	"	2	"	"	"	400	68.1	0
"	"	Outsid	Sawdst	1	May 5	"	May 5	400	44.8	0
"	"	"	"	2	"	"	"	400	69.8	0
"	"	"	Sand	1	May 6	"	May 6	400	32.9	0
"	"	"	"	2	"	"	"	400	25.5	0
"	"	"	Soil	1	May 7	"	May 7	400	32.7	0
"	"	"	"	2	"	"	"	400	46.6	0
"	Nv-Jan	Cellar	Sawdst	1	"	"	"	240	30.9	3
"	"	"	"	2	"	"	"	240	21.1	2
"	Nv-Mr	"	Sand	1	May 9	"	May 9	400	29.4	0
"	"	"	"	2	"	"	"	400	37.9	1
"	"	"	Soil	1	May10	"	May10	400	28.9	0
"	"	"	"	2	"	"	"	400	31.2	0
"	Apr 2	Gr ho	So-Sa		May 4	"	Jun26	320	39.4	0
"	"	Outsid	"		May 7	"	"	320	39.5	0
"	"	Cellar	"		May 10	"	"	320	55.2	0
"	Mar 2	Field	Soil	1	May4-7	"	"	320	42.4	0
Variety	Jan 1	Cellar	Sand	2	May11	"	May11	550	52.2	6
"	Feb 1	"	"	2	"	"	"	300	57.4	0
"	Mar 1	"	"	2	"	"	"	280	59.9	0
"	Apr 1	"	"	2	"	"	"	300	40.1	0
@-2%	Ja-Mr	"	"	2	May20	Jun 30	May20	570	45.8	1
@-1%	"	"	"	2	"	"	"	490	47.8	1
@-5%	"	"	"	2	"	"	"	340	43.6	2
Check	"	"	"	2	"	"	"	270	41.2	1
@-18hrs	May 2	Tunnel	Soil	1	May21	Jul 3	May21	750	23.2	5
@-24hrs	May 1	"	"	1	"	"	"	700	5.2	0
@-30hrs	May 3	"	"	1	"	"	"	520	1.6	0
Check	May 4	"	"	1	May23	"	May23	545	42.7	0
@-24hrs	Ja-Feb	Ce-Tu	Sa-So		---	May 23	May16	570	28.3	0
Tunnel	Mar 8	Tunnel	Soil	1	---	May 10	May10	320	79.6	9
"	Apr26	"	"	1	May11	Jun 26	May11	430	88.3	0
Mallet	Dec-Mr	Cellar	Sand	2	May 7	"	May 7	100	22.6	0
Lengths	---	---	--	-	May13	Jun 30	---	570	--	-
Split	---	---	--	-	May23	"	---	625	--	-

1 means upright position, 2 means inverted position

@ indicates $KMNO_4$

ROOT FORMATION AND DEVELOPMENT

In studying the root development we find that only forty-five (45) out of sixteen thousand six hundred fifty (16,650) cuttings produced roots, or twenty-seven hundredths of one per cent (.27%). This percentage is so small that it is insignificant and definite conclusions based upon such scanty evidence are hardly tentable. It is possible, however, to point out some results that are at least suggestive and the negative nature of the data is a contribution to the present knowledge of the subject.

Seasonal effects

In studying the effect of the time of making the cuttings upon root development, we find little variation. Of the forty-five cuttings which developed roots five were made on November first, six on December first, twelve on January first, six on February first, none on April first and five on May first. Considering the fact that a greater number of cuttings were made during the winter, there is little significant seasonal variation in the rooting of the cuttings. The fact that five of the November first cuttings and five of the May first cuttings which had been gathered during the winter and stored in the cellar as cion wood until they were made into cuttings developed roots, indicates that the time of gathering and marking the cuttings does not have as great an influence upon the rooting as upon the callusing of the cutting.

Effect of inverted position and storage mediums

In comparing the rooting of the cuttings, which were stored or planted in different mediums with other factors similar, it is to be noted, table 11, that in the greenhouse bench two out of four hundred (400) cuttings rooted in sand and four out of four hundred (400) cuttings rooted in soil. Of the cuttings which were stored in the greenhouse and cellar seven out of eight hundred (800) rooted in sand, two out of eight hundred (800) rooted in soil and five out of four hundred eighty (480) rooted in sawdust. From this, it would seem that the value of the different mediums would be graded thus: First sawdust, second sand and third soil. The value of sawdust as a storage medium at comparatively low temperatures becomes more apparent.

In regard to the position in which the cuttings were stored, we find that out of fourteen cuttings, which developed roots under similar conditions other than position, eight which were stored in the upright position and six which were stored in the inverted position developed roots. This would indicate that there was little difference in the two positions with a slight advantage, if any, in favor of the upright position. This comparison is based upon equal numbers, but the proportion rooting is too small to be of great value.

Effect of temperature

In studying the effect upon root development of location and temperature a more direct influence is found, although

there are decrepancies. Table 12 presents data upon this comparison. It is well to note that of twenty-four hundred (2,400) cuttings which were stored outside none developed roots; twenty hundred eighty (2080) cuttings which were stored in the cellar six developed roots; and of twenty-three hundred eighty (2,380) cuttings which were stored or planted in the greenhouse fourteen developed roots, which shows the advantage of maintaining a higher temperature than that of the cellar and field.

Table 12. Root development as affected by temperature

Temperature	Storage location	No. cuttings	No. rooted	Per cent rooted
Variable 0°-22° C	Outside	2400	0	.00
9.5°-11.5°C	Cellar	2080	6	.29
17°-18° C	Greenhouse	2380	14	.59
22°-25° C	Heating tunnel	320	9	2.81
#26°-28° C	Heating tunnel	430	0	.00

Period of storage only two weeks

With a still higher temperature, which was procured by storing the cuttings over a heating tunnel, the number of cuttings which developed roots was still greater. Of three hundred twenty (320) cuttings, which were stored here for two months at 22°-25° C., nine developed roots. While those

which were stored here for two weeks at the same temperature showed no root development, they undoubtedly would have if they had been left longer. While the relation between root development and temperature is not very clear or specific, it is quite evident that higher temperatures favor root development up to twenty-five degrees centigrade, above which it apparently retards their development.

Potassium permanganate treatments

In studying the effect of the potassium permanganate treatments, it will be noted in table 11, that of fourteen hundred (1,400) cuttings, which were made and stored in sand in the cellar during the winter, and which were treated just before they were planted in the field for twenty-four hours, four developed roots. Of one hundred ninety (190) cuttings (table 10), which were treated for eighteen hours with a five-tenths of one per cent solution of potassium permanganate and stored for three weeks over the heating tunnel at from 26°-28° C., one developed roots. The important point disclosed here is that while the potassium permanganate treatment materially lessened callus formation, it did not totally prevent the formation of roots. However, from the results obtained the value of the treatment seems rather doubtful.

Varietal variability

In looking over the summary in table 13, of the different varieties which developed roots, it will be noticed that the varietal variability is very marked. Of the seventy-eight

varieties used, only twelve showed root development and within these the variation was great. The variety Brier, a sweet crab, showed distinctly the greatest tendency to develop roots.

Table 13. Varieties which formed roots

Variety	No. cuttings	No. rooted	Per cent
Hibernal	1240	7	.57
Virginia	1080	7	.65
McIntosh	870	1	.11
Florence	700	6	.86
Duchess #4	360	1	.28
Brier	220	13	5.91
A. Spaneholms	130	2	1.54
Antonovka	120	3	2.50
Babbitt	100	2	2.00
Whitney	100	1	1.00
Jewell Seedling	80	1	1.25
Bethel	50	1	2.00

Antonovka, Babbitt and Astrachan Spaneholms, all Russian varieties, showed a comparatively favorable tendency to root; and Whitney, Bethel and a Jewell seedling each produced one cutting which showed root development. The six varieties just mentioned were used in relatively small quantities, so that the per cent secured may not be as representative as it would have been if larger numbers had been used. McIntosh and Duchess Number 4, another Russian variety in the Minnesota experimental orchard which greatly resembles the Oldenburg each produced one rooted cutting. The Florence, Virginia and Hibernal which were all used in comparatively large numbers in the major experiment produced six, seven and seven

rooted cuttings respectively. From these results one would gather that crab apples show a greater tendency to develop roots than do varieties of the common apple, and that varieties of Russian origin show a greater tendency than do those of European and American origin. Some nurserymen are of the opinion that varieties of sweet apples will root more readily from cuttings than will subacid varieties. But this belief was not substantiated by these experiments.

Relation between callus and root formation

In comparing the root development with the callus formation by referring to tables 5-9, and others it will be noted that there is little correlation. Referring to table 8, we find that among the varieties which developed roots Brier ranks fourth in callus formation of the crab apple varieties, while Virginia and Whitney rank below the average with Florence well toward the lowest callus index. And by referring to table 5, we find that those varieties which gave the best callus indexes are not necessarily those which showed the greatest tendency to develop roots. It seems that the ability of a variety to callus readily should not be taken as an index of its ability to develop roots. Shaw (27) points out that in his work there was apparently no correlation between callus formation and root development.

The formation of roots

In regard to the manner in which the roots were produced we find that only four of the forty-five cuttings, which pro-

duced roots, developed them from the base of the lower bud. The remaining forty-one developed them at the base of the cutting through the callus. In every case the new root was pushed out from the growing cells beneath the callus or bark, splitting it in a mechanical manner. Forty-four of the cuttings produced their roots at right angles to the cutting, and only one went directly down.

Sachs (25) describes this process thus, "When the young roots begin to grow, they must first break thru its cortical tissue (bark) as may easily be observed in fresh roots; the lateral roots of these protrude from slits, the edges of which are often raised up in a lip-like manner." Corbett (5) after six years of observations on the callusing and rooting of various species concludes that, "It is not from the callus, that the roots formed on cuttings take their origin. In fact the callus has no connection with, and is not necessarily concerned in the origin or development of roots from cuttings", and that, "Roots grow from a fundament formed in the tissue of a portion of a plant used as a cutting, in a manner precisely similar to that of the development of new roots from a root on the plant axis of a seedling." He considers callusing to be only a healing process.

Figure 2 shows a rooted cutting of the variety Virginia. This cutting was planted in soil in the greenhouse on December fourth, 1920 and photographed on May thirty-first, 1921.

It illustrates the fiberous and much branched root system, and also a newly formed white root at the left. These roots are very tender at first and extreme care must be exercised in transplanting or they will be broken. Figure 3 shows more clearly the numerous white newly formed roots of the same cutting.

CONCLUSION

The fact that hardwood cuttings of the common apple callus readily but root with difficulty under ordinary commercial practices was further substantiated by the results of these experiments.

The degree of callus formation varied widely with the different varieties used, with the temperature of the medium and with the time of making the cuttings. Varietal variability was very marked. Other factors had less effect.

The per cent of cuttings which developed roots was so small (.27%) that accurate comparisons of the different factors are impossible. The cuttings rooted under a variety of conditions so that no definite explanation can be offered for the rooting of a few cuttings and the failure of the great majority. There was, however, marked varietal variability. Higher temperatures appeared to favor root development.

Cuttings of various lengths gave decidedly negative results under field conditions and failed to substantiate the accounts by Gould and Shaw (page7). However, in the writer's experiments the cuttings were not planted until May.

More favorable results might have been secured, if earlier plantings had been made.

The treatment of cuttings with potassium permanganate checked callus formation but did not prevent root development. Since there appears to be no correlation between root development and callus formation, and since the treatment tends to preserve the cuttings, the treatment might prove to be of value in encouraging root development. The results of this work indicate that the treatment is of doubtful value. Further experiments may prove otherwise, however.

The results of this investigation further substantiate the fact that the common apple may be propagated by hardwood cuttings. An intensive study of individual factors and specific treatments might bring to light a method of treatment which would be of experimental if not of commercial value.

Since the field for study upon this problem is so large, I believe that further study should be confined to specific physiological factors. If I were given the opportunity to continue the study of this problem, I would prefer to investigate further varietal variability, the effect of temperature and special treatments.

Since we know that hardwood cuttings of the common apple will occasionally root, I believe that further study of the various physiological factors will bring to light a method of handling the cuttings so that roots will be more readily

developed and make commercial propagation by this method possible.

SUMMARY

Cuttings which were made during the later part of the winter callused more readily than did those which were made during the fall or early spring.

Sawdust proved to be a better storage medium than either soil or sand where low temperatures prevailed over long periods. It must be free of fungus diseases if used at 10° C. or above. There was little difference between soil and sand as storage mediums.

Cuttings which were stored in the inverted position produced better calluses than those which were stored in the upright position. This advantage was largely due to the higher temperature which existed in the upper part of the mediums.

Temperature proved to be an important factor in governing callus formation which varied directly with the temperature. The cuttings, however, lost vitality when stored at 26° C. or above.

Varieties vary greatly in their ability to produce callus tissue on hardwood cuttings. There was also great variation within the varieties, encouraged, perhaps, by the varied conditions under which the cuttings were stored.

Treatment with potassium permanganate proved decidedly detrimental to callus formation which varied inversely with

the strength of the solution used and with the duration of the treatment.

Mallet cuttings yielded poorer callus formations than ordinary cuttings. Cuttings of various lengths which were planted directly into the field in the spring formed little or no callus tissue.

Hardwood cuttings of the apple rarely rooted. Only forty-five out of sixteen thousand six hundred fifty (16,650) cuttings developed roots in these experiments.

There was no seasonal advantage exhibited in the cuttings which developed roots.

Sawdust proved to be more favorable for the development of roots than either soil or sand which were of nearly equal value.

Little difference was exhibited between the inverted and upright positions in the storage mediums.

The rooting of the cuttings is loosely correlated with temperature. The higher temperatures favored root development.

Varieties varied greatly in the production of roots. Cuttings of twelve varieties out of the seventy-eight used showed root development.

The potassium permanganate treatment did not prevent rooting; but the treatment appears to be of doubtful value.

There was no correlation between the formation of callus tissue and the development of roots.

In the majority of cases the roots were produced from beneath the callus and at right angles to the cutting.

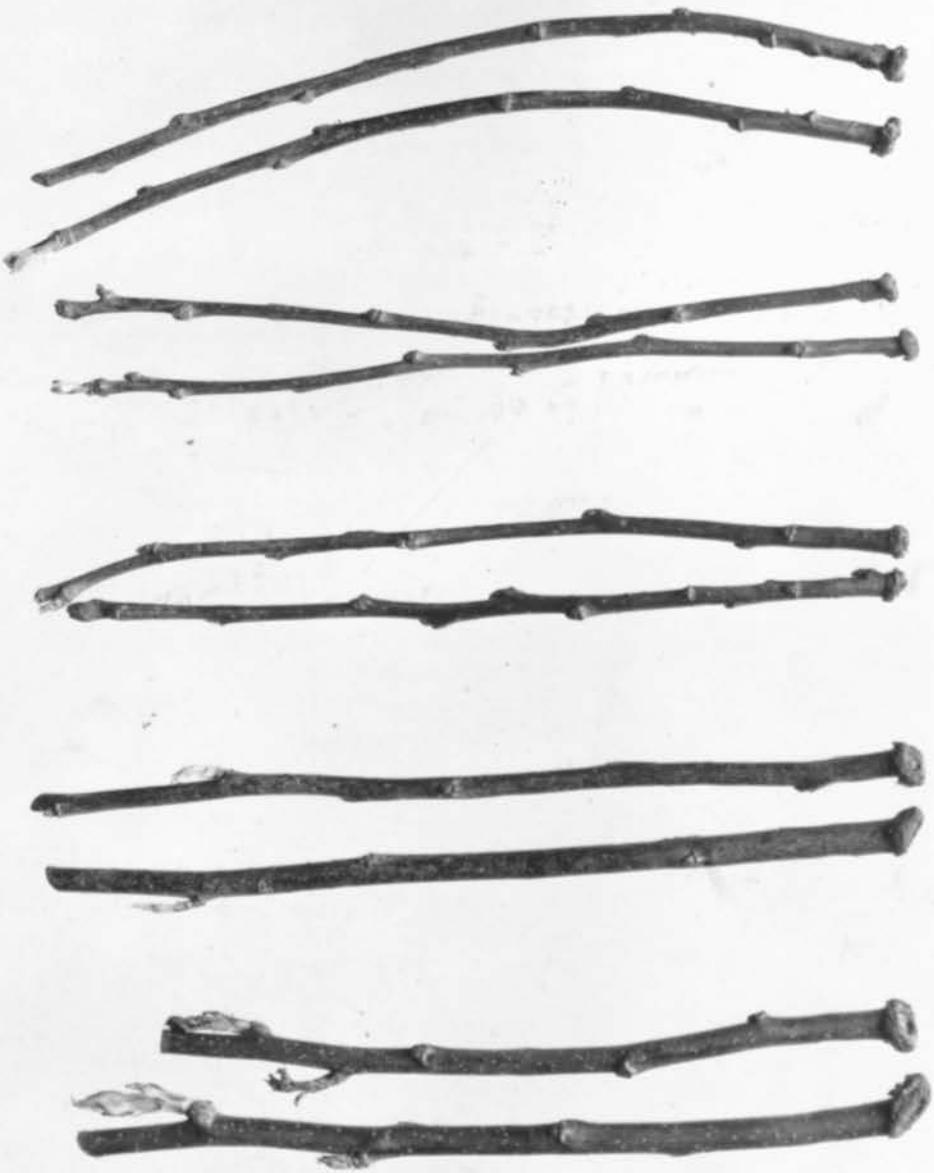

Fig. 1. Well callused cuttings of five varieties, from left to right, Wealthy, Virginia, Hibernal, Berlin and Cross.

Fig. 2. A rooted cutting of the variety Virginia.

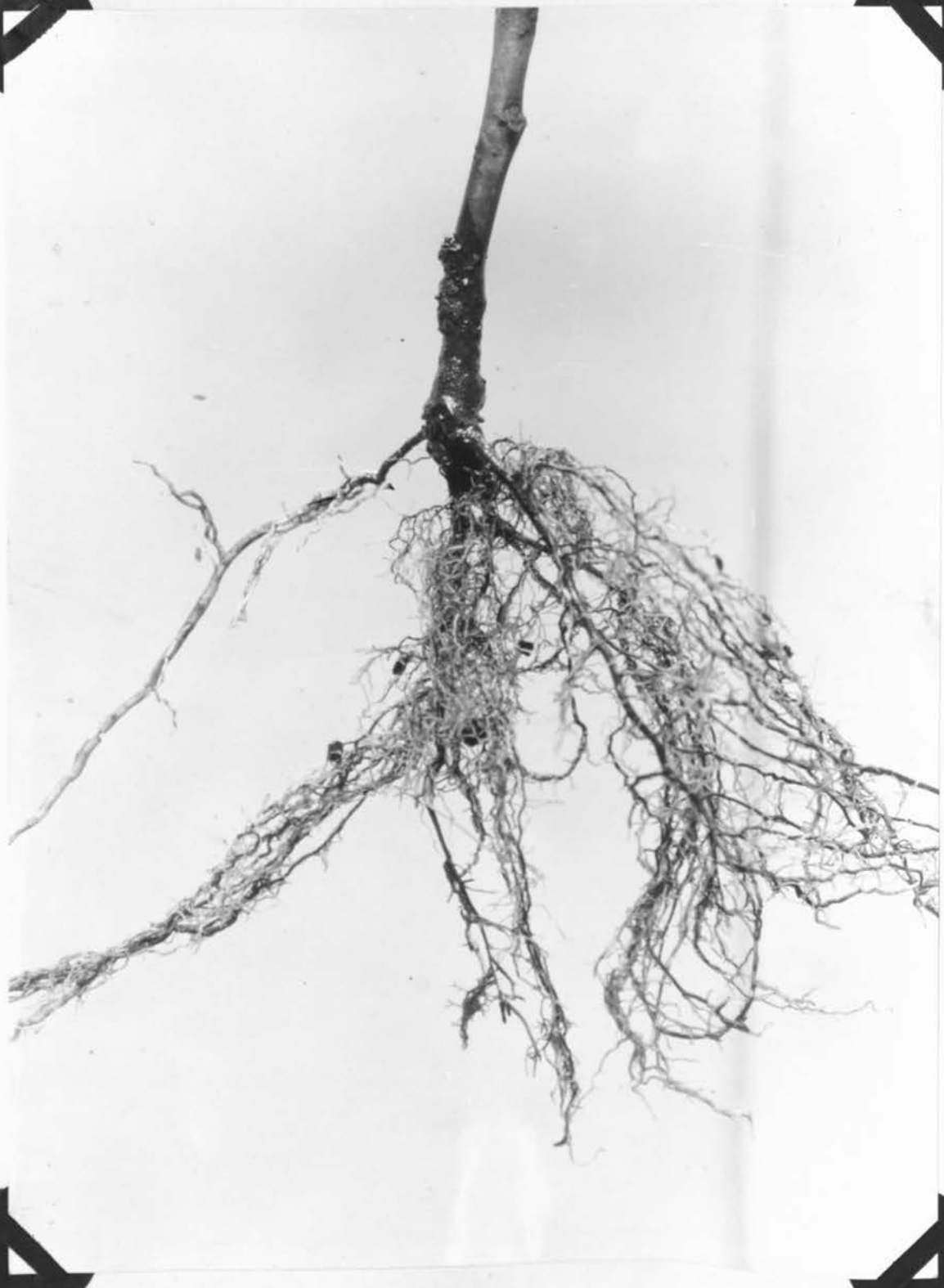

Fig. 3. A closer view of the root system of the cutting illustrated in Fig. 2.

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