October 29, 1968

To the Graduate Council:

I am submitting herewith a thesis written by Jackson B. Jett, Jr., entitled "The Vegetative Propagation of Yellow-poplar (*Liriodendron tulipifera* L.)." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Forestry.

Eyvind Thor
Major Professor

We have read this thesis and recommend its acceptance:

Edward R. Buckner

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Accepted for the Council:

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Vice Chancellor for
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THE VEGETATIVE PROPAGATION OF YELLOW-POPLAR

(*Liriodendron tulipifera* L.)

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Jackson B. Jett, Jr.

December 1968

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ABSTRACT

Survival and height growth were parameters used to evaluate the influence of two methods of bench grafting, two stages of dormancy, and two storage methods on yellow-poplar grafts. Examinations of sectioned grafts were made in order to evaluate graft unions for symptoms of incompatibility.

Dormant versus non-dormant understock, and the interaction of dormancy and grafting method produced significant differences in mortality. Grafts on non-dormant understock and the combination of side graft on non-dormant understock produced the highest levels of graft survival.

Storage treatments produced highly significant differences in height growth. No other treatments or interactions exhibited significant differences in height growth.

Poor height growth was the only external symptom of incompatibility that was observed. Examination of sectioned grafts suggested that graft incompatibility exists in yellow-poplar. Internal symptoms of incompatibility included: (1) heavy accumulation of parenchymatous tissue in the union; (2) reduced mechanical strength of union; (3) severe vascular distortion in the union; and (4) necrotic areas in the union. Grafts with poor height growth exhibited these symptoms of incompatibility.

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CHAPTER I

INTRODUCTION

More than a decade ago the forest industry began directing attention to the selection, breeding, and propagation of trees of superior traits. The majority of this effort was concentrated on the improvement of pine species. Only recently have efforts been directed towards the improvement of hardwood species.

Much of this recent interest in hardwood improvement is a result of technological advances in methods of utilizing these species. McElwee and Jones (1965) state that hardwood pulp production increased approximately 250 percent in the previous ten years. With increased demands for pulpwood coupled with a continued need for hardwood logs of saw-timber and veneer size, they envisioned a shortage of hardwoods in certain species, sizes, and grades. It is apparent that there is a real need for improvement of several hardwood species.

Many of the most important hardwood species have been propagated vegetatively by various methods of grafting or budding (McAlpine, 1965). According to Grigsby (1957) more forest trees are being propagated by grafting than by any other vegetative means. Another important attribute of grafting and budding is that it allows the plant propagator to work with mature material of established traits. Yerkes (1929) stated that one important aspect of grafting is that the period required to obtain flowers and fruit is drastically reduced. This is a prime consideration in obtaining early production from seed orchards.

Whether to field graft or bench graft is a decision often based on personal preferences. McAlpine (1965) reported that bench grafting, if the species lends itself to this method, was preferred because grafting could be done during the dormant season when the work load was light. Bench grafting also lends itself to mass production by concentrating efforts at one location under close supervision and environmental control.

The prime objective of this study is to evaluate grafting methods, dormancy, grafting, and storing yellow-poplar (Liriodendron tupilifera L.) grafts. A second objective is to evaluate graft unions for symptoms of incompatibility and failure by using microtechnique methods. Height growth and mortality were recorded at monthly intervals throughout one growing season. Data were subjected to analyses of variance to evaluate the differences in height growth and survival as influenced by conditions of understock, grafting method, and storage method.

CHAPTER II

LITERATURE REVIEW

Factors Influencing Graft Survival

Several authors have discussed the importance of timing in obtaining successful grafts. Churchwell (1965) grafted dormant yellow-poplar scions to both dormant and non-dormant understock. He reported that only 5 percent of the grafts on dormant understocks survived, while 85 percent of the scions grafted to non-dormant understocks survived. Thor (1965) reported that superior survival was obtained with yellow-poplar when field grafting was done in early April, using dormant scion wood. Excellent results have been obtained by bud grafting yellow-poplar in September (Funk, 1962). Using buds collected in July, he scheduled grafting between 3 and 6 weeks after height growth ended in the forest. Sussex and Clutter (1959), as cited by Hartmann and Kester (1968), state that callus proliferation--essential for a successful graft union--occurs most readily in the spring just prior to and during "bud break." Callus proliferation then diminishes through the summer and into the winter. Increasing callus proliferation occurs again in late winter, but this is not dependent upon breaking of bud dormancy.

Different treatments to improve grafting success have been reported by several authors. Allen and Scarbrough (1961) reported that the application of fertilizer to the understocks increased survival of slash pine (*Pinus elliottii* Engelm.) grafts by 30 percent. The application of mulch in conjunction with fertilizer increased survival an additional 20 percent. The effect of the fertilizer and mulch was to improve the vigor of the understock and thereby hasten healing of the grafts. Johansen and Kraus (1959), working with slash pine, obtained no increase in scion survival when they added a nutrient solution in the bottles of bottle grafts or applied a nutrient spray to cleft grafts. Zak (1955a) states that if necessary, mature trees used as scion sources may be fertilized and watered to obtain vigorous tissue for grafting.

Zak (1955b) states that the success or failure of grafting is largely a matter of water relationships initially in the scion and later in the scion-stock combination. In field grafting shortleaf pine (*P. echinata* Mill.) he found that the use of portable humidity chambers made from 5-gallon containers increased grafting success. Mergen (1955), in grafting slash pine, found that bottle grafts produced the best results. He also noted that use of black roofing paper in humidity chambers on field grafts gave an improvement over uncovered field grafts. Perry (1955), Webb (1961), and Zak (1955a) report improved grafting success with pine by using cellophane or plastic bags. They cautioned that shading should be provided to reduce temperatures. Thor (1965) found that a plastic wound covering was sufficient to prevent dessication of yellow-poplar grafts and that plastic and kraft bags were unnecessary. Black walnut (*Juglans nigra* L.) grafts placed in a saturated atmosphere had up to 95 percent callusing versus 52 percent callusing in 25 percent relative humidity (Sitton, 1931). Best results were obtained using completely waxed graft unions.

the importance of careful alignment of cambia of stock and scion in order to obtain rapid healing of graft unions. One exception to this practice is the use of the soft tissue method of grafting. Zak (1955b) states that little care is required in matching cambial zones of grafts made using the soft tissue method. However, he cautions that with all other grafting methods, cambia must be aligned carefully.

Formation of Graft Unions

According to Nienstaedt _et al_. (1958) the sequence of anatomical changes taking place during the healing of a graft union varies between species. Hartmann and Kester (1968) state that the healing of a normal graft union occurs in the following sequence: (1) a contact layer is formed; (2) callus tissue is formed; (3) cambial formation occurs with callus differentiation; and (4) vascular elements form and connect in the new vascular cambium.

In grafts between guayle and sunflower, Artschwager (1951) found that thickness of the contact layer depended largely upon evenness of the cut and pressure holding stock and scion together. If the cut was uneven and the contacting surfaces were not under pressure, the contact layer became broad and irregular and often broke apart. These breaks produced air-filled pockets between stock and scion. Mergen (1954) stated that the contact layer, formed by death and shrinkage of the cells, was most pronounced in the regions of the cortex, phloem, cambium, and pith.

Depending upon the species involved, different patterns of meristimatic activity in callus formation are possible. Studies by Mergen

(1954), Sass (1932), and Crafts (1934) showed callus production not to be limited to primary meristems. Parenchymatous cells of the cortex, the phloem rays, and the pith have the capacity of becoming secondary meristems. In bud grafting roses, Buck (1954) found that the cambium contributed no callus during the healing process. He noted that callus was derived from the secondary phloem and secondary xylem in the immediate vicinity of the scion bud. Sass (1932) described apple grafts of Wealthy scions on French crab understocks. He noted that callus was the exclusive product of tissues outside the phloem cylinder and that any living tissue of the bark, exclusive of the periderm, could produce callus. He further stated that the cambium may contribute very little to callus formation. Early work with cleft grafts of Hibiscus rosa-sinensis by Sharples and Gunnery (1933) showed the cambium to be of little importance in the early development of callus. They found no sign of cambial activity until the callus was completely formed.

Dormling (1963) in studies of graft unions of Scots pine (P. sylvestris L.) and Norway spruce (Picea abies (L.) Karst.), found that after the graft was made, first cell enlargement began in the cells near the wound surfaces. Cell division was found to be most vigorous in areas of nutrient storage and also areas which were best suited for conduction. Cells of rays emerging from leaf gaps were particularly active in the formation of callus.

Although the callus cells derived from stock and scion soon became indistinguishable, it is apparent that the stock and scion do not necessarily contribute equal quantities of callus. Kostoff (1928) in species of Nicotiana, Mergen (1954) working with grafts of slash pine, Sass (1932) studying apple grafts, and Esau (1954) in a general statement concluded that callus contribution is predominantly from the stock. Sharples and Gunnery (1933) stated that both stock and scion in Hibiscus grafts contribute about equal shares to callus formation.

After the gaps between graft components are filled by callus, the formation of a bridging cambium begins. Sass (1932) reported that the callus between stock and scion is bridged by a cambium-like layer. He felt that in view of subsequent activity and structure, these cells constituted a true cambium region. Esau (1965) reported that the calluses of the stock and scion intermingle, and eventually the vascular cambium is formed through mixed callus in line with the cambium of stock and scion. The cambium first appears where the cambia of the stock and scion are in contact with the callus cells. Subsequent divisions, forming a cambium in the callus, progress toward each other until they meet. These new cambial tissues are in continuity with the xylem and phloem of both graft components. According to Sass (1932) the important features of this development of a new cambium are: (1) the cells differentiate from the spongy callus; (2) reactivation begins adjacent to old cambium; and (3) differentiation progresses tangentially until a cambial arc connects the respective cambia of stock and scion.

The newly formed cambial zone in the callus bridge begins typical cambial activity, laying down new xylem and phloem, along with the old cambium of stock and scion (Hartmann and Kester, 1968). The type of cells formed by the new cambium seems to be influenced by the cells stock and scion. The insertion of a mutually compatible interstock overcomes the incompatibility symptoms.

A form of incompatibility in which the graft union grows normally for varying periods of time and then fails is known as "delayed incompatibility." This type of incompatibility appears to be a variation of translocated incompatibility. Graft failures in Douglas-fir (Pseudotsuga menziesii (Mirob.) Franco) are of two main types: (1) failure to make initial union; and, (2) delayed graft failure (Duffield and Wheat, 1964). Delayed failure may not become evident for several years. The authors attributed this type of failure to a stock-scion interaction in which the phloem became blocked. Rogers and Beakbane (1957) stated that incompatibility in trees may not express itself until many years after the graft union was made. This type of incompatibility appears to be associated with aging of the plant.

Graft union failures due to incompatibility can often be correlated with certain external symptoms. Chang (1938) observed that, in general, certain foliage characteristics of grafted plants were symptomatic of incompatibility. Premature autumn coloration of the leaves, early defoliation, and dying back of the young growth were taken as indicators of incompatibility. Defoliation began at the top of the plants in incompatible combinations in contrast to the defoliation from the basal portion of the plants in compatible combinations. Toxopeus (1936), in studies of Citrus, reported that leaves failed to reach normal size and defoliation was early in incompatible unions. He also noted that the foliage had a chlorotic appearance. Similar results were reported by Mosse (1958) in grafts of pear on quince.

Other external symptoms of incompatibility include: (1) a smooth break at the union; (2) excessive suckering; (3) unequal growth rate of scion and stock; (4) high percentage of graft failures; and, (5) over-growth at, above, or below the graft union (Hartmann and Kester, 1968). Lapins (1959) found a highly significant correlation between smoothness of break at the union and incompatibility of apricot varieties grafted on peach seedling understocks. Unequal growth at the union and smooth breakage of pear scions when grafted on quince were associated with incompatibility (Mosse, 1958). Copes (1967) and Duffield and Wheat (1964) reported that scion over-growth is a reliable symptom of graft incompatibility in Douglas-fir. Scion over-growth, as a symptom of incompatibility, is probably a species phenomenon since Bradford and Sitton (1929) found no correlation between incompatible combinations and scion over-growth. The most incompatible combination (pear on apple) produced no swelling. However, the most compatible combinations of apple frequently exhibited swelling.

Chang (1938) listed the internal symptoms of graft incompatibility as: (1) bark and wood fiber discontinuity; (2) heavy accumulation of parenchymatous cells at the union; (3) reduced mechanical strength at the union; (4) some form of vascular obstruction at the union; and, (5) abnormal starch distribution. Proebsting (1928) stated that compatible unions exhibited good continuity by the way in which xylem elements were dovetailed together to form a well-knit, mechanically strong juncture. Discontinuity of the bark is not in itself a symptom of incompatibility but may precede a more definite discontinuity in the wood (Bradford and Sitton, 1929). Mosse and Herrero (1951), in studies of pear and quince grafts, also noted that discontinuity of tissues at the union was not always directly correlated with incompatibility.

Herrero (1951) considered the presence of parenchymatous tissue in the wood, either as isolated areas or a continuous layer, as symptomatic of incompatibility. Proebsting (1926; 1928) reported that the most common type of structural defect in *Pyrus* grafts was that involving the deposition of parenchyma by the cambium of stock and scion at the line of union. They stated that presence of this parenchyma interrupted the vascular connection. Eames and Cox (1945) described a graft failure in white fir (*Abies concolor* L.) that seemed to have been in part caused by a rapid covering of the cut surfaces by masses of callus. On both stock and scion the callus almost completely enclosed each wound area separately, with little intermingling of callus cells.

Mosse (1958) stated that the quality of the union depended on the relative area with normal vascular connection as compared to the area where non-lignified parenchymatous tissue separated the xylem of the stock and scion. Severe distortion of the vascular tissue, indicated by whorls and loops of vessels, may cause difficulties in translocation (Proebsting, 1928).

Chang (1938) and Mosse and Herrero (1951) noted that incompatible unions were frequently associated with abnormal starch distribution, particularly in the root system. Chang (1938) noted heavy starch accumulation above the unions in incompatible grafts of *Pyrus* and *Prunus*.

Buchloh (1960) found that the processes which were involved in lignification of cell walls were mainly responsible for the formation of strong unions in grafted pear-quince trees. Reactions which inhibited lignification of cells gave rise to weak unions. He felt that lignification of cell walls in the union line was one of the most important processes responsible for the compatibility of grafts.

It is well established that genetic variation between species of the same genus and within the same species often plays a role in graft incompatibility. Armstrong and Brison (1949) reported that in incompatible unions of post oak (Quercus stellata Wengehn.) and live oak (Q. virginiana Mill.) phloem fibers were continuous across the graft unions and that breaks occurred in the xylem. It was noted that the xylem of the post oak understocks became differentiated at times when there was no corresponding differentiation of the xylem of the live oak scions. Results reported by Pauley (1948) showed that there is apparently a high degree of compatibility between the various species of poplars (Populus L.). Funk (1962) felt that there were differences in compatibility between clones of yellow-poplar when budding non-dormant buds. Individual trees of western white pine (P. monticola Dougl.) have shown considerable variation in graftibility and different degrees of early compatibility (Hanover, 1962). Knowledge concerning graft compatibility of forest trees is of great concern, but the information available is limited (Nienstaedt et al., 1958).

CHAPTER III

PROCEDURES

Scion Wood and Understock

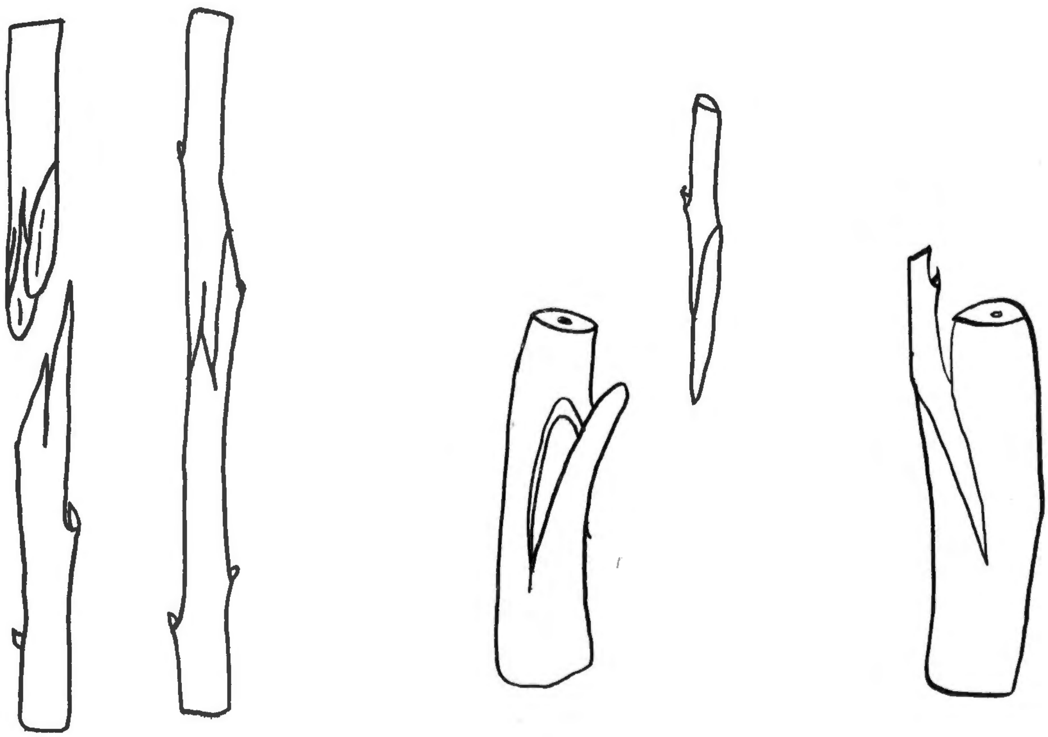
Scion wood was collected from three dormant widely scattered trees located in the U.T. Cherokee Forest, near Knoxville. Collections were made in February and March, 1967. The trees were at least ten inches in diameter at breast height and a minimum of 30 years of age. Scion wood was taken only from the upper two-thirds of the tree crown. These criteria were used to insure that the scion wood was physiologically mature.

All material was bundled and labeled at time of collection and placed in plastic bags. A small amount of damp sphaghnum moss was placed in each bag prior to cold storage at 40 degrees Farenheit.

Two thousand, one-year-old, dormant yellow-poplar seedlings were obtained from the Tennessee Valley Authority nursery at Norris, Tennessee in February, 1967. One-half of the seedlings were placed in a greenhouse mist bench and the remainder in sawdust in a shadehouse. Supplementary artificial lighting was used to increase day length and break the dormancy of the seedlings in the mist bench.

Grafting Methods and Treatments

Two methods of grafting were used in this project: whip graft and side graft. The whip grafts were made at or near the root collar and side grafts were made several inches above the root collar, depending upon the scion fit. The grafting methods used are presented in Figure 1.



(a) (b)

Figure 1. Schematic representation of grafting methods. (a) Whip graft; (b) side graft.

Source: J. P. Mahlstede and E. S. Haber. 1957. Plant Propagation. John Wiley and Sons, Inc., N.Y.

Grafts were made only on the larger more vigorous seedlings. Care was taken to insure that the cambium of understock and scion was well matched. All grafts were wrapped with standard grafting rubber bands and thoroughly coated with melted grafing wax.

A total of 1,200 grafts were made; 600 on dormant understock and 600 on non-dormant understock. A further division of the above main treatments consisted of dividing each main treatment into subtreatments consisting of 300 grafts made using the whip graft method and 300 using the side graft method. One hundred scions from each of the three trees were used in making the whip grafts and the same number were used in making the side grafts. Grafted seedlings were tagged for identification of understock dormancy, clone and graft type, and kept in the mist bench until all grafting was completed. Approximately 11 days were required to complete the grafting.

The final treatment consisted of storage in the mist bench and in a cooler. Half of the grafts from each treatment-clone combination were packed in plastic-lined cardboard boxes filled with damp sawdust and placed in a cooler at 50° F. The relative humidity was maintained at 90 percent in the cooler by using an automatic electric humidifier. The sawdust used to pack the grafts was moistened as required to prevent the dessication of the roots. The remaining 600 grafts (50 of each treatment-clone combination) were left in the mist bench. A breakdown of treatments and subtreatments is presented in Figure 2.

Outplanting and Measurements

Prior to outplanting, all surviving grafts were tagged with

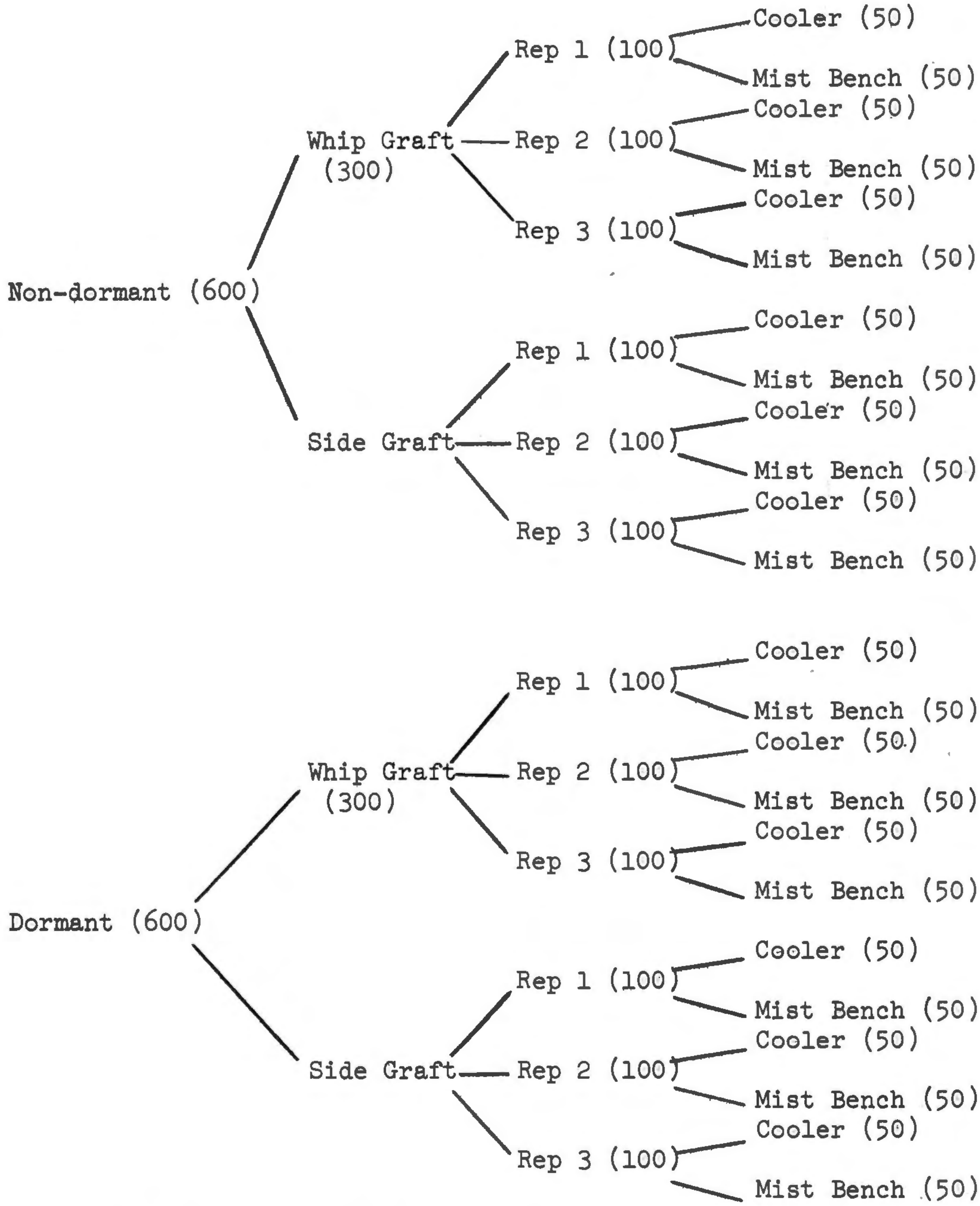


Figure 2. Types of treatments and subtreatments with number of grafts shown in parentheses.

consecutively numbered metal tags and all dead grafts discarded. Out-planting in the nursery was started April 18 and completed April 19, 1967, using a planting bar. Irrigation was started immediately upon completion of planting and was further applied as required.

After planting was completed each scion was pruned to a single bud. All branches from the understock which overtopped the scion were removed. Subsequent pruning of the understock was performed as required throughout growing season to keep the scion dominant.

At the time of planting, length of each scion was determined with a yard stick and recorded to the nearest full inch. Remeasurements were made at monthly intervals until October, 1967.

Collections, Sectioning and Observation of Graft Unions

Four types of graft unions were selected for sectioning and observation: (1) side graft--vigorous growth, (2) side graft--slow growth, (3) whip graft--vigorous growth, and (4) whip graft--slow growth. Vigorous growing scions had a mean growth of 34 inches for the entire growing season and slow growing scions had a mean growth of 3 inches.

Ten graft unions of each type were collected in early April, 1968, while the unions were still dormant. Immediately following collection, the unions were tagged with the tree number and placed in a formal-acetic acid-alcohol solution (FAA) for fixing and preserving the tissue.

Prior to sectioning, graft union types (1) and (3) were placed in a Soxhlet extractor for 48 hours to remove FAA from the tissue.

Preparation of graft union types (2) and (4) consisted of placing them through a series of 70, 85, 95, and 99 percent ethyl alcohol and embedding in paraffin. Graft union types (2) and (4) were too small and poorly knitted together to section without being embedded in paraffin.

A sliding microtome was used to cut full length 30 micron sections from the center of the graft unions. The whip grafts unions with vigorous growth were short enough to be held directly in the microtome chuck. Side grafts with vigorous growth, due to their large size, had to be held in a chuck adapter made by the author (Figure 3). The materials embedded in paraffin were held for sectioning by sticking the paraffin blocks to blocks of wood which would be clamped in the microtome.

Stains were prepared according to Johansen (1940). Sections were stained using a modified form of the safranin-fast green double stain schedule presented by Sass (1958). The first modification consisted of placing about 3 drops of hydrochloric acid in the 95 percent alcohol solution immediately preceding the fast green in the staining schedule. The HCl provided rapid bleaching of the excess safranin from the sections. A second modification was the use of diaphane solvent instead of xylene following the 95 percent alcohol wash. Use of diaphane solvent prevented cloudiness in the sections when mounted on the slides using diaphane green adhesive.

After mounting, the slides were air dried for 48 hours, then oven dried at 45° C for an additional 48 hours. The slides were then cleaned and labeled.

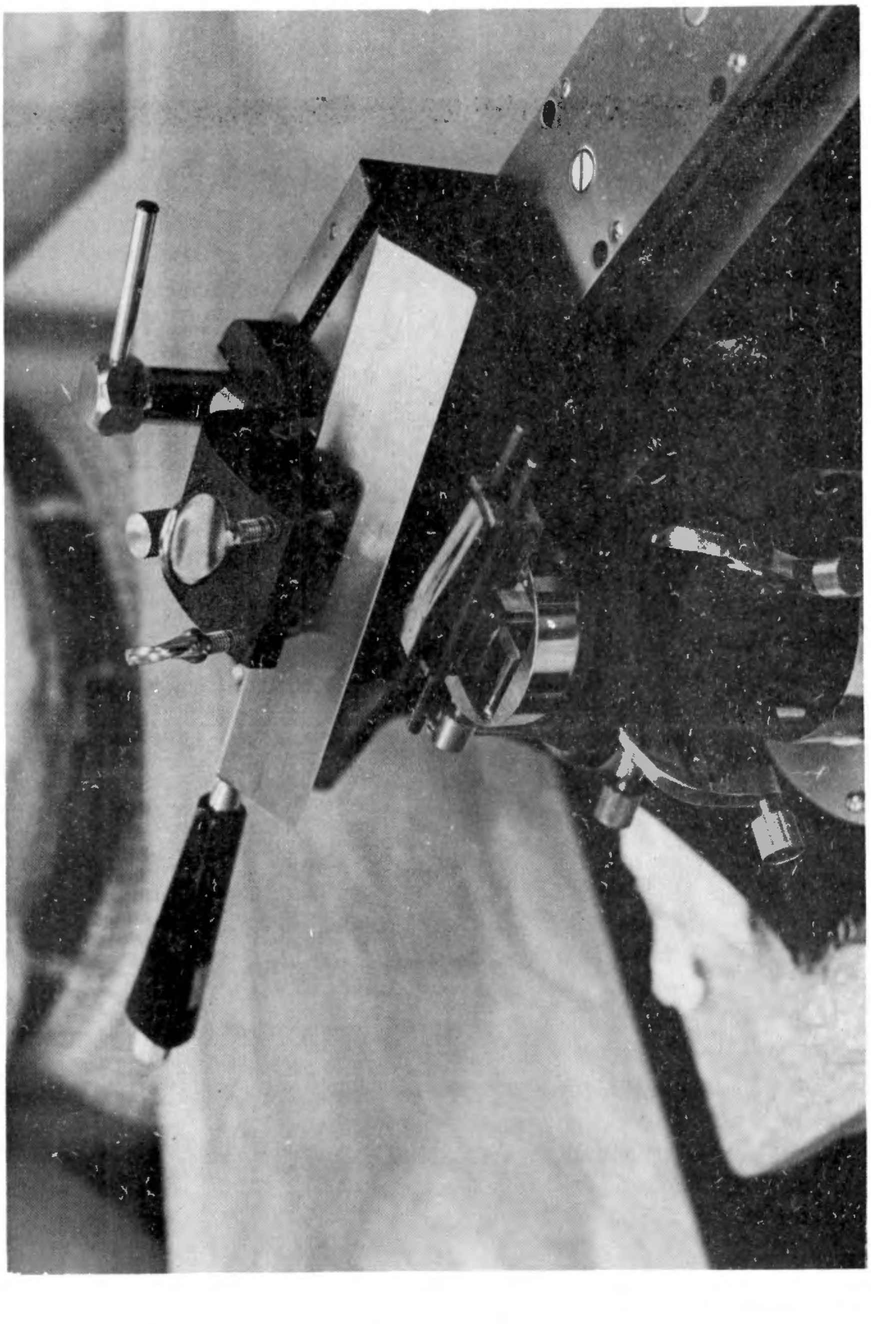


Figure 3. Chuck adaptor in use.

Vascular continuity in the sectioned grafts was observed using a microprojector at 43 magnifications. The microprojector was used in conjunction with a 35 mm camera to make photomicrographs of the graft unions. In this technique, slides were projected on the frosted glass screen of the microprojector and photographs were taken of the projected image.

Statistical Analyses

A Chi-square test showed no differences in mortality between clones and this permitted the use of clones as replications for the analyses of variance. A split design was used to determine differences in height growth and graft survival. Since plot values for survival percent were not normally distributed, an arcsin transformation was used for this analysis.

CHAPTER IV

RESULTS AND DISCUSSION

Mortality

Eighteen percent of all side grafts survived the first growing season as compared to 11 percent of all whip grafts. Twenty-two percent of the grafts made on non-dormant understocks survived as compared to 9 percent of all grafts made on dormant understocks. The summary of percent mortality is presented in Table 1. Thor (1965) and Churchwell (1965) obtained similar results in grafting yellow-poplar when grafts were made on non-dormant understocks. The best result, 38 percent survival, was obtained by using the side graft method on non-dormant understock and storing the grafts in the mistbench.

The analysis of variance of percent mortality as affected by dormancy of the understock, grafting methods, and storage treatments is presented in Table 2. At the 95 percent level of probability no significant differences were found between main treatments, sub-treatments, or in the interaction between treatments. However, two of the treatments, dormant versus non-dormant understock, and the interaction of dormancy and grafting method were significantly different at the 90 percent level of probability.

The scions of grafts in the mistbench broke dormancy and leafed out completely. Wilting of leaves and death of scions were readily observed for these grafts. Scions of grafts stored in the cooler did

Table 1. Summary of percent mortality with mean percent mortality shown for treatments

	Dormant Understock				Non-Dormant Understock			
	Side Graft		Whip Graft		Side Graft		Whip Graft	
Reps	Mist	Cooler	Mist	Cooler	Mist	Cooler	Mist	Cooler
1	77	90	100	100	71	86	100	92
2	94	96	80	100	50	88	89	96
3	95	88	77	98	65	78	43	84
$\bar{X}$	89	91	86	99	62	84	77	91

Table 2. Analyses of variance of percent mortality affected by dormancy, grafting methods, and storage treatments[a]

Source of Variation	df	Mean Square
Dormancy	1	754.21 N.S.
Error a	4	126.98
Graft Method	1	468.52 N.S.
Graft x Dormancy	1	1,241.78 N.S.
Error b	4	216.58
Storage	1	523.04 N.S.
Storage x Dormancy	1	2.52 N.S.
Storage x Graft	1	10.04 N.S.
Storage x Graft x Dormancy	1	241.07 N.S.
Error c	8	270.30
Total	23	

N.S. = Nonsignificant.

[a]Percent mortality analyzed using arcsin $\sqrt{\text{percentage}}$ transformation.

not break dormancy and any mortality that might have occurred could not be readily determined.

The mortality in the mistbench prior to outplanting was 39 percent for grafts on dormant understock. For non-dormant understocks this mortality was 61 percent. The apparent superiority of dormant understocks in the mistbench was probably due to their not being in an active state for as extended a period as non-dormant understock. The extended period of activity of graft unions on non-dormant understock may have allowed more necrotic tissues to develop within the callus line. Dormant understock had to go through a period of breaking dormancy before callus formation could begin. Consequently these grafts had a shorter period during which necrotic tissue could develop in the union. The presence of a necrotic layer in the graft union would reduce the already limited water transport through the callus and could therefore increase graft failure.

Other significant differences between dormant versus non-dormant understocks were not evident until after outplanting. Grafts made on non-dormant understocks had a higher percentage survival at the end of the growing season than did the dormant understock grafts. The lower survival of grafts made on dormant understock may have been caused by the period of inactivity that preceded the formation of callus and union while in the mistbench. It is possible that this period of inactivity allowed the contact layer to deteriorate and become partially necrotic. This would severely weaken the subsequent union by limiting the area which could successfully knit. In addition to causing poor knitting of graft components, the necrotic tissue would greatly reduce moisture and nutrient movement between graft components.

Since the grafts on dormant understock had less mature unions than did the non-dormant understock grafts, it is likely that moisture stress encountered under field conditions was above the water conducting capabilities of immature unions. This resulted in the failure of many unions of this type.

Side graft probably survived better because of the relatively greater contact area provided by this type of union and more opportunity to match cambial surfaces than in the whip graft. Since only a limited amount of water movement takes place through the callus bridge, increasing the area of this bridge would assist in increasing the amount of water available to the scion. This increase in available water would aid in early survival of grafts in the period prior to callus differentiation and during the establishment of vascular continuity between understock and scion.

A prime requisite for any successful graft union is the matching of cambial surfaces of understock and scion. Increasing the opportunity to match these cambial surfaces helps to insure more successful graft unions.

Prior to outplanting, 47 percent of the grafts in the mistbench were surviving. Survival of these grafts dropped to 10 percent at the end of the first growing season. This represents a 78 percent loss due to outplanting. Factors other than those already mentioned which appeared to have an effect on survival after outplanting included:

(1) planting depth, and (2) water stress. Outplanting was done by hand with a planting bar. It was observed that some seedlings were not planted as deep as they had been in the nursery bed. This resulted, in many cases, in death of the entire seedling. Water stress, brought about by a disturbed root system and transpirational pull on the scion leaves, may have been responsible for a large part of the field losses. Several methods could be utilized to alleviate this type of loss: (1) pruning; (2) bagging grafts with paper bags; (3) shading; (4) latex sprays or dips for the foliage; and (5) outdoor mist systems. Pruning was used in this investigation, but it may be necessary to use additional treatments to increase survival.

Height Growth

The mean height growth for grafts stored in the cooler was 28.0 inches as compared to 17.0 inches for grafts stored in the mistbench. A summary of height growth is presented in Table 3.

The analysis of variance of height growth for dormant versus non-dormant understock and storage treatments was limited to side grafts only because of the almost complete lack of data for whip grafts on dormant understocks (Table 4). No significant differences were found for understock condition or storage treatments.

The analysis of variance of height growth for graft methods and storage treatments on non-dormant understocks is summarized in Table 5. Differences in height growth for the storage treatments were highly significant. No other treatments or interactions showed statistical significance.

Table 3. Summary of mean height growth[a]

	Dormant Understock				Non-Dormant Understock			
	Side Graft		Whip Graft		Side Graft		Whip Graft	
Reps	Mist	Cooler	Mist	Cooler	Mist	Cooler	Mist	Cooler
1	13.7	20.5	---	---	19.0	24.7	---	40.0
2	---	---	27.5	---	23.2	39.0	5.2	---
3	---	20.8	25.8	---	7.0	24.2	10.9	38.4
$\bar{X}$	---	20.6	26.6	---	16.4	25.9	8.1	39.2

[a]Means computed only where a minimum of four observations were available.

Table 5. Analyses of variance of height growth for grafting methods and storage treatments

Source of Variation	df	Mean Square
Replications	2	39.60 N.S.
Graft Method	1	69.75 N.S.
<u>Error a</u>	2	56.72
Storage	1	875.01**
Storage x Graft Method	1	104.39 N.S.
<u>Error b</u>	4	43.16
Total	11	

N.S. = Nonsignificant.

** = Highly significant (P = .99).

In addition to different rates of growth, grafts in the two storage treatments also differed in the time when height growth took place. For the grafts stored in the mistbench, 33 percent of the total height growth was completed by July. Forty percent of these grafts had only grown one or two inches by this time and ceased growing for the remainder of the season. In contrast, less then 1 percent of the grafts stored in the cooler had finished height growth by July. Grafts taken from the cooler delayed appreciable height growth until August when 44 percent of growth occurred. Grafts stored in the cooler had a mean height growth of 12.8 inches in August as compared to 4.9 inches for grafts stored in the mistbench. Grafts stored in the cooler continued to grow more than did grafts stored in the mistbench for the remainder of the growing season.

The differences in amount of height growth may have been due to different levels of reserve carbohydrates. Kozlowski (1958) reported that height growth of many trees is made at the expense of stored carbohydrates rather than products of current photosynthesis. Since both understock and scion were non-dormant in the mistbench, the rate of metabolism would have been greater than that of the grafts in the cooler. Temperatures in the greenhouse would have increased metabolism, while temperatures in the cooler would have reduced the rate of metabolism.

High metabolic activity, resulting from high temperatures and completely active graft components could have drastically reduced stored carbohydrates in grafts stored in the mistbench. If this is the case, height growth potential of these grafts would have been reduced. The lower metabolic rate of grafts in the cooler would not have reduced the amount of stored carbohydrates to a much lesser extent and would result in a greater height growth potential.

Graft Failure

Several external symptoms such as abnormal defoliation, smooth break at the union, overgrowth of stock or scion, and excessive suckering, have been correlated with graft failures due to incompatibility. None of these symptoms were observed in this investigation. Hartmann and Kester (1968) state that high percentages of graft failure are often indicative of incompatibility. Although high graft failure was experienced in this study, factors other than incompatibility may have been responsible. Since observations were only made for one growing season, external symptoms of incompatibility may become evident in subsequent growing seasons.

Unlike external observations, internal examination of graft unions provided several clues to the reasons for variable performance of grafts and high mortality. Internal symptoms of incompatibility include: (1) heavy accumulation of parenchymatous tissue at the unions; (2) reduced mechanical strength of union; (3) severe vascular contortion in the union; and (4) necrotic areas in the union (Chang, 1938).

Figure 4 shows extensive areas of undifferentiated callus tissue in a side graft which exhibited slow field growth. In Figure 5 the same type of abnormality appears in a whip graft with slow field growth. Proebsting (1926) stated that this parenchymatous tissue served to interrupt the vascular connections. The limited movement of water and

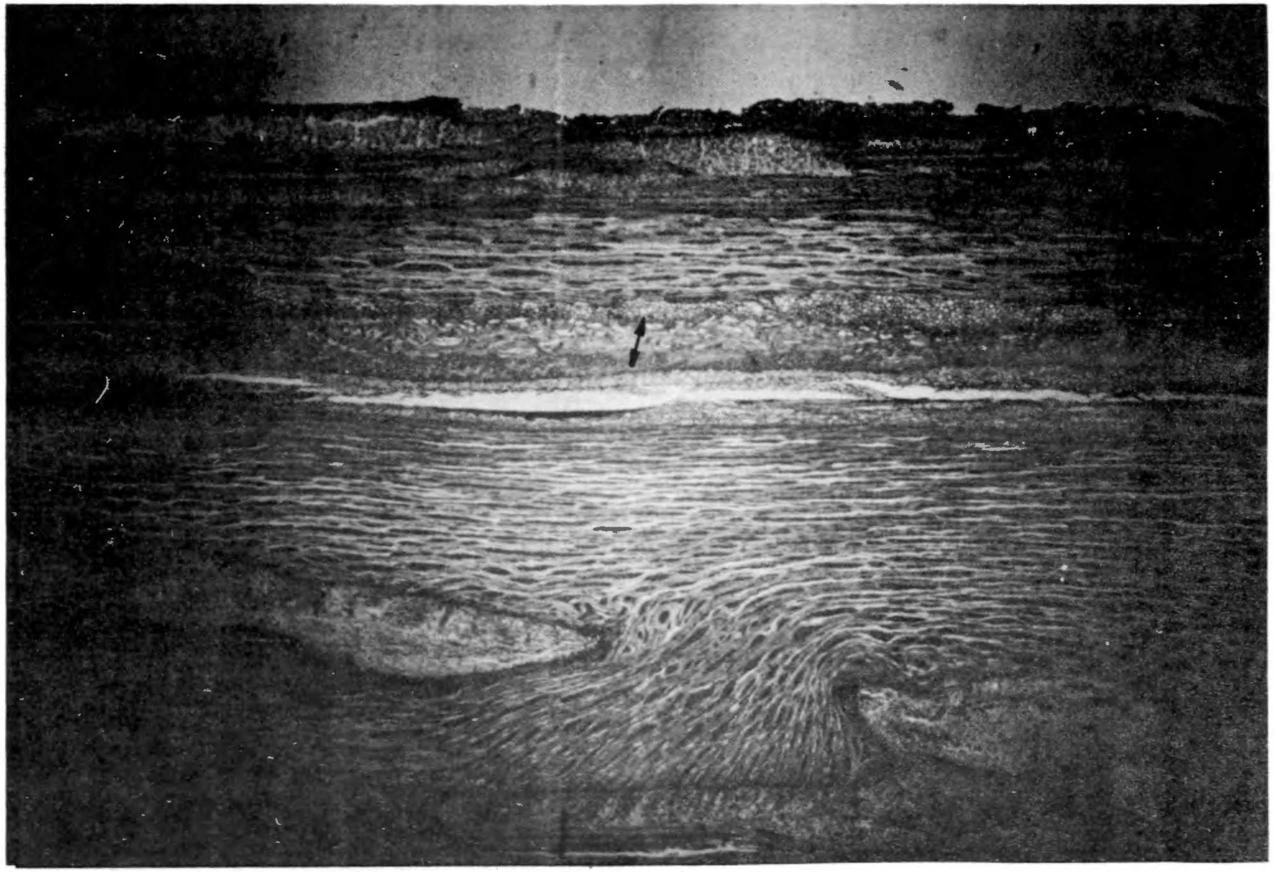


Figure 4. Longitudinal section of a side graft with poor height growth showing areas of undifferentiated callus tissue.

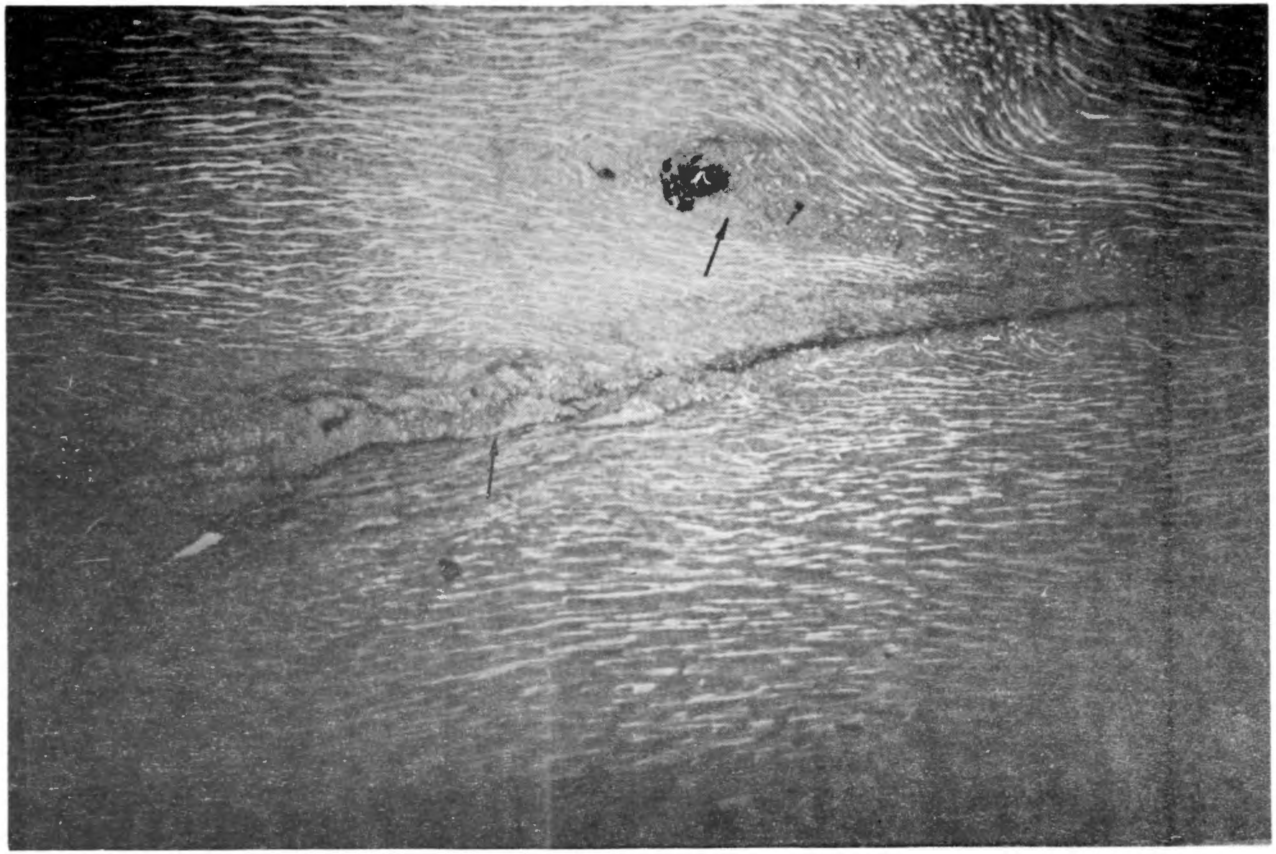


Figure 5. Longitudinal section of a whip graft with poor height growth showing areas of undifferentiated callus tissue.

nutrients brought about by this parenchyma would reduce the growth capabilities of scions.

In both side and whip grafts of vigorous growth, no extensive areas of callus tissue were observed. Figures 6 and 7 show the high degree of vascular continuity in rapidly growing side and whip grafts. These unions appear as a fairly distinct line with only moderate distortion of vascular tissue.

Reduced mechanical strength of graft unions was apparent in many grafts of slow growth. Knitting of tissue between graft components was spotty and the cut surfaces remained remarkably distinct and often unseparated by any callus. These unions were especially difficult to section and graft components often separated when cut. The absence of callus, which offers some mechanical support to the unions, places all physical stress on the limited area of vascular continuity. The distinctness of cut surfaces of stock and scion in a weak side graft, are shown in Figure 8. Figure 9 shows the lack of callus tissue in a whip graft union with the components becoming separated during sectioning.

Severe distortion of the vascular tissue may cause difficulties in translocation (Proebsting, 1928). This defect, although not as prevalent as other defects, was observed in a few cases (Figure 10). Distortion is here in the form of loops and whorls of vascular tissue in the union. It is doubtful that this type of defect commonly presented a serious problem to graft success.

The fourth defect observed in graft union sections was the presence of necrotic areas (Figure 11). Whether these areas will expand and

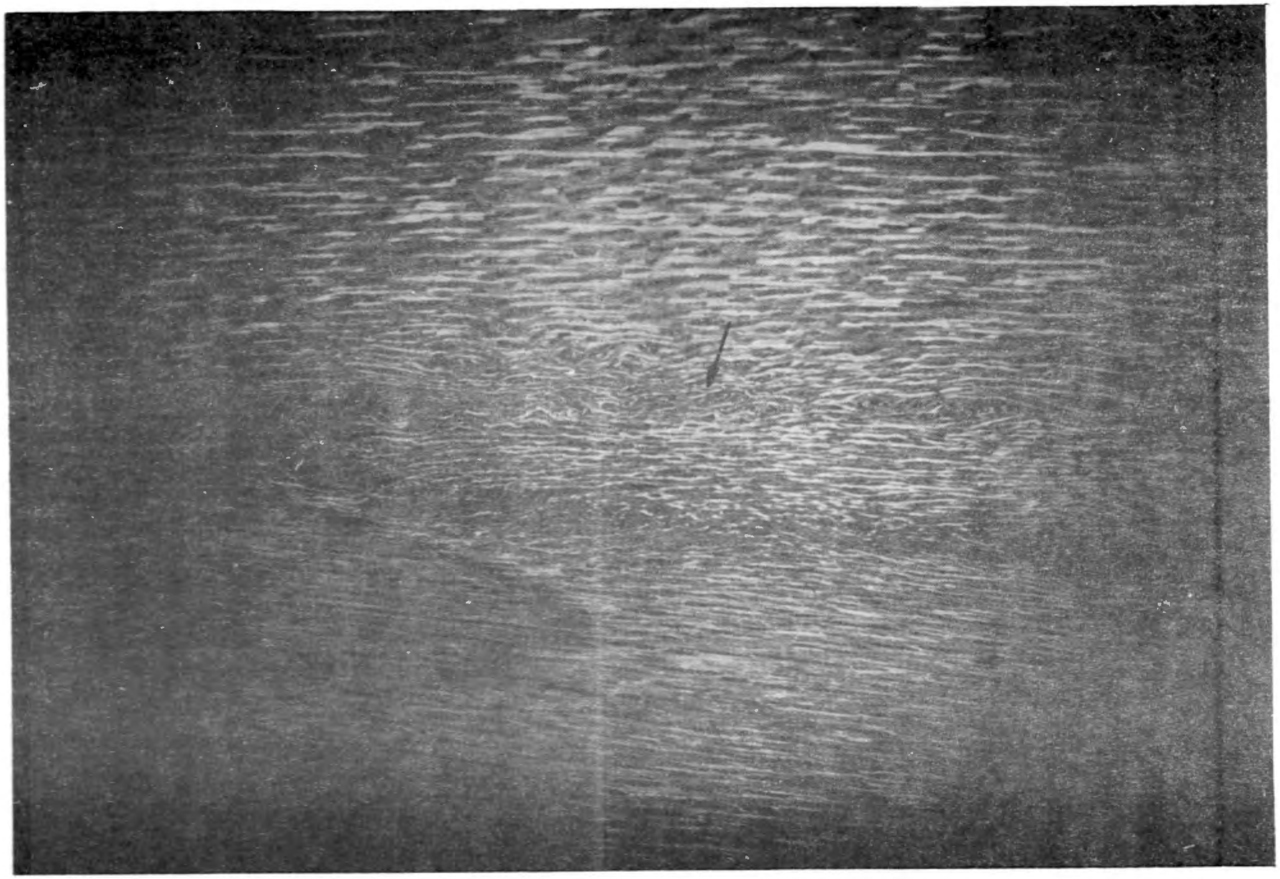


Figure 6. Longitudinal section of a side graft union with vigorous height growth showing a high degree of vascular continuity.

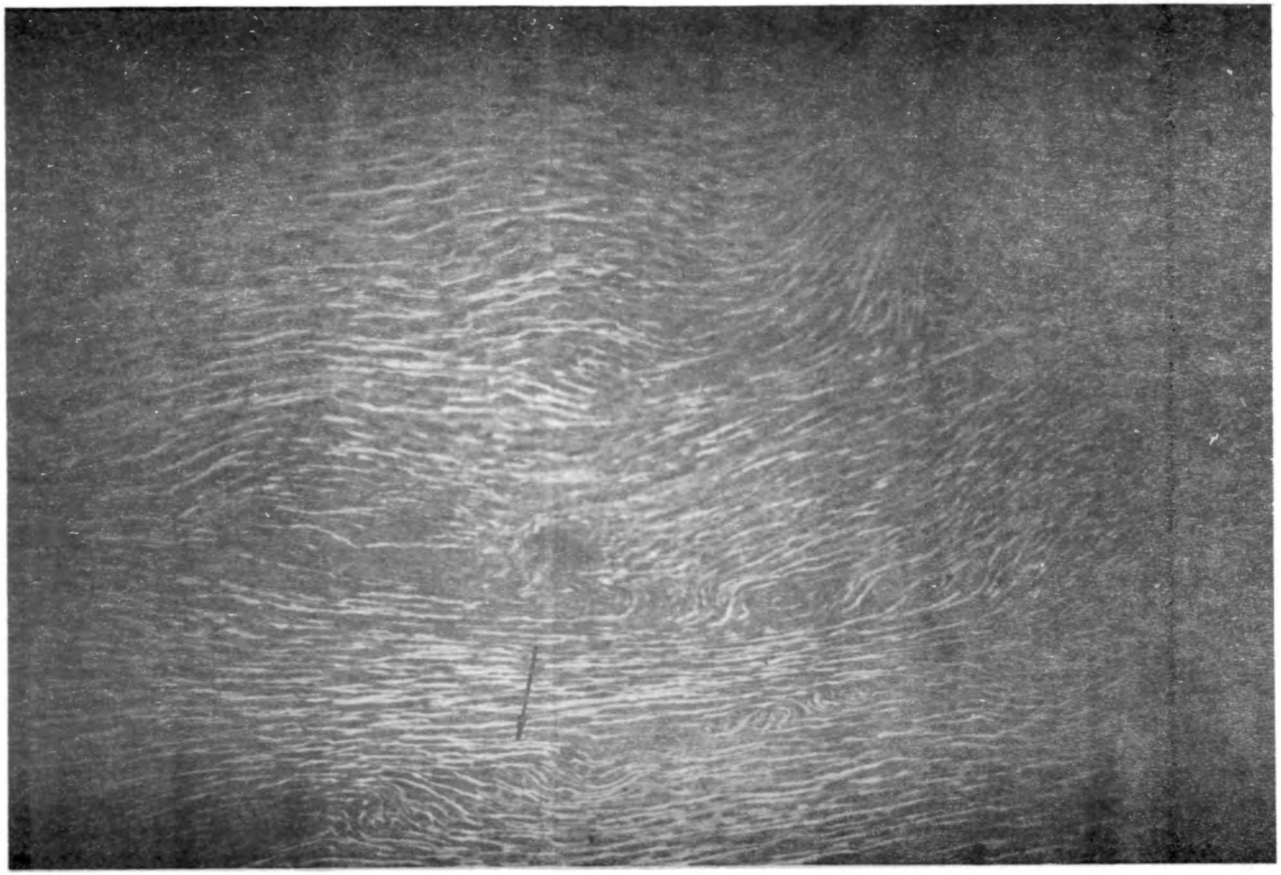


Figure 7. Longitudinal section of a whip graft union with vigorous height growth showing a high degree of vascular continuity.

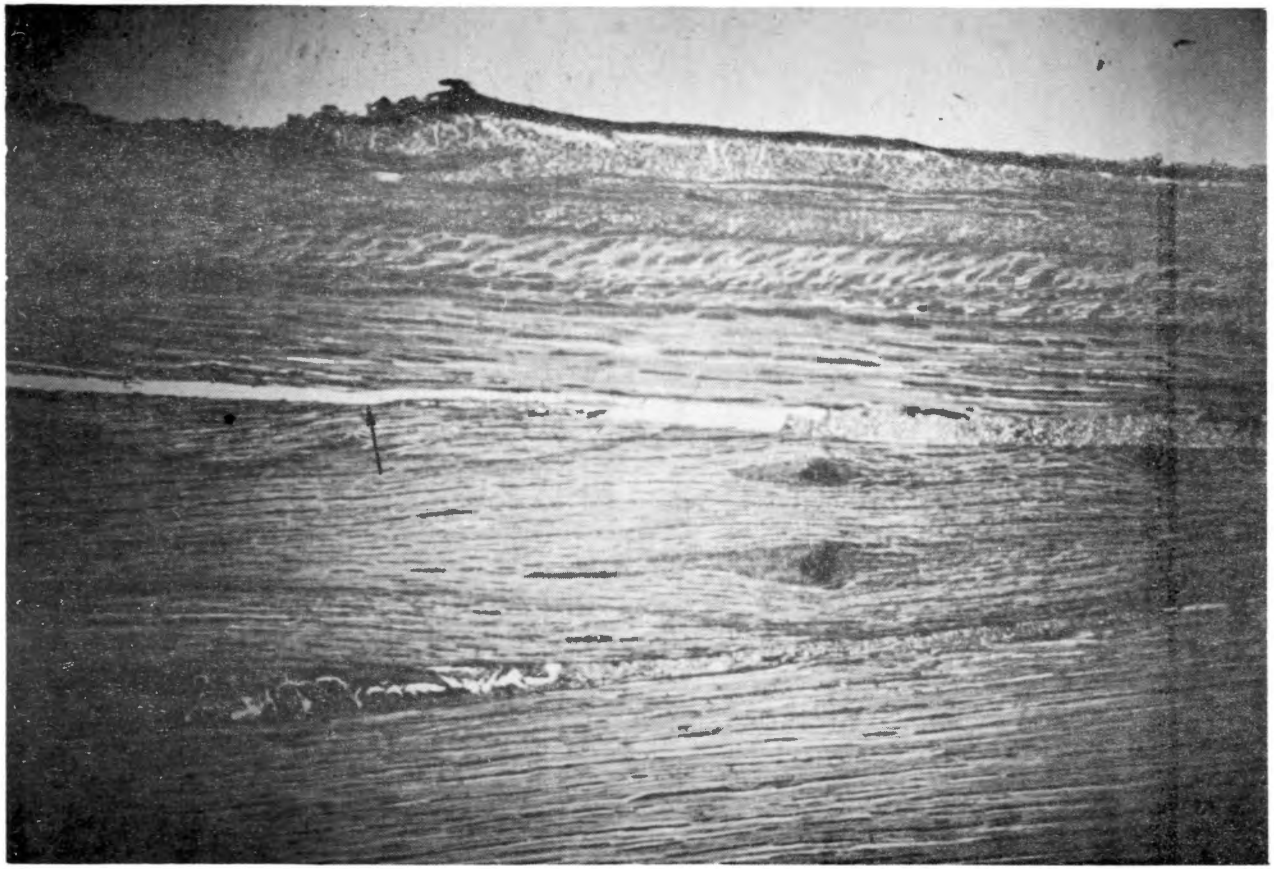


Figure 8. Longitudinal section of a side graft showing lack of callus between graft components.

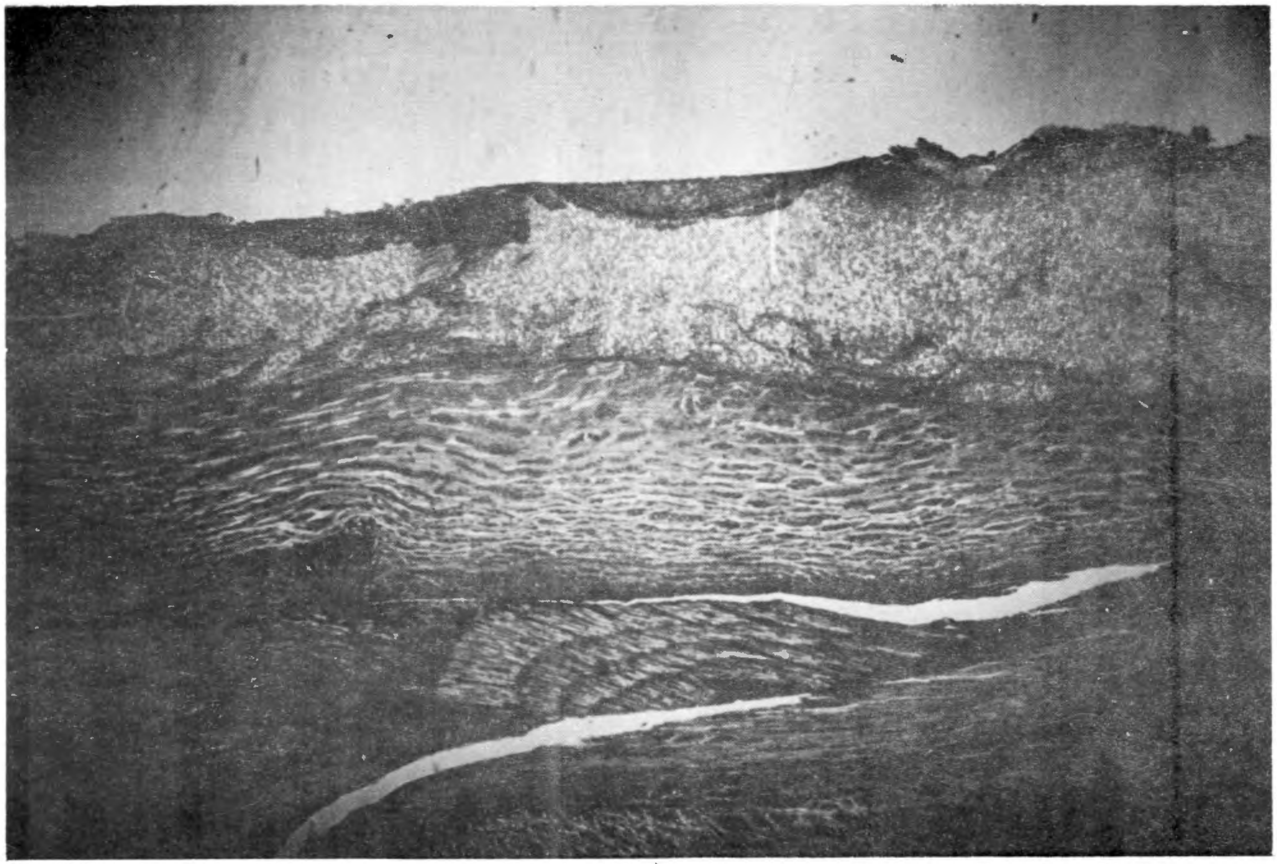


Figure 9. Longitudinal section of a whip graft showing lack of callus between graft components. Components separated during sectioning.

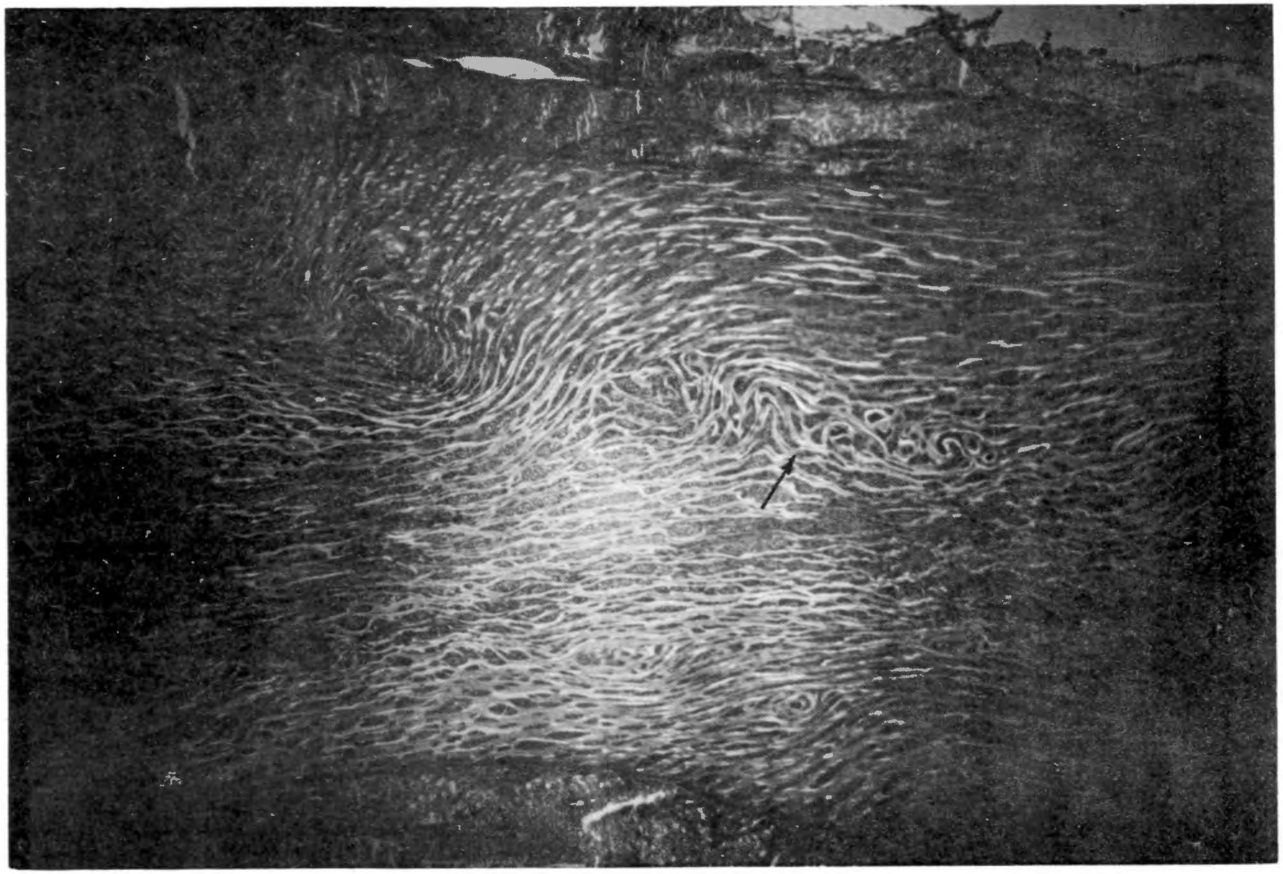


Figure 10. Longitudinal section of a whip graft showing distortion of vascular tissue.

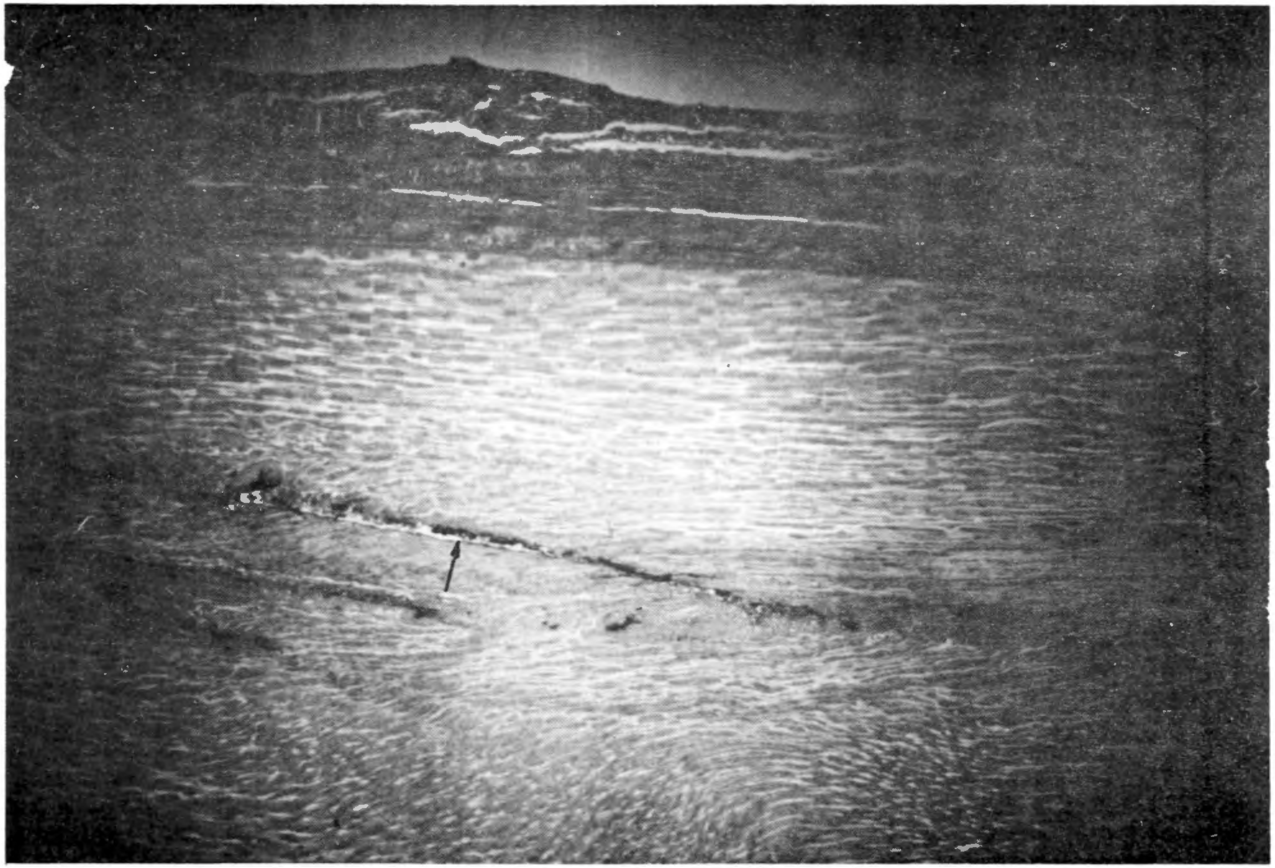


Figure 11. Longitudinal section of a ship graft showing necrotic areas in graft union.

further weaken the union is unknown. Hartmann and Kester (1968) suggested that this layer of dead cells consisted of cells crushed in making the grafting cuts and is gradually resorbed and disappears. However, this defect was regularly seen in both side and whip grafts with slow field growth. It may be that the necrotic layer has only a transitorily inhibiting effect on water and nutrient movement through the union.

CHAPTER V

SUMMARY AND CONCLUSIONS

Methods of bench grafting and storing yellow-poplar were evaluated in this study. An examination of sectioned grafts was made to evaluate graft unions for symptoms of incompatibility. Although clonal differences in graftibility have been reported for yellow-poplar, no significant differences were observed between the clones used in this study.

One of the treatments, dormant versus non-dormant understock, gave statistically significant differences in mortality. Twenty-two percent of the grafts made on non-dormant understocks survived the first growing season as compared to 9 percent of grafts made on dormant understocks. These results are similar to those reported by other investigators. The interaction of understock dormancy and grafting method was significant at the .05 percent level. The combination of non-dormant understock and side graft resulted in the best survival--27 percent.

Observable graft survival prior to outplanting was 34 percent for grafts stored in the mistbench. Percent of surviving grafts dropped to 22 at the end of the first growing season. This dramatic loss of grafts points out weaknesses in methods of outplanting and caring for the grafts. Care should be taken that seedlings are planted as deeply as they were in the nursery bed. Some method or a combination of methods should be used after outplanting to reduce water stress.

Several methods could be utilized to alleviate losses due to water stress: (1) pruning; (2) bagging grafts with paper bags; (3) shading; (4) latex sprays or dips for the foliage; and (5) outdoor mist systems. Pruning was used in this investigation, but it is apparent that this treatment alone was not sufficient.

Grafts stored in the cooler had a greater mean height growth than did grafts stored in the mistbench. Differences in height growth for the storage treatments were highly significant. No other treatments or interactions exhibited significant differences in height growth. It was noted that 40 percent of the grafts stored in the mistbench grew only one or two inches during the entire growing season. Less than 1 percent of the grafts stored in the cooler behaved in this manner. Poor growth was the only external symptom of incompatibility that was observed in this investigation. It is possible that other external symptoms of incompatibility might have appeared had this study been carried through more than one growing season. Future studies similar to this one should be conducted over more than one growing season in order to determine whether incompatibility in yellow-poplar can be related to other external symptoms.

Internal symptoms of incompatibility that were observed in sectioned grafts included: (1) heavy accumulation of parenchymatous tissue in the union; (2) reduced mechanical strength of union; (3) severe vascular distortion in the union; and (4) necrotic areas in the union.

In both side and whip grafts which exhibited poor field growth, extensive areas of undifferentiated callus tissue were observed. Neither side nor whip grafts exhibiting vigorous field growth had extensive areas of callus tissue. Reduced mechanical strength in many grafts exhibiting poor growth was disclosed by spotty knitting of tissue and often a lack of any callus between graft components. This type of defect was frequently associated with grafts of poor growth.

Severe distortion of the vascular tissue, although not as prevalent as other defects, was observed in some cases. A fourth defect observed in graft union sections was the presence of necrotic areas. These necrotic layers were frequently observed in conjunction with extensive areas of callus in the graft unions exhibiting poor field growth.

Further investigations of the vegetative reproduction of yellow-poplar should be directed towards developing methods of reducing high mortality, prior to outplanting, of grafts stored in the mistbench. Proper timing of grafting may have a very pronounced effect in obtaining high graft survival. Since side grafts on non-dormant understocks were found to produce best results, this method could be used in making grafts at intervals throughout the year. Graft unions should be sectioned frequently to describe the developmental stages in formation of unions.

The effect of cold storage on height growth also needs investigation. Techniques should be developed for hardening off the grafts stored in the mistbench before outplanting in the spring.

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