STUDIES OF APPLE GRAFT UNIONS

by

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TABLE OF CONTENTS

INTRODUCTION

Since the severe freeze of November 11, 1940 damaged many of the fruit trees of north and central Kansas, the selection of hardy apple stocks has become one of importance. It was made evident at that time that commercial apple varieties should be topworked to hardy stocks in order to prevent a recurrence of cold unjury. Resistance to the cold was exhibited by the known hardy stocks of Virginia Crab and Hibernal. However, these varieties of stocks do not have the ideal characteristics required for Kansas conditions. This led to the selection of the K-series, a group of French Crab seedlings which exhibited cold hardiness by living through the 1940 freeze. These seedlings were transplanted in the nursery row and propagated vegetatively until enough plants were available to plant into the field.

After two years growth in the field, the seedlings were topworked to Jonared and Winesap. A modified whip graft was used in grafting. These trees were then allowed to grow and observations were made on their growth characteristics, climatic adaptation, insect and disease resistance, precosity of bearing, and compatibility of stock and scion.

As the trees grew older, symptoms of a possible incompatibility began to appear at the graft union of many of the combinations. The probability of defective grafting was eliminated by the consistency of certain stocks to be associated with abnormal unions.

The most common abnormality was that of the stock outgrowing the scion, and in only one combination did the scion outgrow the stock. The resulting tree growth was somewhat slower with the abnormal graft unions, indicating a dwarfing reaction caused by the incompatibility.

The purpose of this study was to make anatomical studies on graft unions of the K-series combined with either Jonared or Winesap, and determine if anatomical characteristics can be associated with the cause of incompatibility. Studies were also made on the possibility of the abnormality being a weakness in the framework of the tree, and on young grafts to see if characteristics of abnormality would show up at early stages for use in selecting stocks for the future.

LITERATURE REVIEW

The anatomy of the graft union has been under discussion for a number of years, due primarily to the characteristics of growth influences realized from certain grafted combinations.

These characteristics, plus the curiosity of how the stock and scion were knitted together were of interest to men long before the advent of equipment capable of seeing the microstructure.

Bradford and Sitton (1929) referred to Duhamel (1758), Goeppert (1841) and Sorauer (1875) in reporting the historical background of the study of graft unions. Duhamel made observations with a magnifying glass and outlined in a general way some of the processes involved in the graft union. He noted the extensive development of callus tissues filling the vacancies incident to grafting and connecting the stock and scion. Goeppert established the existence of a parenchymatous union between the stock and scion and reported that in the second year, the union was completed through cambium continuity. Traces of union tissue persisted in tangential section long after they had become indistinguishable in transverse section.

One of the early questions was the source of this parenchymatous or callus tissue which aided in the formation of the union. Goeppert considered this parenchyma to arise from the xylem rays while others of that day considered it to arise solely from the cambium.

The cambial theory continued unabated until Sorauer showed that any tissue capable of forming parenchyma could assist in establishing union. In describing the method of uniting, he said that the actual contact of cambium layers is not essential; that in practice it is rarely achieved. It is the contact of cambium derived callus that is important. He proceeded to divide the kinds of grafting into groups based on the method of establishing union.

Sharples and Gunnery (1932) reported the formation of callus had its derivation in the medullary ray system in the Rosa sinensis L. and Hevea brasilienses. An extension of these rays as an origin of callus tissue would indicate that the phloem rays are the source of callus initiation.

Sass (1932) said that callus may be derived largely from the primary cortex of the scion, or from the secondary phloem of the stock or scion. He stated further that the tissues of the bark may contribute variable portions of the total callus, but that the periderm does not seem to produce any callus. Other workers have reported on the origin of callus, but the derivation given by Sass is generally accepted as correct.

The principal source of the callus tissue is the scion, although both the stock and scion contribute variable portions. Kostoff (1928) reported that the ability of the stock to take up water enabled it to furnish the initial joining tissue. Following this union, the accumulation of elaborated food above the union gives the scion a greater capacity for growth. Shippy (1930) found that most of the callus tissue could be attributed to the scion.

The bridgingby cambium of tissues formed subsequent to grafting is accomplished shortly after the laying down of callus tissue. Bradford and Sitton (1924 reported that Herse (1908) observed that in a well-matched graft a new cambium appears, bridging the intervening callus between members in 10 weeks. Other evidence indicates that the time of cambial bridge formation is dependent upon the callus produced. Sass (1932) and Sharples and Gunnery (1933) cite that there is no sign of cambial activity until the callus cushion is completely laid down. It appears that the initiation of this cambium is at the point of contact with the cambium of either the stock or scion.

In summarizing the formation of a normal graft union, Argyles (1937) said that the callus production is initiated in cells which have remained meristematic, but is later assisted through a resumption of the embryonic state by mature tissues. Where there is no unobstructed contact between respective callus of stock and scion, the conditions favor a mingling of parenchumatous cells and continued proliferation of mixed callus. He goes on to say that the amount of callus produced by each member varies with the conditions prevailing. Following the intermingling of callus as reported by Waugh (1904), Proebsting (1926), and Sass (1932), cambial continuity is developed by differentiated callus cells. The completed cambial layer subsequently lays down a vascular cylinder.

With many abnormal graft unions developing from certain combinations of stocks and scions, studies were directed toward the cause of such abnormalities. These abnormal unions were characterized by a large mass of callus or a gall at the union or by the unusual increase in size of the stock over the scion or of the scion over the stock. These characteristics were frequently associated with weakness at the point of contact.

The report of Ohmann (1908) as described by Bradford and Sitton (1929), was the first in the study of abnormal graft unions. He stated that when the calli of completely uncongenial plants meet, periderm develops in the callus and closes all communications between the stock and the scion. He gave no cause for the formation of the peridermal layer. Waugh (1904) closely followed Ohmann. He studied the unions after several growing seasons and observed that the wood of the original stock and scion never unite. The new wood that forms is in layers, visible to the naked eye, but indistinguishable under the microscope. He believed as did Ohmann, that the cambium zone was the origin of the callus, and that it frequently produced so much callus that it prevented the formation of continuous layers of wood and renders the union mechanically weak.

Proebsting (1926) is in agreement with Waugh in that the large amount of parenchymatous material causes a severe distortion of vessels lying in the region of the union resulting in a discontinuity of the xylem. He also found a bark layer extending to the point at which the cambium layers of the stock and scion were placed in contact, indicating that it forms a line of mechanical weakness.

Bradford and Sitton (1929) indicate that a swelling at the union does not necessarily indicate incompatibility, but is very likely to be caused by defective grafting methods or conditions. They believe that some contact between the cambial zones of the stock and scion are necessary and that sufficient pressure needs to be applied to attain a complete graft.

Riker and Keitt (1926) and Riker, Keitt, Hildebrand, and Banfield (1934) found that poor workmanship causes excessive callus formation but that it may be reduced by proper care in grafting, storage, and care in the field. It was found that a tight wrapping with a tape strong enough to hold during callusing but rotting shortly after, would reduce excessive callus formation in most combinations. This method of control failed to restrain the callus formation in some grafts and after further research, they concluded that there were genetic differences between the stock and scion.

Sass (1932) believes that in addition to poor mechanics in grafting, the conditions surrounding the work also influences the proper union. He said that the early formation of a necrotic condition in the outer layer of callus cells is due to mechanical injury or unsanitary conditions. This is very similar to a peridermal layer in that it prevents the intermingling of the callus cells.

In observing poorly matched grafts in which some union occurred, Crafts (1934) noted that the vascular strands which are differentiated from the callus parenchyma are long and crooked, resulting in various patterns of cambial initials. This is in contrast to the short and straight strands produced in as few as five days in well-matched grafts, resulting in normal cambium formation. Sass indicated that these crooked vascular strands mentioned by Crafts merely twist around and are discontinuous.

Distortion or disruption of vascular continuity may affect the translocation of elaborated food material down the plant. This was mentioned by Riker and Keitt (1926) and Shippy (1930). It is believed, however, that their material was the reverse of that used in this problem, that is the overgrowth of stock by the scion rather than the scion by the stock.

Closely associated with the overgrowth of one or the other element is the sharp angle of the vascular tissue in the region of the graft union. Bradford and Sitton (1929) have noted this condition and concluded that this degree of distortion has no apparent influence on the life of the tree and its behavior. Proebsting (1928) found that when the distortion reaches the degree of forming whorls and loops, and showing vessels in both the longitudinal and transverse section within the space of a millimeter and when the continuity of the vascular system appears to be definitely broken by these contortions, it is difficult to believe that there is not a functional disturbance as well as affecting the life of the tree.

There is some indication that a parenchymatous accumulation at the line of union causes growth abnormalities. Waugh (1904) and Proebsting (1926) noted this in their early studies.

Chang (1937) reported parenchymatous cell accumulations to be correlated with mechanical weakness of the graft unions of pears and plums. He found abnormal cell accumulations and enlargements present ten weeks after grafting. Although there is some accumulation of parenchymatous cells in a normal union, they possess regular strands of medullary ray cells across the union after four weeks.

Growth enlargement shortly after grafting was found also by Herrero (1951). However, he noted that in compatible combinations, the second years growth was normal and showed no variation in the amount of new growth.

Only a few investigations have been made on the anatomy of the stock and scion for the purpose of correlating growth characteristics and compatibility. Although they concluded that most abnormal grafts are due to defective grafting, Bradford and Sitton (1929) suggested that uncongenial unions may be due to differences in the size of the cambial cells of the stock and scion.

Adapted techniques for studying the histological characteristics of the graft union were devised by Beakbane and Thompson (1939). This method consists of photomicrographing the material and using the photomicrographs to determine relative areas of each histological character.

This method was used by Herrero (1951) to study nine histological characteristics in transverse sections of different parts of stems and roots of peaches, plums, and pears. Although differences in histological structure between the components of the grafted trees were found in many combinations, they could not be correlated with the degree of compatibility of varieties grafted. His conclusions were that although other histological characteristics may possibly correlate with incompatibility, the negative results obtained on the nine studied probably indicate that the basic causes of incompatibility are not due to differences on the structure of the varieties grafted.

From these studies it is observed that the method of union of stock and scion has been determined.. However, the cause of abnormal graft unions has not been satisfactorily explained. Amatomical study is one of the possibilities in solving this problem and more research is needed in this field.

MATERIALS AND METHODS

Grafting Study

A study of the success in grafting of congenial and uncongenial combinations was made both in the greenhouse and in the field. The soil was essentially the same in that the soil for the greenhouse bench was secured from the horticulture farm near the growing trees. Soil tests did not reveal any mineral deficiencies.

Greenhouse facilities were used to hasten the procurement of material for laboratory research. A total of 97 stocks consisting of 10 varieties of the K-series were used. Of these 10 varieties, K1, K22, K24, and K40 were considered congenial; K5, K21, K23, K28, K29, and K43 were considered uncongenial.

One-year old stocks had their dormant period broken by leaving them in the field until January 4. They were dug on that date and placed in cold storage until planting in the greenhouse on January 12. All tops were cut back to 8-10 inches and all roots were left on. Watering was done daily to provide optimum growing conditions.

Grafting was begun when the stocks showed signs of growth in the vegetative buds, The first grafts were made on February 14 and continued at intervals of one week until all stocks were grafted. Forty-nine stocks were grafted with Jonared and 48 stocks were grafted to Winesap. The last grafts were made on March 10.

Grafting technique consisted of a whip graft followed by wrapping with rubber strips, and thoroughly waxing the union and the entire scion. The same procedure was used in making the original grafts in the orchard in 1945. In stocks having two or more main branches, each was used for grafting to increase the number of graft unions available for study.

A seven week period for observing the callusing and growing together of the stock and scion was allowed before cutting for laboratory study.

Grafts were also made on nursery stock left in the field at the horticulture farm. This consisted of five varieties of stocks, eight plants of each and one-half grafted to Jonared and one-half grafted to Winesap. Grafting was done on April 23 and May 7, at which time the stocks showed signs of vegetative growth. The method of grafting was the same as that used before. Cutting of the graft unions was done seven weeks after grafting.

A similar group of seven-year old trees in the orchard were grafted but injury and breakage by farm implements resulted in a low percentage of success.

Results of the grafting study were broken into two categories. Combinations showing good scion growth and normal unions were considered to be successful. Both failure to grow and failure after growth initiation occurred were considered to be unsuccessful.

Microtechnique and Microstructure Studies

A study of the microstructure of the greenhouse and field graft unions and segments of graft unions from nine-year old trees was made. The graft unions secured from the grafting experiment were used as seven-week old graft unions and the older material was secured from growing trees in the horticulture farm orchard.

Treatment of the seven-week old graft unions consisted of cutting and removal to the laboratory for trimming to the desired size (Plate I, Fig. 1). They were then placed in F.A.A. for one week for killing and fixing of tissues. This was followed by softening in 50 percent hydrofluoric acid for two weeks, washing with water for two days, and placing in a solution of glycerin and 95 percent ethyl alcohol for storage until further research could be done.

Research was resumed in January, 1952, at which time the graft unions were removed from the glycerin-alcohol solution and dehydrated with Cello-solve (Ethylene glycol monoethyl ether acetate). This was followed by embedding in celloidon with Cellosolve as a solvent. A period of 48 hours at 53° C. in each of 2, 4, 6, 8, 10, and 12 percent concentrations was allowed. While in the 12 percent concentration, the material was allowed to cool to room temperature and then mounted on wooden blocks. The celloidon was hardened in chloroform.

This material was stored in glycerin and alcohol until sectioning.

Sectioning was accomplished with a sliding microtome, making sections of 15-20 microns in both tangential (Plate 1, Fig. 2) and transverse areas. The sections proved to be too fragile to handle by the "freehand method" as recommended by Sass (1940). Haupt's adhesive was used to hold the sections to the slide during the staining process. Hematoxylin and safranin were used as staining compounds. The celloidon matrix was dissolved out during the dehydration process by a 50 percent solution of ether and absolute ethyl alcohol. Mounting in balsam was the final step.

Slides of tangential sections were studied to determine the methods of knitting together the stock and scion and photomicrographs were taken to record the steps in vascularization through the graft union. It was hoped that variation in knitting structure between congenial combinations and uncongenial combinations could be seen.

Transverse sections were used to get a clearer picture of the growth pattern of the union of a stock and scion. It was this type of material which has been studied by most investigators. However, there is no record of counts being made of a histological character similar to that made in this study.

Numerical counts of the number of vessels in a unit area were taken. This was accomplished by using a binocular microscope equipped with an ocular micrometer. Magnification of 440 times and an area of 57.058 square microns was used. Two fields were counted on one-year old wood of the stock and one field on one-year old wood of the scion. The amount of area available for counting was small and there was little chance for randomization of fields. The results were combined with those obtained from sections from seven-year old graft unions and subjected to statistical analysis.

Segments of graft unions from the orchard were from trees that were planted in 1943 and grafted in 1945. The modified whip graft previously described was used to topwork the trees to Jonared and Winesap. Six to eight trees of each variety of stock were available for use, of which one-half were topworked to Jonared and one-half were topworked to Winesap. Each tree had five to eight graft unions.

In order not to sacrifice the trees for this experiment, small wedge-shaped pieces were removed from each graft union. This was accomplished by using a four inch circular saw on a flexible shaft which was driven by a one and one-half horsepower gasoline engine (Plate II, Fig. 3). Two vertical cuts three-fourths inch apart, three to four inches long, and one inch deep were made (Plate II, Fig. 4) and this piece was removed with a hammer and wood chisel (Plate III, Fig. 6). These wedges were placed in cellophane bags until they were removed to the laboratory.

A fine-toothed band saw was used to trim the wedges into segments approximately one and one-fourth inches long and from one-fourth to three-eights inches thick. Care was taken to get areas of both the stock and scion on the same segment.

In addition to these segments, some material was secured from graft unions which were removed from the trees in their entirety. They were sawed in half (Plate IV) and then subdivided into segments similar in size and shape of those mentioned above.

Killing and fixing of the living tissues was accomplished by immersing in F. A. A. solution for five days, followed by a two-week softening process in 50 percent hydrofluoric acid. Following the softening process, the segments were washed in running water for 48 hours to remove the acid.

A sliding microtome was used to make tangential, radial, and transverse sections from these segments. The tangential and radial sections were cut from the area of two-year old wood. These were handled as freehand sections, stained with safranin and fast green, and mounted in balsam. The transverse sections were made from one and two-year old wood. Instead of treating as freehand sections, they were held on the slide with Haupt's adhesive and then stained and mounted similarly to the tangential and radial sections.

Observations were made on the tangential sections with a binocular microscope at 25 magnifications, at which power the entire section could be seen at one time, and yet the arrangement of the vessels, tracheids, and rays could be ascertained. All slides were subjected to this analysis in search for any unusual graft combination.

The same slides were used to study more critically the histological characteristics of the graft union. Magnification of 100, 440, and 900 times were used to determine if possible, the line of division between stock and scion cells and also study any abnormal histological character that may be present.

Magnification of 100 times was used to make counts of the number of rays present in 1040.4 square microns. Counts were made on both the stock and scion end of the slide. Two areas on the stock end were selected by beginning 1.04 millimeters from the end (determined by an eyepiece micrometer), and taking the second count at 4.16 millimeters from the end. If the two counts differed materially, a third count was taken 3.12 millimeters from the end. One count 1.04 millimeters from the end was made on the scion. These counts were used as a basis for comparing the structure of congenial and uncongenial stocks.

Counts were made on the number of vessels per unit area in transverse sections. The magnification and field used was the same as that used in the transverse sections of the seven-week old graft unions. The areas counted were one-year old wood and three counts were made on each slide. The results here were combined with those obtained previously.

It was anticipated that the counting of histological characteristics would be easier from photomicrographs and many pictures were taken. However, microscope counting proved successful and the photomicrographs served as a basis for comparing structures and also to record the microstructure of the graft unions.

EXPLANATION OF PLATE I

Fig. 1. View of seven-week old graft union after preparatory trimming for analytical work. New bark has formed over the callus-filled areas. 4x

Fig. 2. View of a seven-week old graft union following tangential sectioning. Note the complete callusing within the graft and the bridging of callus over the stock and scion lip. 4x

Plate I

Fig. 1   4x

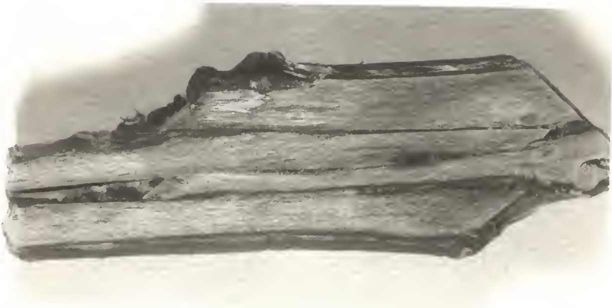


Fig. 2   4x

EXPLANATION OF PLATE II

Fig. 3. Circular saw on flexible shaft driven by one and one-half horsepower engine. The wheelbarrow served as a method of transporting the unit from tree to tree.

Fig. 4. Method of cutting into graft union prior to removal of the section.

Plate II

Fig. 3

Fig. 4

EXPLANATION OF PLATE III

Fig. 5. Method of sawing into graft union. Two vertical cuts were made side by side in this manner and chiseled out.

Fig. 6. Method of removing the section after sawing. A hammer and wood chisel were used to cut the ends of the section.

Plate III

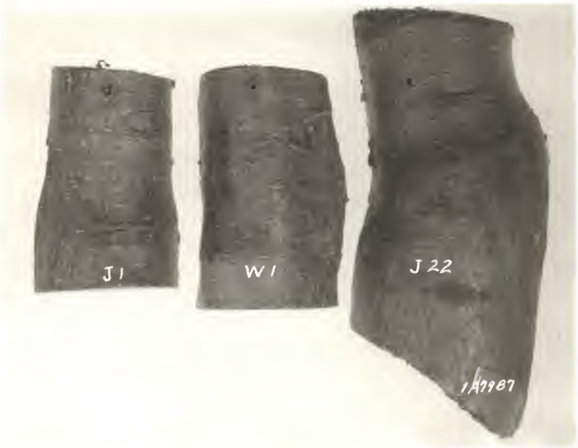
Fig. 5

Fig. 6

EXPLANATION OF PLATE IV

Congenenial graft unions prior to division for microtechnique. Note the smoothness of union, especially in J1 and W1.

Plate IV



RESULTS AND DISCUSSIONS

Grafting Study

Grafts made during the experiment were for observation and analytical work. There were daily observations on the development of the grafts made in the greenhouse and weekly observations of the grafts made at the horticulture farm.

New shoot growth was considered to be the result of union of the stock and scion. However, some of the greenhouse grafts exhibited this characteristic, only to die after two or three weeks. There were no apparent trends of this characteristic in either the congenial or uncongenial stocks and the cause was probably due to weak rootstocks. This condition was not observed under field conditions, where all but one graft was successful.

At the time of cutting the graft unions for laboratory work, final observations were made and the combinations were classified as successful or not successful. The results are presented in Table 1.

In analyzing the results of the congenial stocks by the Chi squared test (Table 2), it was found that a probability of greater than 20 percent existed. Since anything greater than five percent can be considered non-significant, it was assumed that there were no significant differences in grafting success between the congenial stocks.

Table 1. Grafting success in the greenhouse.

	Successful	Not successful
	Congenial combination	
J1	4	1
W1	4	0
J22	6	0
W22	5	1
J24	3	0
W24	3	0
J40	1	1
W40	5	2
Total	31	5
	Uncongenial combination	
J5	5	4
W5	6	0
J21	1	2
W21	3	1
J23	2	0
W23	0	4
J28	2	0
W28	2	2
J29	5	2
W29	6	0
J43	4	4
W43	6	0
Total	42	19

Table 2. Test for stock differences as regards successfulness of grafts for con enial stocks.

Stock	Successful	Not successful
1	8	1
22	11	1
24	6	0
40	6	3
Total	31	5

χ^2 equals 4.25 for three degrees of freedom
$P > .20$

Uncongenial stocks analyzed for differences between stocks resulted in no significant differences between stocks (Table 3). The Chi squared test gave a probability of greater than 30 percent, which indicates that there is no difference in grafting success between the uncongenial stocks.

Table 3. Test of stock differences as regards successfulness of grafts for uncongenial stocks.

Stock	Successful	Not successful
5	11	4
21	4	3
23	2	4
28	4	2
29	11	2
43	10	4
Total	42	19

χ^2 equals 5.43: five degr es of freedom.
$P > .30$

In applying the Chi squared test to the totals of Tables 2 and 3, one degree of freedom gave χ^2 to equal 3.61 or a probability of six percent. While this is approaching the level of significance, it cannot be so considered because of the small expected number.

From the above discussion, it can be concluded that there are no significant differences within or between congenial or uncongenial stocks, as regards success in grafting.

Microstructure Studies

Comparative studies were made possible through the use of photomicrographs. Various stages of callusing and vascularization were present in sections from both congenial and uncongenial stocks. A series of photomicrographs recorded the progressive steps in the vascularization process.

Plate V, Figs. 7-10 presents sections from the congenial graft unions beginning with callus formation and continuing through complete vascularization. There is a regular continuity of the vascular elements, especially in Fig. 10. If this were continued through to a mature graft union, it would be found that there would be little or no distortion in this stage. The slight angle at which the vascularization occurs is normal, being a result of the horizontal as well as the vertical transfer of food materials at the point of union.

Distortion and irregularity are presented in developmental stages in Plate VI, Figs. 11-14. Evidently the distortion begins immediately after callusing is completed (Figs. 11, 12) and continues throughout the vascularization process. At the seven-week stage (Fig. 14), the process is nearly complete, with the presence of twisting and curling of the xylem elements.

Comparative studies of Plates V and VI indicate some difference in the relative smoothness and completeness of the vascularizations process. This distortion early in the stage of the graft union is one of the chief components of the swelling which is visible in some top-worked trees. There is an absence of a line of union which would serve to identify the cells of the stock and the cells of the scion.

New growth in tangential section is illustrated in Plate VII, Fig. 15. The cavity between the stock and scion wood is filled with callus tissue, and will never be vascularized. There is a slight amount of callus developed at the margin of the original stock and scion, and this persists through resultant growth. However, normal growth eventually occurs and it is believed that much of the swelling the unions in uncongenial combinations is due to this original layer of callus tissue.

A line of division between the stock and scion was noted in grafts on K28 (Plate VII, Fig. 16). This stock was the only one of the group of 10 stocks which produced a line of division. Previous investigators have used differentially colored stocks and scions to secure similar results, but here the color of the wood was homogenous. The small number of unions available with this stock made it impossible to tell whether this line of division is a constant factor. However, advanced characteristics were noted in seven-year old grafts as illustrated in Plate X, Figs. 21 and 23.

Such a condition is not merely the presence of a line as commonly conceded, but an area of irregular or undeveloped tissue which forms a line of weakness between the stock and scion. This condition persisted throughout the new wood produced by this stock. The cause of this irregular area is undoubtedly due to a variation in the size or number of histological characters between the stock and scion (Tables 5 and 7), or to a toxic substance produced by one or the other member.

Transverse sections through young grafts gave a different aspect from the tangential sections. In this study, the transverse sections were used to better learn the mechanics of the union of the stock and scion and to get another view of the degree of congeniality (Plates VIII and IX). These photomicrographs represent the result of congeniality or of uncongeniality rather than the cause of either.

An abnormal or uncongenial union as represented by Plate VIII, Fig. 18 is typical of graft unions in which there is a degree of uncongeniality. In this section, distortion is present where the callus tissues of the stock and scion meet. This distortion remains in force for a short period of growth and eventually disappears. It is possible that the tissues overcome whatever is the cause of the distortion and can unite as completely as the tissues of congenial unions.

Poor mechanics in grafting often cause necrotic conditions similar to those in Plate IX, Fig. 19. This could be due either to the placement of the stock and scion or to the lack of sanitary conditions during grafting. Uncongeniality may also be a factor, especially since this combination is considered to be uncongenial. Graft unions resulting from this type of growth are bound to be distorted and uneven due to this internal disruption of continuity. A graft of the same age, but of a congenial combination is pictured in Plate IX, Fig. 20. In this combination, normal callusing and differentiation has occurred, resulting in a good union and continuous vascularization.

Numerical counts of the number of vessels per unit area in the transverse sections of the seven-week old graft unions were combined with counts made on similar sections of seven-year old graft unions. All counts were made on one year old wood and were similar in nature, Table 4.

Table 4. Number of vessels per unit area of stock and scion.*

Congenial stocks			Uncongenial stocks		
Stock	First count	Second count	Stock	First count	Second count
K1	33	37	K5	33	30
	22	26		25	28
	35	28		22	26
	32	39		31	28
	36	25		27	32
	39	35		29	24
	27	31		31	35
	37	39		28	27
	32	35		27	34
K22	37	31	K21	25	22
	30	33		19	23
	26	32		27	26
	25	29		17	20
	31	28		21	33
	36	34		29	21
	31	36		18	21
	29	28		18	21
K24	28	31	K43	25	18
	23	19		22	24
	27	20		29	31
	32	33		26	23
	18	21		32	27
	22	23		21	26
	25	17		26	28
	22	21		33	21
	18	23		26	29
K40	32	36			
	35	29			
	34	33			
	27	24			
	34	40			
	25	30			
	33	28			
	32	31			
	37	32			
	33	37			

*Unit Area = 57.078 sq. microns

The statistical analysis of data in Table 4 are presented in Table 5.

Table 5. Analysis of variance on stock differences secured by counting of vessels.

Source of variation	Degrees of freedom	Mean square
Stocks	6	276.61***
Slides, same stock	54	25.562**
Duplicate counts	61	12.139

***Statistically significant at the 0.1 percent level
**Statistically significant at the 1.0 percent level

Differences in the number of vessels between stocks are highly significant. With the exception of K24, this difference can be noted between the congenial and uncongenial stocks. However, it is impossible to consider this difference in number of vessels a cause of uncongeniality if K24 is considered to be congenial.

Evidence indicates a significant difference in the number of vessels within a stock. This no doubt is due to the counts having been taken from one year old wood, and making no distinction between seasonal growth areas, or the exact age of the wood. The difference between counts on the same stock would indicate that two or more samples of a stock should always be taken.

Observations of orchard grafts with a magnification of 25 times did not reveal any serious or unusual graft unions. It was possible to see the waviness or distortion present and to note those combinations which presented no distortion. It might be noted here that the zone of union in K5, K21, K29, and K43 could be identified while sectioning with the microtome. Callus-derived tissue from these stocks would crumble before the knife and it was difficult to obtain tangential sections from these stocks. This was uncommon in the congenial stocks.

Stocks producing normal grafts in external observations gave the largest number of straight unions. Even so, some distortion was found in the congenial stocks, indicating that distortion may be present, but without being expressed strongly enough to be seen without a microscope.

K43 was the only uncongenial stock to show straight unions under the microscope. It is possible that the size of the xylem elements between the stock and scion are different. No measurement of the size of elements was made, but the number of rays per area in both the stock and scion are about the same.

Counting of the rays was accomplished by using a checked ocular micrometer. The principle objective of this type of study was to compare the number of rays in the varieties producing normal grafts as compared with the number of rays in the varieties producing abnormal unions. Occasional slides would produce two counts that differed more than 10 rays per unit area and a third count was taken (Table 6).

Table 6. Number of rays per unit area of stock and scion.*

Congenial stocks				Uncongenial stocks			
Stock	First count	Second count	Third count	Stock	First count	Second count	Third count
K1	59	71		K5	97	101	
	69	78			98	97	
	72	74			82	88	
	62	55			57	58	
	87	77			42	55	45
	78	84			97	97	
	97	88			86	83	
	96	97			82	79	
	74	78			67	71	
	77	69			87	84	
K22	81	91		K23	72	86	76
	59	64			88	83	
	77	53	67		65	81	81
	59	59			66	66	
	89	85			80	82	
	61	66			84	81	
	78	73			69	66	
	80	78			85	78	
	64	81	80				
	61	56		K28	102	98	
					101	103	
K24	96	87			100	85	88
	76	77			104	107	
	83	75			87	94	
	65	69			79	86	
	85	77			105	103	
	68	82	76		99	102	
	88	84			95	104	
	95	88			105	96	
K40	103	102		K29	75	92	76
	75	81			104	98	
	107	107			79	86	
	106	102			89	95	
	82	79			84	99	93
	103	91	94		103	95	
	81	98	83		85	88	
	92	93			93	98	
	95	101			93	89	
	104	92	89		86	92	

Table 6. (concl.)

Congenial stocks				Uncongenial stocks			
Stock	First count	Second count	Third count	Stock	First count	Second count	Third count
				K43	77	75	
					81	81	
					69	76	
					87	82	
					66	83	79
					76	83	
					89	91	
					82	87	
					75	71	
					66	70	

*Unit area = 1.0404 sq. microns

The statistical analysis of data in Table 6 are presented in Table 7.

Table 7. Analysis of variance on stock differences secured by counting the rays.

Source of variation	Degrees of freedom	Mean Square
Stocks	8	1728.52***
Slides, same stock	77	210.62***
Duplicate counts	86	12.83

***Statistically significant at the 0.1 percent level.

There is a highly significant difference in the number of rays per stock just as in the number of vessels. In this test for congeniality or uncongeniality, the significance if void because of the dispersion of the congenial and uncongenial stocks. There is no trend in the number of rays for either type of stock.

There is also a significant difference in the number of rays between slides within a stock. This would be secured in using wood produced throughout the years growth rather than one arbitrary period. It is difficult to determine the age of wood in the tangential sections and no effort was made to distinguish the difference between spring and summer wood. Table 7 indicates that at least two and possibly more slides of each stock should be used for making histological counts.

Photomicrographs of tangential sections from seven-year old graft unions provided an easy method of comparing and recording the tissue structure of the component parts of the graft union, as well as that of comparing with other stocks and scions.

Little difference between stocks could be noted by this method. However, two of the uncongenial stocks reflected the result of uncongeniality as shown in Plate X, Figs. 21, 23, and 24. Differences are visible by a close study of the histological characters, rather than by color alone. Two outstanding characteristics in these uncongenial unions are the difference in number and size of rays per area and the irregularity of the vessels in the union area.

In Fig. 22, which is a congenial combination, a line is detected which is due to a difference in stain only. There is a continuous arrangement of the vascular tissues through this line and no evidence of distortion or uncongeniality.

It has been stated that when both longitudinal and transverse areas are seen within the area of a few millimeters, it is difficult not to believe that there is not uncongeniality present. This condition was found in radial sections of K5 (Plate XI, Figs. 25 and 26. Once a ain it can be assumed that this is the result of uncongeniality rather than the cause.

Radial sections were made of many grafts but were found to be poor material for the counting of histological characters (Plate XII, Figs. 27 and 28). However, they were used for completing the overall picture of graft unions and in the classifying of the various combinations into normal or distorted tissue.

EXPLANATION OF PLATE V

Fig. 7. Tangential section through J40. (A) Cavity between stock and scion filled with callus tissue. 54x

Fig. 8. Tangential section through W40. The cells of the callus are differentiating into vascular elements. 54x

Fig. 9. Tangential section through J40. Further development of the process of normal bridging of the gap between the stock and scion. 54x

Fig. 10. Tangential section through J24. Completion of the vascularization between the stock and scion in a congenial graft union. 54x

Plate V

Fig. 7

Fig. 8

Fig. 9

Fig. 10

EXPLANATION OF PLATE VI

Fig. 11. Tangential section through W43. Callus tissue with the beginning of vascularization. (A) Irregular vessels. 54x

Fig. 12. Tangential section through new wood of J1. Vascularization is occurring throughout the callus tissue and is bridging the gap between the stock and scion. Vessels and tracheids are irregular. 54x

Fig. 13. Tangential section through W1. A more advanced stage of vascularization. 54x

Fig. 14. Tangential section through W1. The gap between the stock and scion is completely bridged by vascular tissue. Some twisting and curling of the vessels and tracheids present. 54x

Plate VI

Fig. 11

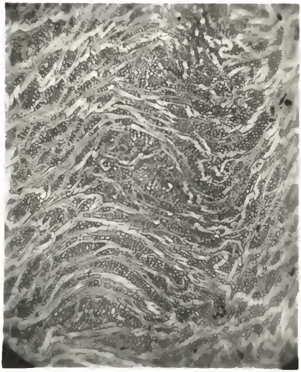


Fig. 12

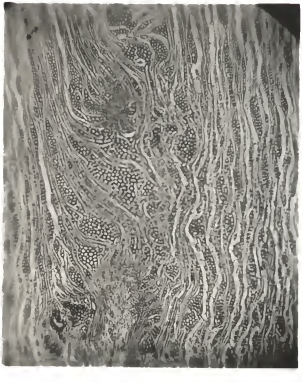


Fig. 13

Fig. 14

EXPLANATION OF PLATE VII

Fig. 15. Tangential section through W21. (A) Callus tissue differentiating at the stock lip. A slight irregularity is appearing. 54x

Fig. 16. Tangential section through W28. (A) Line of union between (B) stock and (C) scion. 54x.

Plate VII

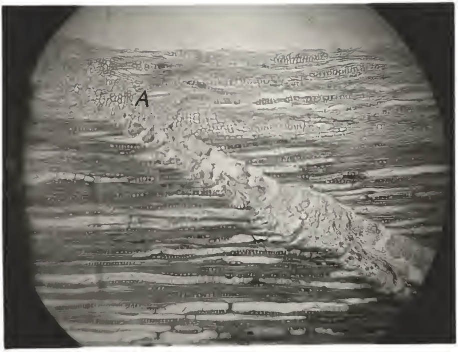


Fig. 15

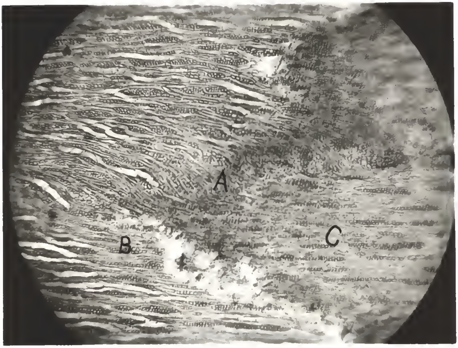


Fig. 16

EXPLANATION OF PLATE VIII

Fig. 17. Transverse section through W5. (A) is stock; (B) is scion. Normal growth, as indicated by the homogenous tissue at (C) where the tissues of the two components are intermingled. 54 x

Fig. 18. Transverse view of J5. (A) is stock; (B) is scion. Abnormal union as indicated by the distorted tissue in the newly-formed area (C). 54x

Plate VIII

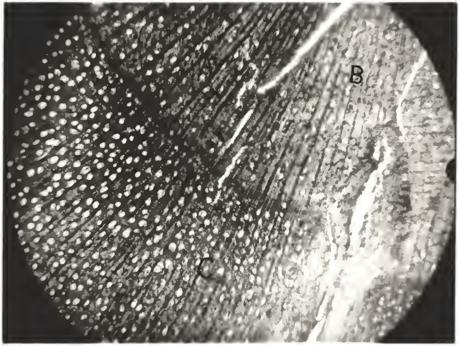


Fig. 17

Fig. 18

EXPLANATION OF PLATE IX

Fig. 19. Transverse section through W43. Poor union of tissues plus an inclusion of a necrotic area (A). (B) is stock tissue. 54x

Fig. 20. Transverse section through W5. Development of normal tissue across the gap created by grafting (A) and in new tissue where the two components meet (B). (C) is stock. 54x

Plate IX

Fig. 19

Fig. 20

EXPLANATION OF PLATE X

Fig. 21. Tangential section through W28. Line of union between stock and scion can be seen in the histological characters at (A). (B) is stock tissue and (C) is scion tissue. 54x

Fig. 22. Tangential section through J1. Line of union visible, but only as a result of the staining process. 54x

Fig. 23. Tangential section through J28. The line of union is visible both by color and by difference in histological characteristics. (A) is stock tissue. 54x

Fig. 24. Tangential section through J28. Differentiated callus tissue is an uncongenial graft combination. Note the distorted xylem elements as well as the heavy accumulation of rays throughout the section. 54x

Plate X

Fig. 21

Fig. 22

Fig. 23

Fig. 24

EXPLANATION OF PLATE XI

Fig. 25. Radial section through W5. Both radial and transverse areas are seen in the same plane. An example of severe distortion with probable mechanical weakness. 54x

Fig. 26. Radial section through W5. (A) Severe distortion in the xylem tissue; (B) irregular growth in the cambial area. 54x

Fig. 27. Radial section through W43. Curvature of xylem elements and oblique angle of rays are indicative of uncongeniality. 54x

Fig. 28. Radial section through W40. Nearly normal tissue, as taken from a congenial graft combination. 54x

Plate XI

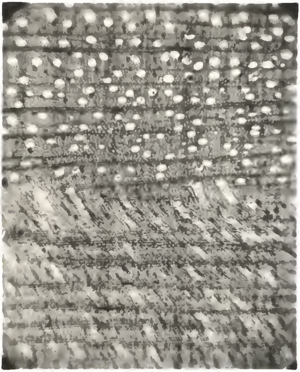


Fig. 25

Fig. 26

Fig. 27

Fig. 28

SUMMARY AND CONCLUSIONS

Congeniality between stock and scion may be expressed early in the life of a graft union. This expression may be either the presence of an abnormal amount of callus tissue or may be the failure of the two to unite. The grafting studies made in this experiment were to see if the expression of congeniality was strong enough to prevent the union of stock and scion, and which stocks would produce this condition.

Grafts made in the greenhouse gave a slight hint of uncongeniality by beginning to grow and then dying. However, statistical analysis showed no difference between the congenial and uncongenial stocks in causing this condition. This cessation of growth can be attributed to the stocks rather than the graft combinations, as all such specimens were found to have negligible root systems at the time of investigation. Initiation of growth was possible due to the reserve food material in the root system, which was unable to sustain growth over a long period of time.

Further evidence leading toward the above conclusion was the fact that all grafts except one made in the field were successful. There was no expression of uncongeniality of any type on these grafts. An undisturbed root system provided all the nutrients and water necessary for a successful graft union.

Crooked and abnormal tissue was found early in the life of the grafts, especially from uncongenial stocks. By using photomicrographs for comparative studies, it was possible to trace this distortion back to the time of vascular initiation. It was in this stage that the distinction between congeniality and compatibility was expressed. All the grafts, regardless of the amount of distortion present, were complete enough that they would continue to grow. If incompatibility were present, there would have been a line of division or of breakage at this point.

One stock produced a line at the point of union which was visible after the staining procedure. It was impossible to detect any mechanical defects in the seven-week old graft. Another stock was associated with a line of division within the structure of the wood. In the seven-year old graft, the line was still visible, and was accompanied by a slight degree of distortion of the xylem elements, Although microscopic examination did not detect a specific weakness, it is possible that this distorted area may give way when subjected to severe strain.

The vigor of the tree is not wholly dependent upon the smooth vascular continuity, as can be seen by broken trees in the forests. However, it would seem that the disruption of this continuity does influence the rate of translocation of food materials. Uncongenial stocks of the K-series produced smaller and more straggly trees than do the congenial stocks. This would indicate a hindrance in the process of translocation.

Studies of the early stages of graft unions indicate that the swelling which is visible in macroscopic observation may be due to excessive callus formation early in the life of the graft, with a normal union of tissues later. Any of the causes of uncongeniality could be the cause of this development of excess callus, and this study has not been successful in the exact determination of the effect of uncongeniality.

Observations of sections from seven-year old grafts at 25 magnifications disclosed distortion of tissues in congenial as well as in uncongenial combinations. Distortion may be present in congenial combinations but is not strong enough to be expressed by a swelling visible to the eye. This could be due to normal character of apple wood, which has a twisting method of growth later in its life.

Since one of the possible causes of incompatibility is variation in the number of histological characters, such counts were made. Microscopic counts with the aid of an eyepiece micrometer proved to be more practical than the use of photomicrographs.

Variation was found both in the number of rays and vessels. Statistical analysis indicated that there is a significant difference between the stocks in both characteristics. By taking the vessels alone, it may be possible to classify stocks into congenial or uncongenial groups. Rays however, are diverse in number and it is impossible to segregate the congenial and uncongenial stocks from this character.

If correlation studies similar to those made by Herrero (1951) were made, it would be found that the two histological characteristics are not correlated. This is an agreement with Herrero, who found differences in nine histological characteristics, but could not correlate them in their entirety. It may be that the vessels are more significant in the union of the stocks and scion than are the rays, and that any variation between the vessels of these two units will cause swelling or distortion. Other longitudinal characters should be studied to determine if they too are significant in producing normal graft unions.

It can be concluded that:

1. Grafting success with uncongenial stocks can be anticipated to be the same as that with congenial stocks.

2. Determination of congeniality through comparative studies can be accomplished by making longitudinal sections of seven-week old grafts. The use of photomicrographs facilitates this type of study.

3. Stock K28 produced a line of division when grafted to Jonared and Winesap. This will probably be a line of weakness as the tree increases in size and weight.

4. Transverse sections may be preferable to longitundinal sections for making histological counts. Vessels are easily counted in transverse section and it is possible that future

investigations could prove this method as a means of predicting congeniality.

5. For histological counts, two or more slides of each stock should always be used. One count per slide is sufficient.

6. Significant differences in histological characters between stocks were found, but they could not be correlated into the congenial or uncongenial groups.

7. There remains to be found a satisfactory explanation of uncongeniality or incompatibility.

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STUDIES OF APPLE GRAFT UNIONS

by

CARROLL CHRIS DOLL

B. S., University of Illinois, 1949

AN ABSTRACT OF A THESIS

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Variation in growth characteristics were observed in seven-year old apple trees which were topworked to the K-series, a group of hardy stocks. This variation consisted of differences in rate and amount of vegetative growth and in the formation of smooth or uneven graft unions. Uneven graft unions appeared to be associated with the poorly vegetative trees and this problem was an attempt to find the association between uncongenial stocks and grafting success and also the cause of the abnormal graft union.

One-year old stocks were grafted in the greenhouse and in the field to test grafting ability and also to provide graft unions for macroscopic and microscopic observations. Of the ten varieties of the K-series selected for this study, four were considered congenial and six were considered uncongenial. This was based upon the seven years of records of the original stocks in the horticulture farm orchard.

Ninety-seven grafts were made in the greenhouse of which 73 were successful and 24 were not successful. Many of the failures originally showed signs of union, but later died and were considered to be the result of weak rootstocks rather than incompatibility of stock and scion. The chi squared test found no significant differences within the congenial or uncongenial groups or between the two groups, and it was concluded that all stocks are similar in respect to grafting ability.

A similar experiment consisting of 40 one-year old stocks in the field were grafted. All grafts but one were successful and this could be attributed to poor mechanics in grafting.

Microstructure studies were made on seven-week old and seven-year old graft unions. Tangential sections were the predominant type and thorough microscopic observations were made. Comparison and recording of the various stages and conditions found were accomplished through the use of a microscope and photomicrographs.

Complete callusing and bridging were observed in the seven-week old graft. Vascularization of the callus tissue begins immediately after the callus tissue is complete. Distortion of tissue also begins at this stage of growth and in uncongenial combinations it frequently continues throughout the life of the graft. Evidently this original layer of callus is the extent of swelling in some combinations of grafts. A line of union was found in K28, an uncongenial stock. This was characterized by differences in the density of histological characters and by a distortion of these characters. One congenial stock produced a line of union, but only in color differences after the staining procedure.

Transverse and radial sections were analyzed for a better understanding of the structure of graft unions and to see the effect of uncongeniality. Normal or congenial unions can be associated with no distortion and straight xylem elements. The effect of uncongeniality of one stock and scion was so great that both the radial and transverse sections could be seen on one plane.

Counts of vessels were made on the transverse sections. Magnification of 440 times and a field of 57.058 square microns on the last year's wood was used to make two counts per section. Significant differences in vessel numbers between stocks were found but there was no correlation between the congenial and uncongenial stocks. Significant difference was also found between sections of the same stock, indicating that two or more slides of a stock should always be used for histological counting.

Counts of rays were made on the tangential sections. Two and sometimes three fields of 1040.4 square microns on last year's wood and a magnification of 100 times were used. Highly significant differences were found between stocks, but as in the vessel counts, the congenial and uncongenial stocks could not be correlated. Similarly, evidence shows that two or more slides of a stock should always be used in making these counts.