ANATOMICAL AND HISTOCHEMICAL VARIATIONS IN MALUS ROOTSTOCK CLONES AS FACTORS IN ROOT INITIATION

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Robert F. Carlson
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ABSTRACT

ANATOMICAL AND HISTOCHEMICAL VARIATIONS IN *MALUS* ROOTSTOCK CLONES AS FACTORS IN ROOT INITIATION

I. Effects of stem anatomy, etiolation and starch reserves on adventitious root formation in layered shoots

II. Effects of stem anatomy, wounding, and starch reserves on adventitious root formation in softwood cuttings

By

Steven L. Doud

Section I

Dwarfing *Malus* rootstock clones showed a range of rooting success when propagated as layers. Root emergence was largely confined to a nodal position near the lateral buds. Anatomical studies revealed nodal rooting to be closely associated with the parenchymatous bud and leaf gaps of the stem. The highest starch concentration was also noted in these tissues and in the outer ring of pith. Etiolation during the layering process increased stem starch content significantly and decreased the degree of sclerification, expressed by the ring of cortical fibers. Rooting success was negatively correlated with degree of sclerification. Etiolated stem cuttings produced roots in a seven day period of time in the mist propagation bed, while non-etiolated cuttings largely failed to send forth roots. Etiolation provided a stimulus to root initiation in the non-differentiated, starch-rich gap areas.

Steven L. Doud

SectionII

Dwarfing Malus rootstock clones propagated as softwood cuttings exhibited a range of rooting response. Root initiation was found to take place in meristematic tissues, such as the bud and leaf gaps, phloem rays, lenticels, and callus proliferation. Rooting success was inversely proportional to the degree of development of cortical fiber tissues. Shallow lateral wounding of cuttings improved rooting performance, most notably in the shy-rooting clones. A seasonal stem starch level fluctuation was noted, with maxima in late autumn and early spring and minima in mid-winter and during greatest extension growth. This pattern coincided with reported rooting response of hardwood cuttings during the dormant season.

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Steven Lorne Doud

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SECTION I

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LITERATURE REVIEW

A. Introduction

Plant propagators have worked for many years to develop various techniques for improving the rooting capabilities of desirable clones. As early as 1537, mention was made of the benefits of etiolation used as an aid to fruit tree propagation, according to Edbjerg (26). Etiolation results from light exclusion from a plant, or portion of a plant. The term "etiolation" is not uniformly defined in literature on the subject. Propagation techniques usually involve the normal development of leaves in the light above a darkened area of the stem. It is in this context that this investigation was undertaken in an effort to elucidate some of the effects of stem anatomy and of etiolation on rooting during the layering process. Layering, the primary method of propagation of clonal Malus rootstocks, was first demonstrated to be practical in 1927 by Knight, et al. (50).

The increased propensity of etiolated plant material to root has been well established. A concomitant inhibition of the rooting process by light has been noted by several researchers. Mevius (56) reported inhibition of rooting in Tradescantia when bases of the cuttings were exposed to light. Galston (29) found similar results in excised asparagus stem tips. Hackett (39) was able to root shoot tips of adult Hedera helix in darkness and low light, but not in high light intensity. Ryan (67)

reported light inhibition in several plant species and pointed out that roots may grow normally in light after formation. Of course, a number of plants appear to be completely light tolerant, as in aerial rooting, and rooting of cuttings in a jar of water.

Frolich (28) noted that rooting inhibition was not limited to light of a certain color. Smith (75) reported successful rooting of Clematis from nodes after exclusion of light by wrapping with black paper. Clematis is normally considered to be an internodal rooter. Several light-excluding techniques have been employed in reported experiments, making comparison of etiolated material difficult in some instances. It is generally reported that the greatest beneficial effect from etiolation can be gained by exclusion of light during early stages of shoot development. Knight et al. (50), in describing the stool method of propagation, found the greatest number of shoots produced when the plant crown was left uncovered until growth began. However, greatest rooting occurred when the crown was covered and shoots grew out through a layer of soil. Similarly, Gardner (30) found that the covering of Malus cultivar shoots from very early development was necessary in order to gain rooting performance when the shoots were taken as cuttings.

B. Etiolation and Plant Anatomy

Most early studies dealing with etiolation concentrated on anatomical relationships, and certain researchers continue to explore this area of interest. Most work has centered on

investigation of lignification and sclerenchyma tissue, stem internal organization, formation of root primordia, and root emergence from the stem.

1. Sclerification

The much-discussed theory of stem sclerification as a barrier to root production or emergence is rather closely tied to tissue juvenility and so is difficult to consider in a purely anatomical sense. However, correlation between extensive sclerification and loss of rooting capacity has been noted by several researchers. The presence of a sclerenchyma sheath has been confirmed in a number of difficult-to-root species.

Ciampi and Gellini (18, 19) investigated the mechanical impedance to root formation in Olea by a nearly complete ring of fibers and sclereids in the cortical region. Much better rooting was obtained from young shoots with little lignification. Mahlstede and Watson (53) in a Vaccinium rooting study noted that roots were bent, stopped, or emerged far from point of origin by a fiber sheath in the cortical region of the stem. Snyder (76) reported that the continuous ring of fibers present in Dianthus cuttings caused roots to grow downward and emerge at the basal cut end. Beakbane (7,8) found a close correlation between the degree of blockage of phloem rays by sclerenchyma tissue and the rooting capacity in Malus and Pyrus. However, the simple explanation of mechanical blockage was not thought to fully explain the observed rooting differences in this report.

An opposing view was taken by Sachs et al. (69). No simple relationship between rooting and sclerenchyma sheath was found in tests with Olea, Pyrus, and Prunus. Shy-rooting cultivars were thought to form fewer and slower developing root initials, which were not unduly constricted by fibers. Unfortunately no explanation of methods or literature review was given to elucidate these findings. Girouard (35) concluded that fiber formation was not the main cause of poor rooting in mature Hedera helix.

A reduction in sclerification due to etiolation has been reported in a number of research projects. Priestly and Ewing (64) noted less xylem and sclerenchyma development in etiolated stems of Phaseolus and Solanum. Reid (66) reported less sclerification and improved rooting in etiolated cuttings of Cinnamonmum. A reduction in thickness of individual cell walls with a corresponding increase in protoplasmic content was also illustrated. Selby (73) reported a decrease in sclerification in a variety of etiolated material. In a recent study, Herman and Hess (42) noted less sclerification and differentiation in etiolated Phaseolus tissue.

2. Internal Organization

Priestly and Ewing (64) and Priestly and Swingle (65) reported actual changes in internal structure of etiolated Vicia and Solanum stems. Certain root-like characters such as development of an endodermis and Casparian strip were seen in etiolated stems. However, roots grown in light retained their usual

anatomical characters. Other researchers have taken exception to these reports. No endodermis was noted in etiolated Phaseolus and Hibiscus by Herman and Hess (42), although there was indication of a starch sheath. Frolich (28) reported no evidence of an endodermis in etiolated Persea shoots and no striking anatomical differences between shoots that would root and those that would not.

3. Root Primordia

In any discussion of rooting procedures it is important to analyze the point in the rooting process which is being adversely or beneficially affected by a given technique. It would be desirable to ascertain whether a technique such as etiolation promotes root primordia formation or merely provides optimal conditions for elongation of pre-formed primordia. Some evidence suggests that the main effect of etiolation is promotion of root initiation. Of course, a number of readily-rooted plants have been shown to produce latent primordia under normal growth conditions. The propagator, however, is mainly concerned with improving the performance of shy-rooting clones.

Gardner (30), using difficult-to-root Malus cultivars, concluded that the large improvement in rooting response of etiolated stem sections taken as cuttings was due to the presence of preformed primordia. The prompt three-to-six day root appearance suggested that primordia were formed during early development in the etiolated portion of the stems and removal of the cutting

from the mother plant provided conditions favorable for primordia elongation. Herman and Hess (42) found root primordia present in etiolated Phaseolus stem tissue, with none observed in non-etiolated tissues. Etiolation not only strongly predisposed the plants to root formation, but root initials were actually formed prior to supplying the conditions favorable to root elongation. In both of the above experiments root primordia could be visually noted as slight protuberances on the etiolated stem surfaces.

4. Root Emergence

Actual root emergence from layered shoots and cuttings is a subject which merits attention in examining the effects of etiolation. Several researchers have reported a characteristic pattern of appearance of adventitious roots at nodes and from bud and leaf gaps in etiolated material. This phenomenon appears to differ from the usual response in cutting propagation, where roots often appear from callus proliferation or at other points near the base of the cutting. This point was raised by Knight and Witt (49), who noted that etiolated Prunus shoots produced most roots laterally along the stem, with very little callus formation, while non-etiolated tissue typically callused and produced roots at the cutting base. One theory holds that etiolation causes root initial formation at a very early stage of development, while cutting propagation forces initial formation in new meristematic tissue. Under this scheme, layering as a form of

propagation would not consitute true regeneration, but the extention of already-differentiated initials.

Sudds (81) showed that most of the root primordia in Rubus cuttings arose from the branch and leaf traces of the buds or in the vascular supply of the leaf subtending the bud before petiole abscission. Priestly and Swingle (65) reported the leaf axil or "leaf insertion" to be the point of root emergence in etiolated tissue. Carlson (12) concluded that pre-formed, dormant root primordia in Salix characteristically formed in phloem ray tissue in any of five positions, all centered around the node and bud trace area. Mahlstede and Watson (53) reported that adventitious roots in Vaccinium usually emerged from bud gaps at the node. Gellini (34), in an experiment using etiolated Ficus shoots, reported that rooting was fastest and most prolific from basal nodes, with no rooting noted from internodes. Cummins and Fiorino (22) found "root germs" developing as visible swellings just above axillary buds on MM 106 rootstock treated with Ethrel to promote leaf abscission.

C. Etiolation and Plant Physiology

The selection of plant material is of prime importance to the propagator, in that he must be aware of the physiological status of the mother plant to be sure of obtaining material with optimum rooting potential. Such factors as juvenility, meristematic activity, degree of tissue differentiation, active top or root growth, and water stress may affect the rooting

performance. It is obviously not possible to divorce the subject of juvenility and tissue differentiation from the chemical and hormonal balance of a plant, but the two topics will be considered separately for the sake of clarity.

1. Juvenility

It has been well established that juvenility of plant tissue results in improved rooting performence. Young, vigorous plants should provide more propagation success than mature speciments. Grace (38) observed that even areas within a single plant vary in rooting capacity, as Picea cuttings from the more juvenile lower portion of the tree were greatly superior in rooting to those from the top portion. Hess (44) reported similar results for Thuja, Juniperus and Fagus. The juvenile form of Hedera helix rooted more readily than the mature form. Also, one, two, and three year old Pinus seedlings showed a steady trend toward decreased rooting capacity. Further information was provided by Hess (43) in determining juvenility patterns. Euonymus coloratus in juvenile form produces brilliant autumn foliage, while much less color is evident in plants which are allowed to become mature. Fagus expresses juvenility by retaining leaves well into the winter on the more juvenile lower portion of the tree. Scions taken from the mature portion will retain mature characters after grafting. Heavily pruned Malus will produce juvenile "water sprouts" from the base of the tree. These observations suggest the possibility of rejevenating mature plants to produce material which would root more readily.

Muzik and Cruzada (59) offered a chemical explanation to the juvenility question by noting that repeated budding of juvenile Hevea onto mature shoots caused a gradual acquisition of rooting capacity in the normally non-rooting mature shoot. A possible accumulation of root promoting substance was thought to account for these results. Beakbane (8) reported a gradient in ease of rooting of shoots in ten year old seedling Malus. The lower shoots produced a growth habit, leaf shape, and cell structure typical of the juvenile condition, while growth from the topmost, fruit bearing branches resembled that of adult trees. Baldini and Mosse (3) reported the superior rooting characteristics of juvenile shoots arising from sphaeroblasts - small nodules with meristematic capacity in the cortex area on mature Malus.

2. Tissue Differentiation

Rooting success may vary on a seasonal basis, being partially dependent on stem tissue differentiation and maturation. The presence of a quantity of non-differentiated cells may provide a source of meristematic activity, leading to root initiation. Etiolation helps to maintain a condition of lower differentiation of stem tissues, as well as the previously-noted reduction in lignification.

Argles (1) noted that the highest root-forming capacity is present when there is activity among lateral or intercalary meristematic tissue in plants with relatively low cell differentiation. Girouard (35) outlined four distinct phases in

formation of adventitious roots: dedifferentiation or remeristematization; initiation or inception of root initials through cell division; differentiation as tissues give rise to root primordia; and elongation or extension. Stoltz and Hess (79), in a study of the effect of girdling upon root initiation, attributed a portion of the beneficial effect to an increase in the amount of parenchyma tissue above the constriction. Herman and Hess (42) reported that etiolation caused an increase in the amounts of parenchyma cells, making a greater meristematic potential available for root initial formation.

D. Etiolation and Plant Nutrition

Considerable evidence suggests that the nutrition of the stock plant exerts a strong influence on root initiation and development of the propagules. The level of carbohydrate reserve, and starch in particular, has been correlated with rooting capacity by a number of researchers. This seems reasonable in that root initiation and proliferation would be totally or partially dependent upon stored energy reserves in each of the various propagation methods. For example, Doud and Carlson (24) noted that excessive root elongation of hardwood cuttings prior to planting led to a poor survival rate as compared with cuttings with minimum root elongation. Nutrient depletion may account for this phenomenon. Few results have been published as to the effect of etiolation on starch levels in the stem.

As early as 1918, Kraus and Kraybill (51) demonstrated that the number of roots produced by Solanum cuttings was related to the carbohydrate-nitrogen ratio. Cuttings with high C/N ratios produced the most roots. Starring (77) found similar results with Solanum and Tradescantia. Carlson (11) correlated level of starch present with rooting success in two cultivars of Rosa. However, Brandon (9), in a similar experiment with several Rosa cultivars, could find no simple correlation between starch content and ease of rooting.

Preston et al. (62) reported that a high level of nitrogen in comparison to carbohydrates was detrimental to rooting in Azalea softwood cuttings. Kelley (47) noted that the C/N balance is influenced by applied fertilizer and stage of development of current season's growth. The carbohydrate reserve can be increased by more efficient photosynthesis (illumination), reducing nitrogen supply, and/or withholding water. Priestly (63) studied the seasonal changes in carbohydrate resources of Malus cultivars on clonal rootstock and concluded that starch and sugars form a reserve "pool" which is capable of supplying both energy and structural materials.

Samananda et al. (70) reported a positive relationship between carbohydrate content and ease of rooting in different clones of Chrysanthemum. Using Chrysanthemum clones of markedly different rooting capacity, Stoltz (78) found an excellent positive correlation between carbohydrate content and root

initiation. No differences in internal auxin levels were noted and the reduced root initiation in difficult-to-root clones was attributed primarily to a low level of total carbohydrates.

Webster and Van't Hof (90) cultured meristems of Pisum root, which failed to proliferate in the absence of a carbohydrate source. It was suggested that the carbohydrate source increased rates of RNA and protein synthesis, both of which were prerequisites for DNA synthesis and cell division in the root initials. Molnar and LaCroix (58) found a positive correlation between starch content and rooting success in Hydrangea. Starch was found to disappear from the phloem and xylem rays in close proximity to the developing root primordia. It was theorized that starch plays an important role in adventitious root development, providing the energy and substrate required for the execution of the metabolic processes of root initiation.

The literature is not clear as to the effect of etiolation on the starch status of the plant stem. Herman and Hess (42) reported etiolated Phaseolus tissue to be low in starch. However, the entire plant was grown in the dark, making a low starch level entirely predictable. Smith (75) reported lowered starch levels in etiolated Clematis tissue. This determination was apparently made by means of a simple phloroglucin staining technique, not a quantitative method. No conjecture was given as to how these findings contribute to the noted rooting increase in the etiolated tissue. Reid (66) speculated that internal food

reserves become more available in etiolated tissue, but gave no data which would apply to the issue in question. Beakbane (6) reported a higher starch level in below-ground tissue of mounded clonal rootstocks of Malus.

Stoltz and Hess (79) and Carlson and Yu (15) reported a major increase in carbohydrate reserves above a girdling cut on Hibiscus and Prunus stems, respectively. These results would seem reasonable in light of the disruption of transport through the phloem elements. However, girdling in the light was found to enhance the ability of a cutting to intiate roots but not to cause formation of root intials while the shoot remained attached to the mother plant. These findings would suggest that carbohydrate reserves are not wholly responsible for root intiation. Since root initials are reported to be formed during the etiolation process, another factor must be produced which contributes to the actual formation of root initials.

D. Etiolation and Chemical Balance.

Much recent work in rooting technique has centered on the chemical and hormonal balance in various plant materials. Of course, the efficacy of endogenous auxin in adventitious rooting and many other plant functions has been well established. Much work has been devoted to identifying chemical cofactors - promotive and inhibitory - which affect the performance of auxin. Studies have attempted to explain the notable differences in ease of rooting among species and clones on the basis of differing

endogenous levels of rooting cofactors. Much work remains to be done in devising more definitive laboratory techniques to isolate and identify the minute quantities of compounds in question. Etiolation has been shown to affect chemical balance in plant tissue and from this area of interest may come results which will resolve the largely-unexplained effects of etiolation in root promotion.

Herman and Hess (42) reported that etiolated and non-etiolated tissues of Phaseolus contained approximately the same levels of endogenous auxin, but rooting proved to be dramatically higher in the etiolated tissue. The most likely explanation advanced was that the etiolated tissues contained a higher level of auxin synergists. Although not sensitive, the techniques showed a slightly higher cofactor level in some etiolated plants. Hackett (39) found no difference in methanol-extractable rooting cofactor between etiolated and non-etiolated tissue of either juvenile or adult ivy, and there was no rooting response of adult shoot tips to extracts from etiolated shoots. It was suggested that a suitable extraction technique had not been perfected to affect the cell fraction containing the cofactor(s). Frolich (28) found no evidence for transmission of rooting stimulus up or down from an etiolated section of stem. This was shown by growing a shoot with a ring of tissue from which light had been excluded. When the shoot was placed in favorable rooting conditions initials formed only in the area which had the dark treatment. Several researchers have noted that etiolated portions of plants show

effects of light exclusion, even though other parts of the same plant are not etiolated.

Van Overbeek and Gregory (88) found that the combined action of applied auxin and a factor or complex from leaves was necessary in order to induce rooting in a difficult-to-root variety of Hibiscus. The rooting factor could only be supplied by the leaves of an easy-rooting variety which had been grafted onto the difficult-to-root variety. The factor was thought to be hormonal, nutritional, or both. In a later paper, Van Overbeek, et al. (87) reported that the leaves of the easy-to-root variety exerted their influence in the dark as well as in the light. The root-forming factors could be entirely replaced by supplied sucrose and nitrogenous substances in the easy-to-root variety. This piece of information led the authors to conclude that there was not necessarily a special hormonal root-forming substance produced by the leaves of the easy-to-root variety. This does not entirely explain the impotency of leaves of the difficult-to-root-variety, however.

Ryan et al. (68) presented evidence at variance with Van Overbeek, using easy-to-root cuttings of Citrus, Avocado, Camellia, and Hibiscus to see if scion leaves exerted any influence on rooting. The authors found the ease or difficulty of rooting to be determined solely by the variety or species forming the stock portion of the graft. Leaves on scion or stock, or both, appeared to exert no influence on typical rooting

pattern of the tissue from which the roots arose. Mes (55) reported similar findings with Citrus cuttings on which had been grafted rings of bark from difficult- and easy-rooting cultivars. In all cases, the rooting was typical of the original tissue and not influenced by leaves of a different cultivar.

MATERIALS AND METHODS

Four dwarfing rootstock clones of differing size control and rooting potential characteristics were chosen for this study: M.9, M.26, M.2, and MM 106. Rooted shoots were obtained from beds of layered stock of uniform age, growth conditions, and cultural treatment. Mounding of the shoots was accomplished throughout the growing season in order to assure early etiolation of the developing tissue, the bases of which were well rooted by early autumn. Figure 1 shows a view of the mounded layers.

The layered rootstock was located at the Michigan State University Horticulture Research Center at East Lansing. The soil type was sandy loam, and supplemental irrigation was used periodically during the growing season. Mounded shoots of uniform 1.0cm diameter were harvested for use in the experimental procedure. Similar shoots which had not been mounded during the growing season were used for a non-etiolated comparison. Data were collected for the various clones outlining average number of roots per shoot and typical point of emergence of the roots from the stem. A portion of the nonrooted, etiolated stems was

Figure 1. Layered rootstock clones at the Horticulture Research Center.

A) MM 106 Layered shoots in late summer.
B) MM 106 Rooted shoot in field.
C) M.9 Rooted shoot in field.

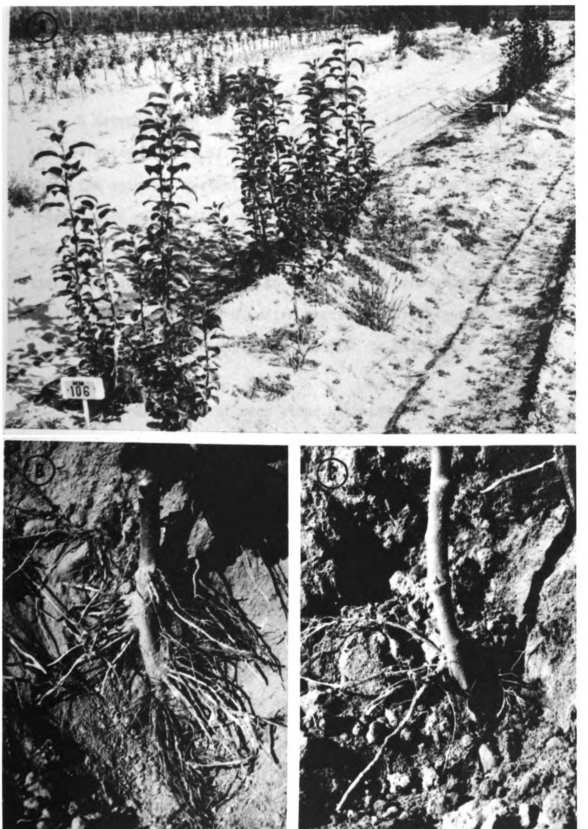

Figure 1

prepared as cuttings to be compared with non-etiolated cuttings of the same clones. The cuttings were placed in a mist rooting chamber.

A determination of internal levels of starch was made for the collected samples of ten etiolated and non-etiolated shoots, using the anthrone reagent method of Clegg (20). One gram of fresh tissue per sample, from the basal 5.0cm of the shoot, was homogenized in ethanol and treated with perchloric acid for starch extraction. The extract was measured chromatographically against a glucose standard, after reaction with an anthrone solution. Absorbance readings were converted to milligrams starch per gram fresh tissue weight.

Three replicates of each clone were used and two readings from each replicate made on a Bausch and Lomb Spectronic 20 Spectrophotometer. The starch test was conducted on two different dates in order to note change in level during the autumn maturation period. A complete stem cross section of tissue was used in each case and the extraction procedure began as soon as possible after collection of plant material.

Anatomical examination of the starch content of etiolated material was accomplished by sectioning of live unimbedded tissue to 40 micron thickness on an American Optical sliding microtome. The sections were killed in boiling water for 30 seconds, decolorized in cold methanol, and stained for starch with dilute iodine solution, after Gates and Simpson (33) and

Molnar and La Croix (58). The mounted sections were examined microscopically and individual stem cross sections photographed by means of an Olympus Photomicrographic system.

Further anatomical evaluation was provided by sectioning stem lengths killed and fixed in formalin-acto-alcohol (FAA). A safranin-fast green double stain, as outlined by Sass (71), was used to differentiate tissue types for microscopic examination.

RESULTS AND DISCUSSION

The rootstock clones showed a large range in number of roots produced per layered shoot (Table 1). Although all the shoots harvested for observation had rooted to some degree, it is obvious that the shy-rooting M.9 and M.26 pose significant problems for the nurseryman in terms of getting sufficient roots to produce a viable tree which is well anchored on all sides.

It is important to note that nearly all roots on the layered shoots emerged at a nodal position near a bud. This phenomenon was consistent with all layered clones observed, although there was a slight trend to more nodal rooting in the shy-rooting clones (Table 1). Roots not counted as nodal typically emerged some distance away from the bud, either above or below, or on the opposite side of the stem.

Visual evidence of nodal root emergence is presented in Figures 2 and 3. Roots typically emerged in close proximity to, or precisely through, the bud and adjacent leaf scar at the

Table 1. Rooting response and degree of sclerification of four Malus rootstock clones propagated by layering.

Clone	Roots per shoot	% at node	% sclerification[y]	
			non-etiol.	etiol.
M.26	13.4 a[x]	91.8	54.6	44.0
M.9	17.5 ab	94.7	56.2	43.4
M.2	27.4 c	88.5	50.5	40.5
MM 106	58.6 d	86.3	34.8	30.1
Ave.		90.3	49.0	39.5*

[x]Means in a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

[y]% of phloem rays blocked externally by fibers.

Figure 2. Nodal rooting pattern of four layered rootstock clones.

A) M.2 and MM 106.
B) M.26 and M.9.

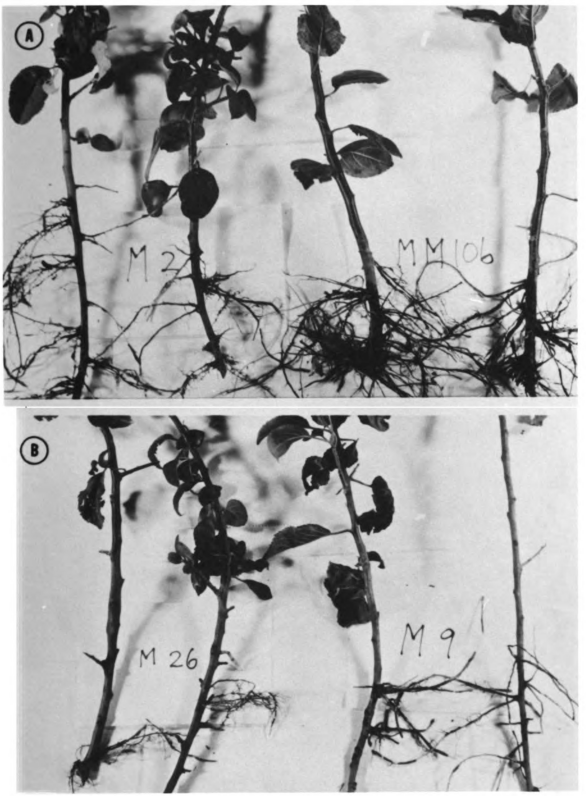

Figure 2

Figure 3. Nodal rooting pattern in layered shoot of M.2.

Figure 3

node, suggesting a strong influence of these stem tissues on root initiation. The same pattern held true for all four clones, with M.2 and MM 106 merely producing a much greater number of roots from the same area.

Propagation by layering employs a number of procedures which differ significantly from those used in cutting propagation. Basal stem tissue from which roots arise is etiolated during the growing season by the mounding process, which also provides optimum environmental conditions for ultimate root emergence and elongation. Shoots remain attached to a mother plant with extensive root system, and with active leaves on shoots above the mound. No exogenous source of root-promoting hormones is available and no wounds are made to promote superficial meristematic activity. Therefore, roots must arise from an internal tissue with sufficient food reserves, hormone supply and meristematic activity present.

It is apparent that some semantic distinction between root formation in layers and cuttings would be helpful. Van der Lek (86) first distinguished two general types of root formation: Morphological roots and wound roots. Morphological roots were defined as forming within intact stem tissues and ordinarily emerging at right angles to the vertical axis of the stem. Wound roots typically arose at basal ends of cuttings and from callus tissue masses. This concept was supported and elaborated by Stoutemeyer (80) and Garner (31). Although it is not certain

that all roots can be catagorized in this fashion, further discussion will make use of this distinction.

It would be of great interest to pinpoint the effect of etiolation on root initiation. Data in Table 2, in a trial of cuttings taken from etiolated and non-etiolated portions of layered shoots, show striking differences in rooting success. Both percentage rooting and number of roots per shoot were higher in the cuttings which had been buried with earth for a portion of the summer, but which showed no visible rooting when placed in the cutting mist bed at the start of a four week period. This would seem to indicate that etiolation causes formation of root initials in the stem, which then may emerge with proper environmental conditions. The cuttings were dormant when placed under mist and some roots appeared within one week -- much sooner than initiation could have occurred in the propagation bed. The great majority of non-etiolated shoots showed no signs of rooting in four weeks. Most roots again emerged from the nodal bud area. No root-promoting hormones were supplied. Figure 4 shows the two types of cuttings.

These results tend to confirm the ease with which etiolated materials have been rooted in several previously-mentioned studies, and elucidate reasons for the usual success of layering techniques. No evidence of pre-formed root primordia in Malus without etiolation has been found, with the possible exception of

Table 2. Rooting response of etiolated and non-etiolated cuttings of two Malus rootstock clones.

Clone	% Rooting	Roots per rooted shoot	% at node
MM 106			
etiolated	100.0 a[X]	12.1 a	93.2
non-etiolated	13.3 b	0.7 b	80.5
M.26			
etiolated	87.5 a	6.7 a	96.1
non-etiolated	6.8 b	0.2 b	100.0

[X]Means in a column followed by the same letter are not significantly different at the 5% level.

Figure 4. Cuttings from layered shoots of M.2, after four week mist rooting treatment.

A) Non-etiolated shoot portion.
B) Etiolated shoot portion with nodal root development.

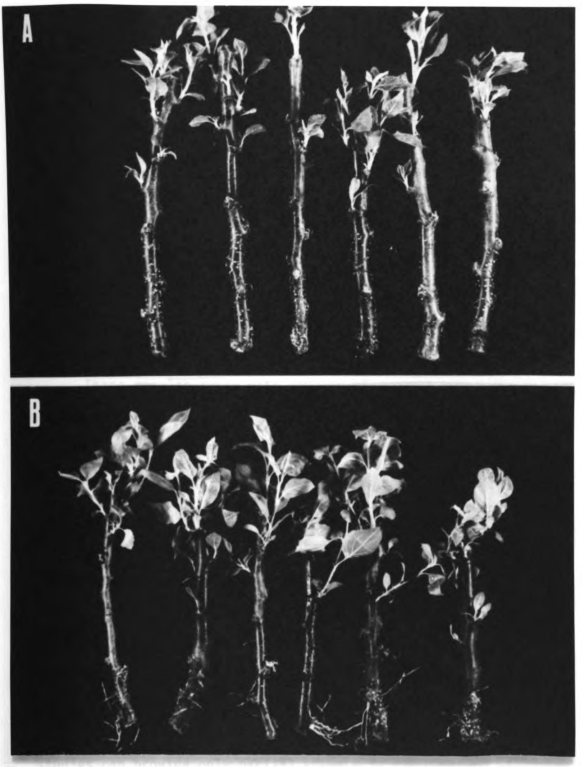

Figure 4

the "burrknot" phenomenon.[1] It is evident that the layering technique involves both the exclusion of light from stem tissue at an early stage of development, and maintaining proper temperature and humidity conditions for root emergence and extension.

Degree of sclerficiation of stems is outlined in Table 1. A definite trend of greater sclerification in shy-rooters can be seen, and percent sclerification proved to be correlated negatively with degree of rooting (r=-0.94). The effect of etiolation on sclerification can also be noted. A significant 10% decrease in sclerification occurred in the etiolated tissue as compared to noncovered shoots grown similarly.

These results agree with studies of several researchers (8, 18, 19, 53 76). It is difficult to determine whether fiber tissue is associated with rooting in a cause or an effect manner. It is rather doubtful that the maximum recorded 56% complete fing of fibers could block root emergence to a significant degree. It must also be noted that the fiber ring is not present across the bud area where most roots emerged. Figures 5 and 6 illustrate the characteristic pattern of fiber development Note the long tapered shape, with thick cell walls.

Further data collected from very difficult-to-root Pyrus and Malus cultivars extended the preceding information, as readings as high as 90% blockage were recorded. Anatomical studies can provide only partial answers to the question of

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[1] Swingle (83) defined burrknots as localized masses of root initials protruding on stems of easy-to-root clones.

Figure 5. Stem sclerification in MM 106. Tangential longitudinal section of stem, double stain, polarized light, x 50.

(Legend: F=fibers, P=phloem, X=xylem)

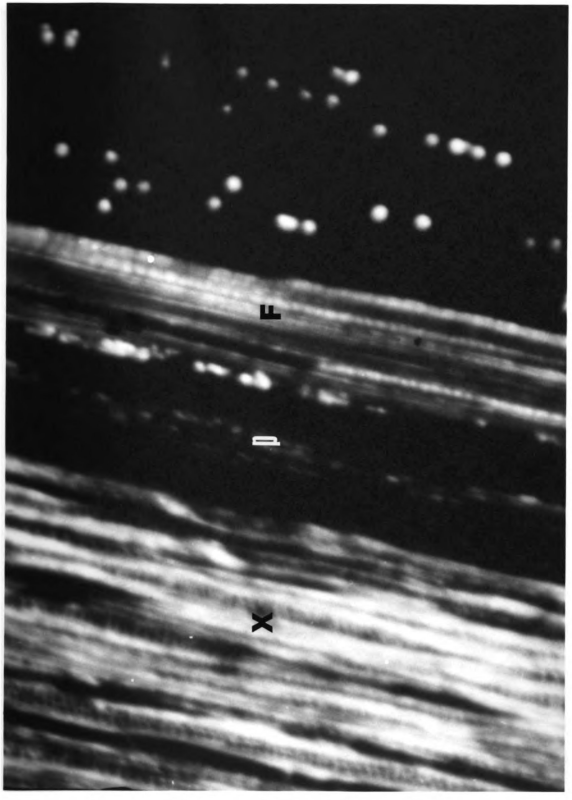

Figure 5

Figure 6. Stem sclerification in MM 106. Transverse section of fibers, double stain, polarized light, x 500.

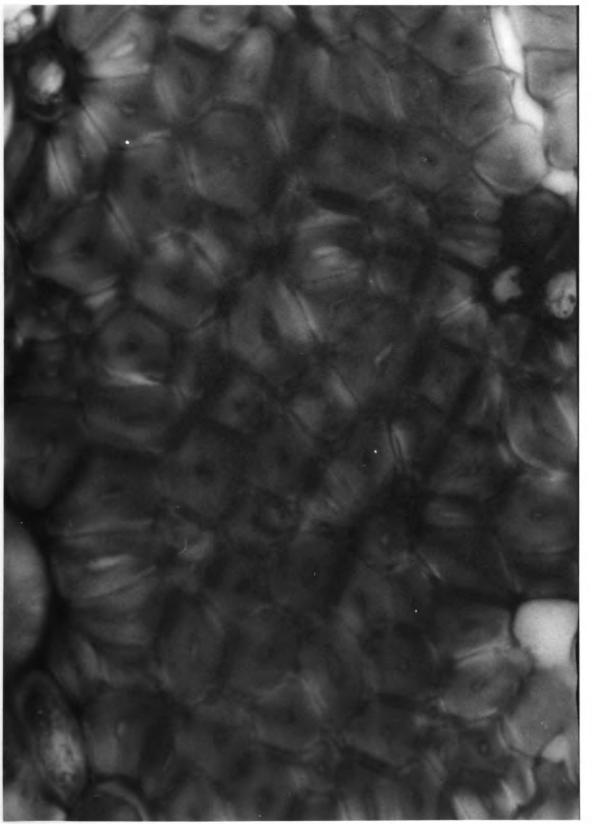

Figure 6

sclerification effect. Biochemical tests might determine whether fiber development is in any way directly related to root development, such as a lignification promoter serving as a rooting inhibitor.

In the same vein, a reduction in sclerification is analogous to a retention of non-differentiation in a tissue. It has been shown that juvenile, non-differentiated tissue generally possesses highest meristematic activity and rooting potential. Etiolation may retard normal maturation and senescence of stem tissue and thereby promote rooting.

Internal starch levels of the layered clones are recorded in Table 3 for two test dates in autumn, 1973. Significant and consistent clonal variation was present, although only M.26 showed a major deviation from the general range of measurements recorded for the other three. There appears to be no simple correlation in this case between clonal starch levels and rooting propensity.

Considerable starch accumulation during the month of October was evident. This suggests the need for healthy, functional leaf surfaces until the cessation of growth activities in order to assure adequate food reserves for winter and subsequent growth of harvested shoots the following growing season. Also, root production continues well into late autumn, and this may be an important period for the propagation success of the shy-rooting clones, which often require a longer period of layering.

Table 3. Internal starch levels in mg starch/g freshweight of four Malus rootstock clones propagated by layering.

Clone	Non-etiolated		Etiolated		Ave.	
	Sept. 26	Oct. 20	Sept. 26	Oct. 20	Sept. 26	Oct.20
M.9	31.88	44.56	49.90	65.61	40.89 a[x]	55.10 a
MM 106	37.22	50.07	63.50	74.06	50.36 b	62.12 ab
M.2	44.90	57.50	60.22	74.25	52.56 b	66.04 b
M.26	49.35	71.22	84.40	93.42	66.88 c	82.31 c
Ave.	40.84	55.85	64.50 **	76.93**		

[x]Means in a column followed by the same letter are not significantly different at the 1% level by Duncan's multiple range test.

The greatest differences in starch levels occurred in the etiolated samples (Table 3). Shoots grown in an etiolated contition showed highly significant increases in starch levels compared to adjacent non-etiolated shoots which had not been mounded. There appears to be an accumulation effect in mounded tissue which has resulted in a 50% increase in starch content when compared to non-mounded shoots, or to above-ground parts of the same shoots.

It is beyond the scope of this study to separate the effect of additional starch on rooting from other biochemical changes which could occur in the tissue. However, it appears that the quantity of food reserves available in stem tissue could contribute markedly to root initiation and development. Several previously-mentioned studies (11, 51, 58, 63, 70, 78, 90) have correlated rooting capacity with starch content, but none has reported the effect of etiolation on starch content. It must be stressed that layering produces etiolation only of lower stem tissue, leaving normal shoot growth above.

Microscopic examinations provided further information about the effects of etiolation and starch reserves on rooting. The extensive nodal rooting pattern was found to be connected with the starch-rich leaf and branch gap areas of the stem. One such area is shown in Figure 7, illustrating the very dark staining for starch in the parenchymatous gap region behind the lateral bud. Areas which consistently showed large concentrations of starch in these studies included the parenchymatous,

Figure 7. Parenchymatous gap region behind bud in M.2. Starch stain, x 16.

(Legend: B=bud, CZ=cambial zone, G=gap, PI=pith, R=rays)

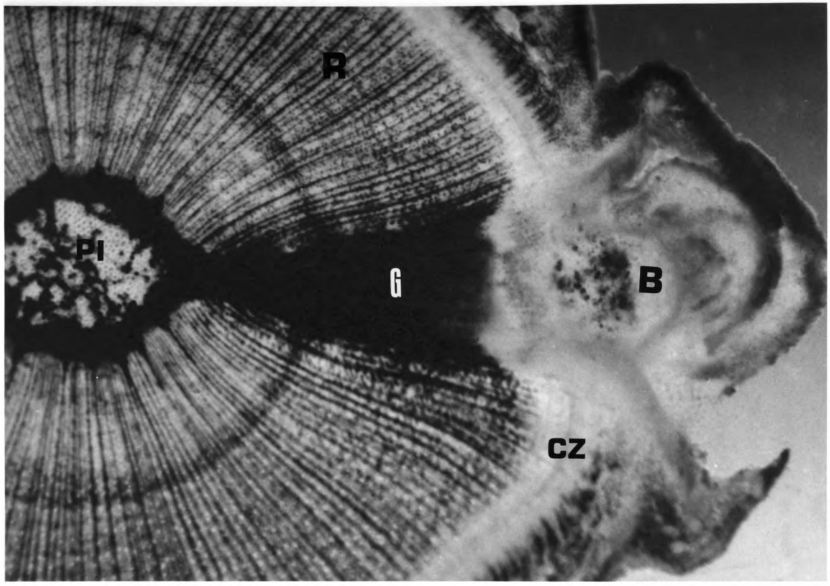

Figure 7

non-differentiated tissues, such as the pith, branch and leaf gaps, and phloem and xylem rays. Meristematic areas such as the cambium, phellogen, lateral and apical buds, and root tips showed little evidence of starch content. The same was true of the vascular elements of the phloem and xylem and fiber tissues in the cortex.

Figure 8, from Eames and MacDaniels (25), clarifies some of the semantic distinctions used to describe these stem tissues. The exit from the stem central cylinder of the vascular supply to leaves and buds causes discontinuities, or "gaps", in the tissues of this cylinder. The number and arrangement of these vascular traces vary among taxonomic groups, but the number is usually three in the case of Rosaceae leaf traces. Traces most commonly depart from that segment of the stele which lies directly beneath the point of leaf attachment, but laterals may arise to the side, or even opposite the leaf.

The net result of gap formation is a band of parenchymatous tissue continuous from the pith to the external bud and leaf area. A distinction is often made between gaps created by leaf traces to the leaf petiole and gaps caused by branch traces, which supply the bud or developing side shoot.

Spacial distinction is often lacking between branch and leaf gaps, as one may merge into the other.[1] The issue of

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[1] In this dissertation branch and leaf gaps are treated as being similar in origin and structure.

Figure 8. From Eames and MacDaniels. Diagrams illustrating leaf and branch traces and gaps.

A) Longitudinal section of node through leaf trace and gap.
B) Similar to A, but with branch trace and gap also present.
C) View of vascular cylinder showing departure of leaf and branch traces, and the gaps associated with each.
D,E, F) Cross sections through stem illustrated in A at levels a-a, b-b, and c-c, respectively.
G) Face view of outside of cylinder shown in C, in leaf and branch traces cut away at the surface of the cylinder.
H) Transverse section of G at a-a.
I) More detailed structure is shown, protoxylem, metaxylem, and phloem being indicated.

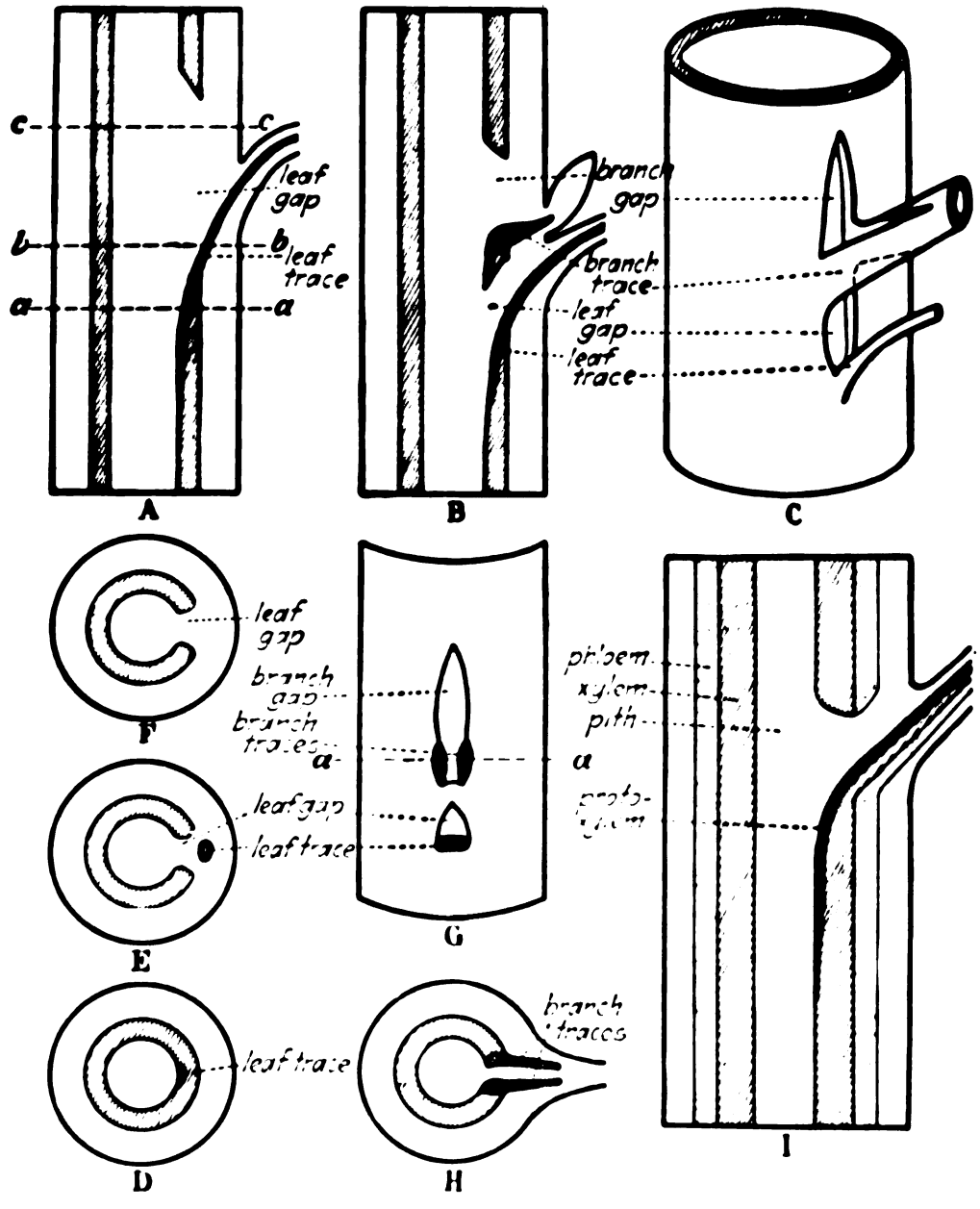
c
c
leaf gap
b
b
leaf trace
a
a
A
branch gap
branch trace
leaf gap
leaf trace
B
C
leaf gap
branch gap
branch traces
a
F
leaf gap
leaf trace
E
G
a
phloem
xylem
pith
proto-xylem
I
leaf trace
D
branch traces
H

Figure 8

importance in this study is the copious starch supply present in the relatively non-differentiated cells of the gap area.

Root emergence from the gap area is illustrated in Figure 9 for M.2. Figure 10 provides an enlarged view of the roots which emerged at right angles from the stem, in very close proximity to a lateral bud. The sequential series of stem sections in Figure 9 C through F shows one of these roots growing out from the gap area slightly below the bud, which is pictured in 9 G and H. Note in the more basal sections C and D that two lateral traces and gaps eventually give way to a wider central gap from which the root emerges. The light cambial and phloem zones become continuous with the root at the point of initiation.

The actual point of initiation would appear to be near the cambial zone, with vascular connections leading into xylem tissue (Figure 11). The dark starch-filled cells of the gap are continuous with the starch-rich outer ring of the pith. The fact that nearly all the roots in layered shoots emerged from near this point of starch deposition provides further evidence that the available starch reserves may provide an energy source for root initiation and development. The bud area also provides a particularly attractive point for emergence, as no external constricting fibers or cortical tissues are present. The production of other etiolation-induced biochemical factors by the bud cannot be ruled out by this study.

Figure 9. Sequential transverse sections of root emergence from gap area near bud in M.2 stem. Starch stain, x 10.

A) Two roots emerging near bud, x 6.3.
B) Two roots emerging near bud, x 10.
C) Below bud with three traces and a root primordium visible, x 10.
D) Below bud at greatest extent of lateral gaps.
E) Bottom of root with cambial continuity.
F) Middle of root showing association with gap area.
G) At bud level between roots.
H) Top of bud near second root.

(Legend: R=root, RP=root primordium.)

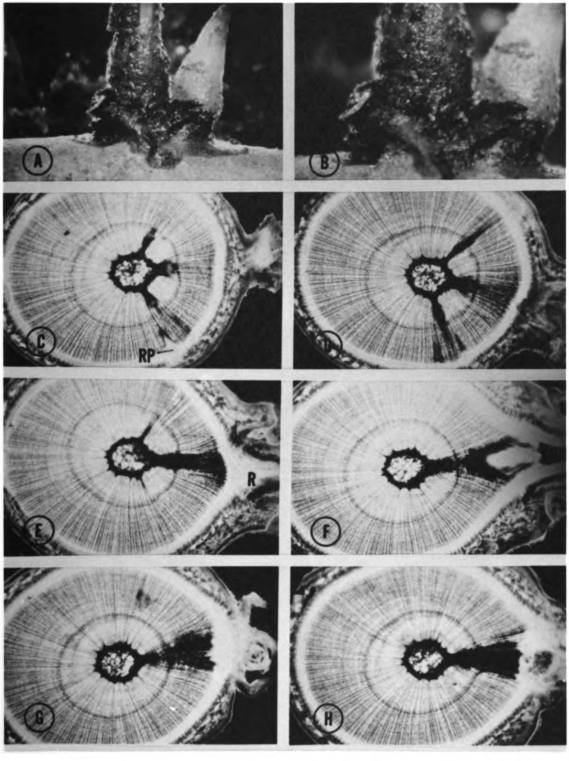

Figure 9

Figure 10. Root emergence near bud in M.2 layered shoot.

(Legend: B=bud, R=root, S=stem.)

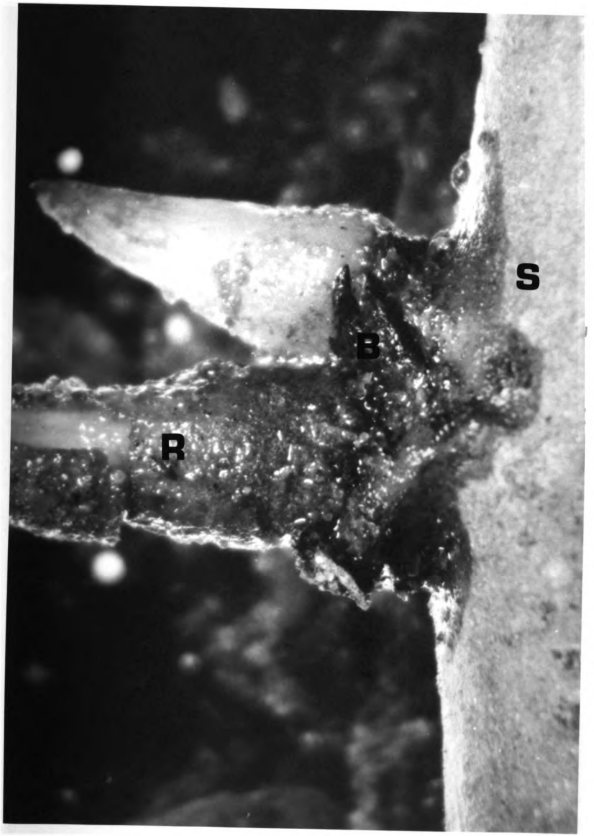

Figure 10

Figure 11. Root emergence from gap area in M.2 stem. Starch stain, x 10.
(Legend: CZ=cambial zone, G=gap, PI=pith, R=root.)

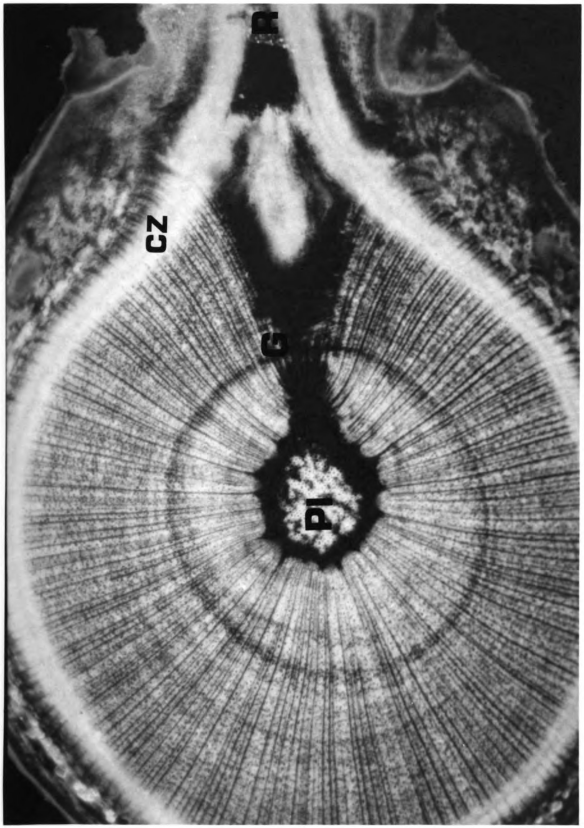

Figure 11

Figure 12 shows a double-stained section similar to that in Figure 11. Note the cambial continuity with the root and the vascular connections to the stem in the gap area.

Figure 13 shows enlarged views of the small root primordium visible in the lower right of Figure 9 C. Note that the primordium was formed at the cambial layer and is connected to the lateral gap-pith area by a particularly wide xylem ray. Figure 13 B (polarized light section) reveals high meristematic activity (black color) in the primordium, which is continuous with the cambial ring. These pictures suggest that the 5 to 14 percent of roots not counted as of nodal origin in the rooted layers, could have been connected internally to the gap and pith starch source.

Serial tangential sections of a M.2 rooted shoot illustrate more clearly the point of root origin and extent of the gap area (Figure 14). Figure 15 provides an enlarged view of two roots emerging just below a bud. These roots can be traced back into the stem in Figure 14 B through F. Note the heavy deposition of starch at the base of the bud in D, a phenomenon which was seen consistently in the clones. The cambial layer can be seen to extend around the roots in the rather external section E and enlarged Figure 16 A. The inner root tissues did not stain for starch to any appreciable extent. Further sections (Figure 14) and enlarged Figure 16 B show the roots to be present into the xylem area and the gap to extend back into the pith.

Figure 12. Root emergence from gap area in M.2 stem. Polarized light, double stain, x 16.

(Legend: CZ=cambial zone, G=gap, R=root.)

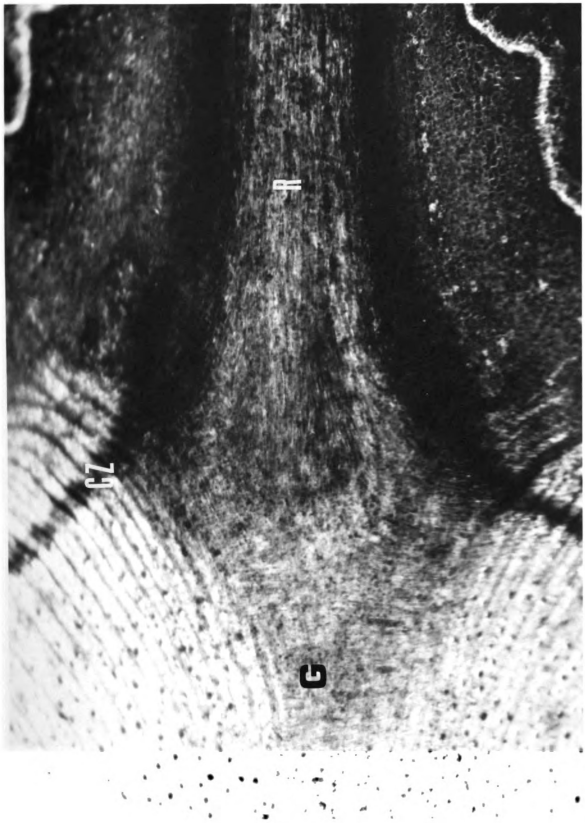

Figure 12

Figure 13. Root initiation from cambial zone in M.2 layered shoot.

A) Root primordium, transverse section, double stain, x 50.
B) Root primordium, transverse section, double stain, polarized light, x 50.

(Legend: CZ=cambial zone, RP=root primordium, X=xylem, XR=xylem ray.)

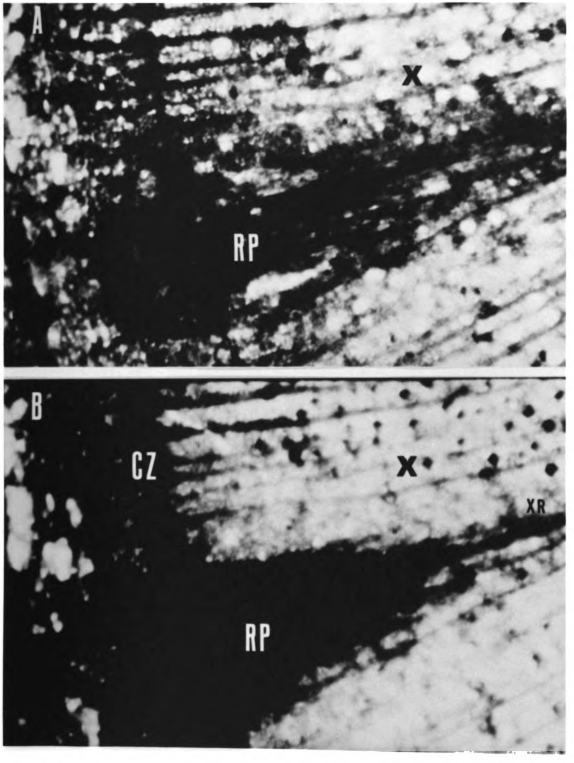

Figure 13

Figure 14. Sequential tangential sections of root emergence from gap area near bud in M.2.

A) Two roots emerging near bud, x 6.3.
B) Root cross sections external to bud, starch stain, x 16.
C) Root cross sections near bud, x 16.
D) Bud longitudinal section, x 25.
E) Gap area and roots internal to bud, x 16.
F) Gap area near point of root initiation, x 16.
G) Trace and gap surrounded by xylem tissue, x 25.
H) Beginning of Pith, x 25.

(Legend: B=bud, C=cambium, G=gap, PI=pith, X=xylem, R=roots.)

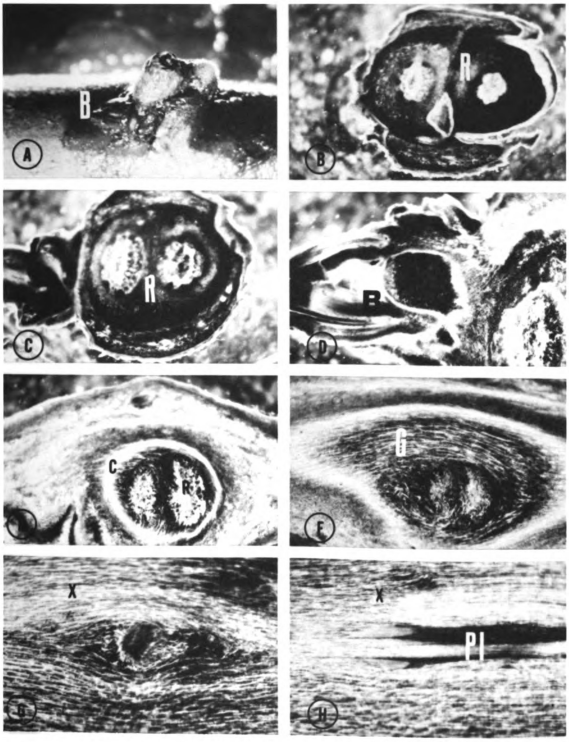

Figure 14

Figure 15. Root emergence near bud in M.2 stem.
(Legend: B=bud, R=root, S=stem.)

Figure 15

Figure 16. Tangential sections of root emergence from gap area in M.2 stem.

A) Roots surrounded by cambium and starch deposition, internal to bud, starch stain, x 25.
B) Gap area and roots near point of initiation.

(Legend: C=cambium, G=gap, R=roots.)

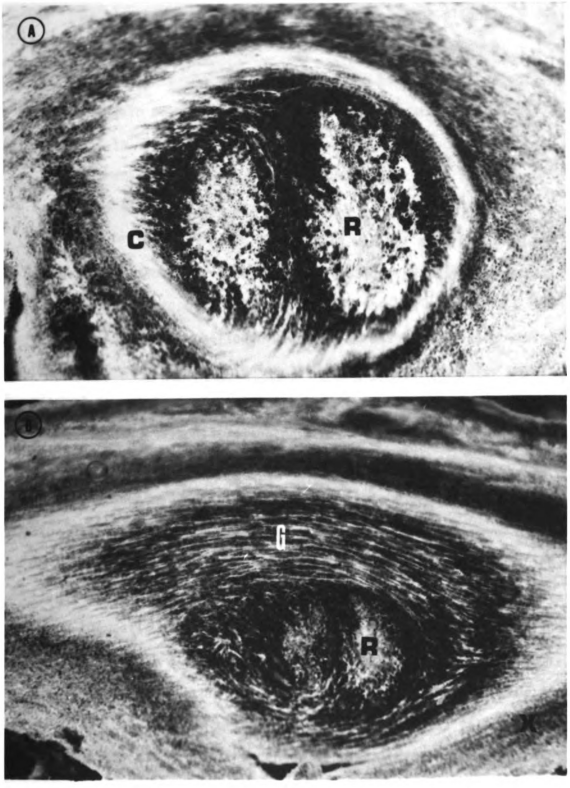

Figure 16

Sections from a M.9 stem show root emergence slightly above the bud (Figure 17). Enlargements in Figure 18 show cellular detail in the gap area behind the bud. Starch deposition can again be noted immediately internal to the bud. Root emergence in Figure 17 E through H can be seen to occur at the upper extreme of the gap, with meristematic activity extending rather far back into the gap.

Figure 19 provides an interesting view of a corollary subject--abscission. Starch staining revealed a sharply-delineated abscission zone to be present on each of the bud scales. A continuation of the phellogen occurred across the scales at this point with starch deposition on the non-meristematic outer portion of the scales. Vascular traces are apparent in the center of the scales. Simons (74) noted that this pattern of meristematic development was typical for bud scale abscission during the growing season and that layering might have hastened this abscission process.

The starch-filled outer cells of the pith and gap area are illustrated in Figure 20. No anatomical differences were evident among the cells of the pith. The darkly stained parenchyma cells were filled with starch grains; the lighter cells appeared empty. Starch-filled cells consistently formed an abaxial ring in the pith, which was continued into the gap area. Starch-filled cells were randomly distributed in the center of the pith. This starch ring pattern may be due to cell age or to manner of deposition of

Figure 17. Sequential transverse sections of root emergence from gap area near bud in M.9 stem. Starch stain, x 10.

A) Bud and gap area, starch stain, x 16.
B) Enlarged bud, cambial zone, and starch deposition, x 25.
C) Enlarged gap area, x 25.
D) Top of bud with extensive cambial development, x 16.
E) Same, x 25.
F) Top of bud with root continuity with cambium, x 16.
G) Same, x 25.
H) Root at top of gap area.

(Legend: C=cambium, G=gap, R=root.)

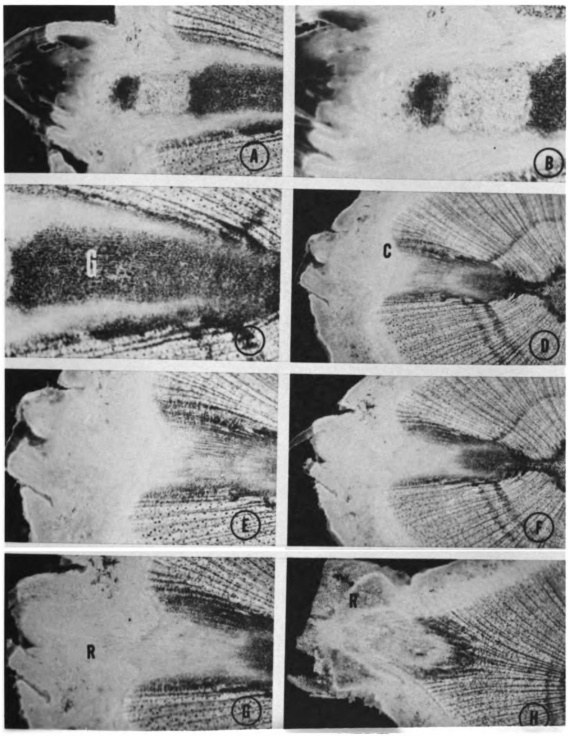

Figure 17

Figure 18. Transverse section of bud and gap area in M.9 stem.

A) Bud, starch deposition and cambial zone starch stain, x 25.
B) Gap area with starch-rich parenchymatous tissues, x 25.

(Legend: B=bud, C=cambium, G=gap, X=xylem.)

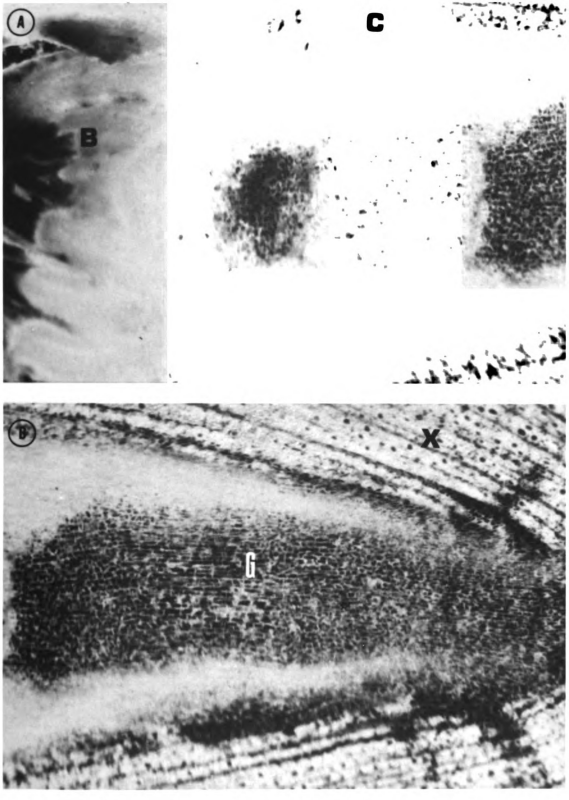

Figure 18

Figure 19. Abscission zone formation in bud scales of M.9 stem. Starch stain, x 16.

(Legend: B=bud, G=gap, PH=phellogen.)

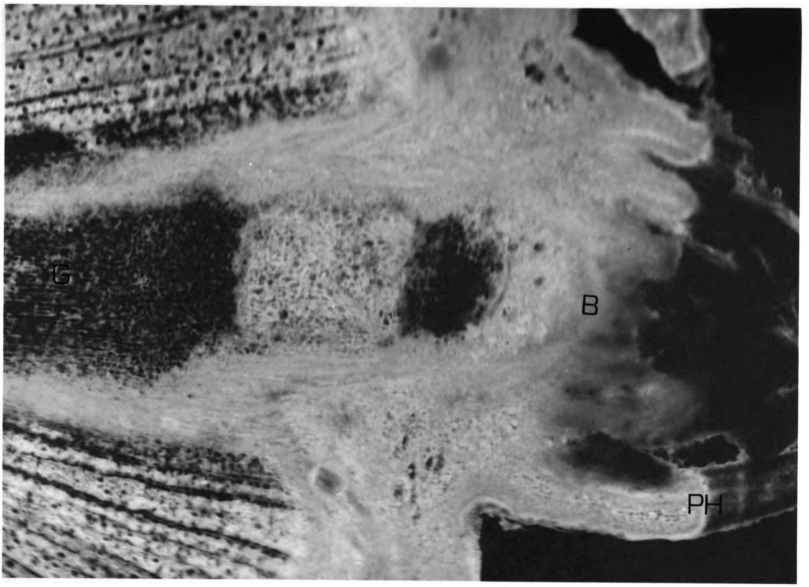

Figure 19

Figure 20. Parenchyma cells of the pith in M.2 stem.

A) Pith area, with ring of starch-filled cells, double stain, x 125.
B) Parenchyma cells, empty and with starch grains, x 500.

(Legend: G=gap, PI=pith, X=xylem.)

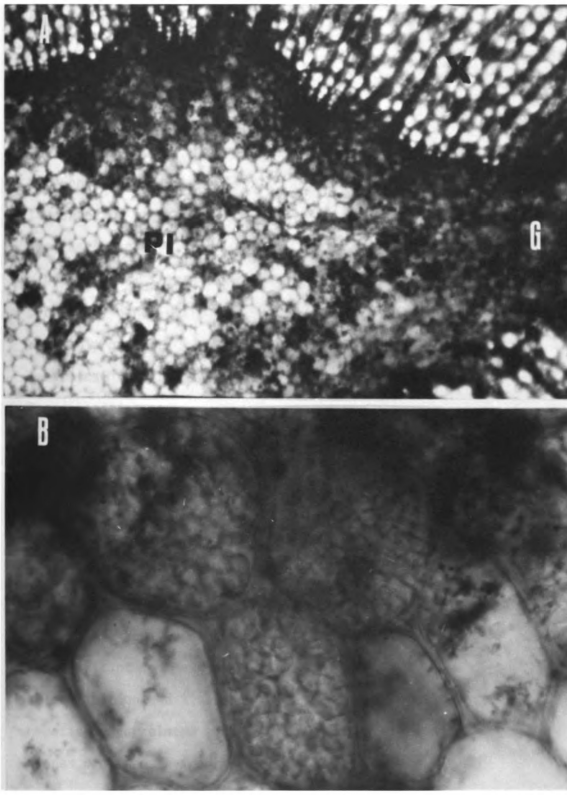

Figure 20

photosynthates. It is not clear to what extent these starch reserves can be transported to centers of metabolic activity.

SUMMARY

This research study investigated adventitious root formation through the layering process in 4 rootstock clones. Interrelationships of stem etiolation and internal nutritional reserves were associated with the rooting process.

Internal starch level among rootstock clones, which is presumably genetically-linked, was not correlated with relative rooting propensity. However, the localization of starch in the anatomical structure seemed to favor positional root initiation. The deposition and increase in concentration of starch during etiolation in all clones implied a role in root production. Roots were produced almost exclusively in association with the starch-rich nodal gap area of the stem. The heavy starch deposition in the non-differentiated parenchyma tissue in the leaf gap and pith areas may have provided optimum conditions for root initiation and development at this site.

The layering process and associated stem etiolation caused a significant increase in starch levels and some decrease in stem sclerification and tissue differentiation. Sclerification was negatively correlated with rooting propensity. Mounding of the clones provided environmental conditions favorable to root emergence. Probably some additional biochemical change is linked

with etiolation. This might include: hormone level adjustments, production of rooting cofactors, or non-production or rooting inhibitors. This change could be influenced by the presence of the lateral bud at each node. The rapid production of roots by etiolated cuttings suggests that the true effect of etiolation is actual initiation of root primordia, which may then elongate under proper environmental conditions.

Future application of these results may provide improvements in the layering technique and more use of etiolation in rooting of hard-to-root plants. Biochemical analyses of etiolated materials may provide more insight into the rooting processes.

SECTION II

EFFECTS OF STEM ANATOMY, WOUNDING, AND STARCH RESERVES ON ADVENTITIOUS ROOT FORMATION IN SOFTWOOD CUTTINGS

LITERATURE REVIEW

A. Introduction

Formation of adventitious roots on cuttings has been a subject of great interest in plant propagation research. The problem of regeneration in detached plant parts with totipotent meristematic cells has been approached from the standpoint of imposing optimum external and internal conditions. However, considerable variation in rooting propensity and requirements between species and cultivars has made it difficult to suggest a standard set of conditions under which the root regenerating capacity of all materials can be expressed to the fullest.

Propagation by softwood and hardwood cuttings offers a number of practical advantages to the nurseryman in the use of those materials which will respond to current techniques. However, due to insufficient response to cuttage techniques, propagation of clonal rootstocks of Malus has historically been accomplished by means of the layering process. This investigation was undertaken to further understand the various conditions necessary to assure successful propagation by cuttings in these rootstocks.

B. Seasonal Plant Carbohydrate Levels

General plant nutrition and level of stored photosynthates has been cited as a factor in rooting success of cuttings. A definite seasonal pattern of plant starch content has been

established in a number of separate investigations. Swarbrick (82) in 1927 showed stem starch level maxima at leaf fall and early spring and minima at the time of greatest leaf growth and in early winter. He reported the cortex and phloem tissues to be most sensitive to changing plant starch levels, with disappearance and re-accumulation proceeding in a basipetal order.

Kraybill et al. (52) reported a maximum content of starch in all parts of mature apple trees in November after leaf fall, with starch being hydrolyzed in December and January, then synthesized from February to April. At bud break a disappearance of starch from tissues was again noted. Brandon (9) found a similar pattern in Rosa cultivars, with the exception of the spring decrease at bud break.

Mochizuki and Hanada (57) provided data from all tree parts to correlate seasonal starch changes with translocation of reserves. The early spring rise in stem starch level was accompanied by a decline in root starch content. Stored reserves in roots appeared to be translocated to aerial parts prior to renewed cambial activity and bud break. Starch level in the tree top was found to increase during the growing season, except in those periods of most active shoot growth.

Priestly (63) derived conclusions generally in agreement with Mochizuki and Hanada, noting starch depletion at bud break and during extension growth, with replenishment when leaves were

present without great meristematic activity. Roots were shown to contain higher levels with more drastic fluctuations than in stem tissues.

C. Starch Effect on Rooting

Whether the level of carbohydrate reserves, and starch in particular, can influence adventitious rooting success has been the subject of several propagation research investigations. In the case of leafless hardwood cuttings, cutting minimum size is governed by its ability to survive and establish. This ability may be determined by the sufficiency of food reserves, as outlined by Browse (10).

Kraus and Kraybill (51) demonstrated that Solanum cutting root number was directly proportional to the plant carbohydrate-nitrogen ratio. Starring (77) found similar trends in studies with Solanum and Tradescantia. Carlson (11) correlated internal starch level with rooting success in two Rosa cultivars. Conversely, Brandon (9) could find no simple correlation between starch content and ease of rooting in a similar experiment with several cultivars of Rosa.

Maire (54) and Kelly (47) noted that plant nutrition plays an important part in cutting propagation and that plant C/N balance is affected by applied fertilizer, water, illumination, and stage of growth development.

In two experiments with Chrysanthemum clones, Samananda et al. (70) and Stoltz (78) found positive relationships between

carbohydrate content and ease of rooting. The latter study found no differences in internal auxin levels among clones and attributed the reduced root initiation in difficult-to-root clones primarily to low total carbohydrate level.

Molnar and LaCroix (58) found a positive correlation between starch content and rooting success in Hydrangea cuttings. The disappearance of starch from parenchyma tissue in close proximity to developing root primordia led the authors to conclude that starch may provide the energy and substrate necessary for the metabolic processes of root initiation. Cuttings taken from plants subjected to a period of darkness, with consequent low starch reserve, failed to root as well as normal cuttings.

Two recent biochemical studies provide information on the possible roles of carbohydrate reserves and auxins in root initiation. Nanda and Jain (61) used cuttings from completely etiolated shoots of Populus to demonstrate the need for carbon sources in root initiation. Rooting was found to be limited primarily by nutritional factors, because segments that failed to root in water or auxin alone, rooted in sugar and starch solutions. The enhanced rooting noted in starch-plus-auxin solutions was due to enhanced activity of hydrolyzing enzymes, especially alpha-amylase. Therefore, exogenously-applied auxim, besides enhancing cell division and differentiation, helps to mobilize reserve food materials. Basu (5) also noted the enhanced hydrolysis of nutritional reserves under the influence of auxin. In

addition, a net synthesis of proteins in the course of regeneration was observed. It was suggested that auxin might act directly to promote the enzyme protein synthesis necessary for successful regeneration.

D. Stem Anatomy and Root Initiation

Much early research work centered on the effect of plant anatomy in propagation and some present researchers have endeavored to use these findings in conjunction with recent biochemical and physiological findings. Of special interest are the subjects of juvenility, meristematic tissues, callus proliferation, and degree of sclerification.

1. Juvenility

Several studies have shown that cutting taken from juvenile plant materials provide superior rooting performance. Several investigations have concentrated on the biochemical basis for this phenomenon, while some have shown actual anatomical differences. Thimann and Delisle (84) showed that the age of the tree from which cuttings were taken determined, in large measure, the rooting propensity in Pinus. Delisle (23) in subsequent work determined that the living cells of the pith, cortex and rays retained protoplasm longer in cuttings taken from juvenile sources. The cortex tissues of juvenile cuttings soon became meristematic, while comparable cuttings from mature stock remained the same or degenerated.

In Malus, juvenility on otherwise-mature trees may be manifested by meristematic development resulting in burrknot root formations on trunk and branches, as noted by Swingle (83). Also the production of shoots from juvenile sphaeroblast tissue has been recorded by Baldini and Mosse (3), Hatcher and Garner (40), and Stoutemyer (80). These small meristematic nodules in the stem cortex produce easy-to-root shoots.

2. Meristematic Tissues

Although an expression of juvenility, the subject of meristematic stem tissues can be considered separately due to the importance attached by many researchers. In the large body of anatomical research on root initiation, most results can be found to agree as to the importance of meristematic, or potentially-meristematic, tissues for successful root initiation. Argles (1) noted that root forming capacity in cuttings is highest when lateral or intercalary meristems are active, a redeposition of food reserves has started, and there is a period of temporary water stress. Waxman (89) showed an ideal cutting to have young cells which can become meristematic, adequate carbohydrate reserves, and a balance of various hormones, vitamins and nitrogenous compounds.

Satoo (72), using many different plant species, reported that adventitious roots arose from several different tissues including the cambium and phloem rays, leaf and branch traces, bud meristem and bud traces, and callus parenchyma. Most of the

adventitious roots arising from the basal cuts came from the meristematic cambium and phloem ray areas. Girouard (36) reported that cuttings of Hedera initiated roots from interfascicular parenchyma or cambial cells. Roots also could arise near the nodal leaf gaps, or externally from callus tissue and hyperhydric outgrowths of lenticels.

Wu and Overcash (92) in a study of root origin in softwood cuttings of Rubus clones varying in rooting difficulty, found a greater degree of rooting from external callus proliferation in hard-to-root selections. Anamolous cambial activity was thought to be present in the callus growth and responsible for root initiation. The easy-to-root clones were found to initiate roots more readily from parenchyma cells in the interfascicular cambium area.

Carlson (13) found pre-formed primordia in Salix stem cuttings which were formed from parenchymatous tissue above a leaf or bud gap at the node. These primordia remained dormant until the cutting was removed from the mother plant. Van Der Lek (85) reported similar primordia in Salix and Ribes, the latter being largely formed in association with lenticels. In older stems adventitious roots arose in association with secondary tissue growth in the medullary ray areas.

3. Callus Proliferation

The phenomenon of callus formation has been studied to determine its effect on, or association with, ultimate rooting of the cutting. Knight (48) analyzed the callus formed by

masses of liquified cells from the vascular cambium and concluded that it was an obstacle to water absorption. Callus formation and root initiation were thought to be two distinct processes bearing no consequential relationship to each other. Carlson (14) and Snyder (76) confirmed these conclusions, showing that root formation takes place from the outer edge of vascular tissue, with developing root tips emerging near the side of the callus formation. Cormack (21) reported that firm compact callus proliferations on Populus cuttings prevented proper root emergence, serving as a mechanical barrier.

Barker (4) found that callus in Tilia was produced from the medullary sheath region between the pith and protoxylem tissue areas in vitro. Latent meristematic potential was found in medullary sheath cells in the center of very old trees. Groups of isolated cells were able to proliferate to form discrete callus nodules.

Jimenez (46) found phloem ray and cortical cells to be the initial source of callus production in Kapok cuttings. Root formation took place on the actual wound callus formation at the basal end. Roots apparently originated from newly-formed phellogen below an external suberized layer.

4. Sclerification of Tissues

The subject of sclerification and lignification of cortical tissues as a potential barrier to root production or emergence has been considered by several researchers, with differing

conclusions. However, the presence of a sclerenchymatous sheath in a number of difficult-to-root species has been confirmed and a correlation established between extensive sclerification and loss of rooting capacity in several investigations.

Ciampi and Gellini (18, 19) and Ciampi (17) provided much of the impetus for research in this area by reporting that root formation in Olea cuttings was mechanically impeded by a nearly complete ring of fibers and sclereids in the cortical region of mature shoots. Much better rooting was obtained from younger shoots with little sclerification or by wounding with a shallow slit through the cortex. Mahlstede and Watson (53) reported that Vaccinium roots were bent, hindered, or caused to emerge far from area of origin by a fiber sheath in the stem cortical region. Beakbane (7) found a positive correlation between the degree of sclereid blockage of phloem rays and rooting capacity in Malus and Pyrus. Goodin (37) reported similar findings in shy-rooting mature Hedera cuttings, with very little fiber development in easy-to-root juvenile stems. It is difficult to isolate these anatomical changes from physiological changes of maturation occuring simultaneously. In both the above cases the simple explanation of mechanical blockage was not thought to fully explain the observed rooting differences.

Sachs et al. (69) could find no simple relationship between sclerification and rooting in tests with Olea, Pyrus, and Prunus cuttings. Girouard (35) likewise concluded that fiber formation was not the main cause of poor rooting in mature Hedera.

E. Wounding

The effect of wounding on root initiation in cuttings is difficult to separate from such factors as juvenility, meristematic activity and lignification. Much discussion in plant propagation literature has dealt with the relative merits of wounding of cuttings to increase auxin absorption, promote callus formation, strengthen root attachment, and speed rooting. Diversity of plant materials and rooting conditions makes standard comparison of all research difficult.

Hsu and Hinricks (45) in a series of experiments with clonal rootstocks of Malus reported that wounding one or both sides of softwood stem cuttings greatly increased percentage rooting in shy-rooting Malling 9, but not in easier-rooting Malling 7. Nahlawi (60) found that shallow wounds on Prunus hardwood cuttings also greatly increased percentage rooting as compared to control. Roots arose both from the base and the wounded areas.

Wells (91) attributed increased numbers of more firmly attached roots, and increased speed of rooting to wounding techniques. He felt the roots might be able to initiate in additional meristematic tissue besides the base and that hormone uptake and coverage might be improved. Ciampi (17) reported a wounding slit to improve rooting in Olea by providing access through thick fiber and sclereid development.

Conversely, Chadwick and Reish (16) found no advantage to wounding in Ilex cuttings.

MATERIALS AND METHODS

Softwood stem cuttings of M.9, M.26, M.2, and MM 106 rootstock clones were selected to test differences in rooting capacity. A cutting hedge located at the Michigan State University Horticulture Research Center at East Lansing was maintained for this purpose by severe annual pruning to promote vigorous shoot growth. Tip cuttings 16 cm in length and 1 cm in diameter were taken at three dates in summer or early fall and placed in an enclosed intermittant mist chamber, with sand as a supporting medium.

Three replicates of 80 cuttings each were used in each trial and placed in a randomized block design. The cuttings were treated with a solution of 1000 ppm. I.B.A., using a shallow 10 second dip. A portion of the cuttings was wounded using a shallow cut to the cambium near the cutting base 3 cm in length to determine the effect of this propagation procedure as shown in Figure 1. Five seconds of mist application every 12 minutes assured humidity conditions optimal for leaf tissue survival.

Another rooting trial was conducted using cuttings from mother plants which had been covered with a black cloth to exclude light for a 10 day period. This treatment provided an opportunity to test the effect of reduced light on starch reserves and of starch on rooting potential. The cuttings were treated with auxin before being placed in the rooting bed.

At the conclusion of each trial, data were collected outlining percentage rooting and number or roots per cutting for each clone.

Figure 1. Wounding technique for cuttings.

A) 3 cm wound at cutting base.
B) Dipping wounded cuttings in IBA solution.

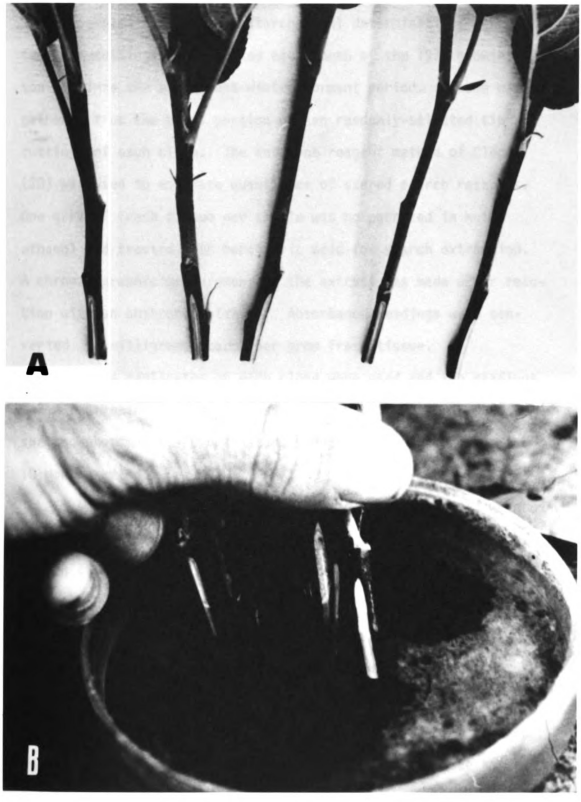

Figure 1

Samples for internal starch level determinations were taken from the cutting hedges during each month of the 1973 growing season and into the subsequent winter dormant period. Tissue was gathered from the basal portion of ten randomly-selected tip cuttings of each clone. The anthrone reagent method of Clegg (20) was used to estimate quantities of stored starch reserves. One gram of fresh tissue per sample was homogenized in hot ethanol and treated with perchloric acid for starch extraction. A chromatographic measurement of the extract was made after reaction with an anthrone solution. Absorbance readings were converted to milligrams starch per gram fresh tissue.

Three replicates of each clone were used and two readings taken from each replicate on a Bausch and Lomb Spectronic 20 spectro-photometer. Clonal starch levels were plotted over the 10 month period.

Anatomical examinations of cutting stem tissue and wounded areas were conducted in conjunction with the chemical analyses. Starch staining methods of Gates and Simpson (33) and Molnar and LaCroix (58) were used to illustrate starch storage patterns in live unimbedded stem sections cut with a sliding microtome. Dilute iodine stain was applied to the killed and decolorized sections. Individual sections were photographed from the microscope.

A safranin-fast green double stain, after Sass (71), was used in further anatomical investigations of stem tissue killed and fixed in F.A.A.

In order to study effects of stem sclerification, counts were made from the sections of percent of phloem rays blocked externally by fiber tissues in the cortex, as outlined by Beakbane (7).

RESULTS AND DISCUSSION

Rootstock clonal rooting response, expressed in percentage rooted cuttings and roots per rooted shoot is outlined in Table 1. As expected, there was a large range of rooting success among the clones, with M.2 and MM 106 approaching commercially acceptable levels. Compared to layering results, the number of roots per shoot is uniformly low. Figure 2 shows typical cuttings of each clone. This may explain the usual problems involved in producing a rooted shoot of sufficiently large caliber to permit budding in the nursery, without allowing an extra growing season. Probably propagation by softwood cuttings would offer sizable advantages over layering only if budding could be accomplished the first summer after rooting of the cuttings. The data show a reduction in both percentage of cuttings rooted and number of roots per cutting in the shy-rooting M.9 and M.26.

The degree of sclerification, determined by percent of phloem rays blocked by cortical fiber proliferation is outlined in Table 1. As with rooted layers, the degree of sclerification proved to be inversely proportional to rooting success. The potential mechanical effect of a fiber ring may be of more importance in cuttings than in layers.

Figure 2. Rooted cuttings of four rootstock clones after six weeks in mist propagation bed.

A) MM 106.
B) M.2.
C) M.26.
D) M.9.

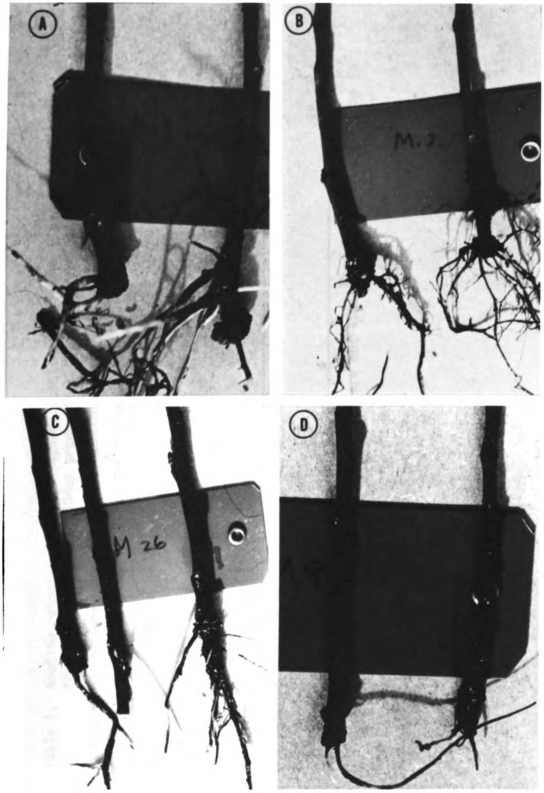

Figure 2

Table 1. Rooting response, degree of sclerification, and internal starch levels of four *Malus* rootstock clones propagated as softwood cuttings.

Clone	% Rooting	Roots per rooted shoot	% Sclerification[y]	Starch level[z]
M.9	38.3 a[x]	2.1	53.1	25.69 a
M.26	38.1 a	2.8	52.4	41.99 c
M.2	69.0 b	3.8	46.5	34.91 b
MM 106	79.2 c	4.3	37.8	27.18 a
M.2 covered	33.5 **	2.5		29.94 **
non-cov.	60.2	3.2		48.77

[x]Means in a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.
[y]% of phloem rays blocked externally by fibers.
[z]mg. starch/g fresh weight.

Propagation by softwood cuttings differs from layering in that cuttings are severed from the mother plant at a fairly early stage of development. No etiolation occurs and an exogenous source of auxin is usually supplied. Wounding of the stem tissues occurs, to a greater or lesser extent, leading to considerable callus proliferation and superficial meristematic activity. Root initiation will likely take place near the cut base in newly-formed tissue or in reactivated internal meristematic areas.

Until root formation occurs the cuttings may be weakened through nutrient leaching by the mist or fog sprays, and by reduced photosynthesis--expecially if leaves cannot be maintained free of fungus problems and ultimate abscission. This type of root formation can be placed in the "wound root" catagory, with most rooting coming directly from newly-cut stem areas.

Table 2 shows data from a study to ascertain the effect on rooting of a deliberate wounding technique, designed to provide greater area for callus proliferation and root initiation. Literature on the subject of softwood cutting propagation has suffered from non-standardization of technique and rooting environment. This is especially true with wounding studies. Rooting success depends to a great extent on environmental conditions. Furthermore, similar plant materials and wounding techniques will not respond the same under different conditions.

However, in this wounding test, an improvement trend in rooting percentage and number was evident, although not significant

Table 2. Effect of wounding on rooting response of four Malus rootstock clones propagated as softwood cuttings.

Clones	Non-wounded		Wounded	
	% Rooting	Roots per rooted shoot	% Rooting	Roots per rooted shoot
M.9	41.2 [X]	1.8	49.7	2.3
M.26	40.0	2.0	46.8	2.4
M.2	73.4	2.6	76.7	2.9
MM 106	87.5	2.4	90.0	2.6
Ave.	60.5	2.2	65.8	2.6

[X]No significant differences.

(Table 2). The differences could not be described as qualitative; however, the technique was most beneficial in the shy-rooting clones, showing a 9% and 7% improvement in rooting, respectively. It should be noted that rooting of wounded cuttings is not necessarily confined to only the wounded side portion of the stem.

Wounding may provide some quantitative benefit by providing additional root initiation sites through increased callus proliferation, meristematic activity, and/or auxin uptake. Also, by wounding, the band of cortical fibers, which could provide a constriction effect, is removed.

Internal stem starch levels averaged for three collection dates for the clones are shown in Table 1. As in the case of the layers, M.26 showed a consistently higher level, and starch content was not correlated ($r = -.53$) with relative clonal rooting performance. Subsequent tests showed starch levels generally dropping during the rooting period in the mist bed, especially if leaf abscission had occurred.

An 11 month study of seasonal starch level fluctuation is outlined in Table 3 and reproduced graphically in Figure 3. The general starch seasonal pattern of the cutting hedge plants follows rather closely that outlined by Mochizuki and Hanada (57) and Priestly (63). Early season trials included an analysis of soluble sugar content, but this was discontinued when very little clonal variation could be noted.

Table 3. Seasonal starch levels in mg/g fresh weight for four Malus rootstock clones.

Clone	May 16	June 7	July 6	Aug. 8	Aug. 23	Sept. 6	Sept. 20	Oct. 20	Nov. 28	Jan. 21	Mar. 10
M.9	15.65	11.29	14.56	18.44	24.20		33.19	44.56	37.61	16.56	22.69
MM 106	17.10	10.76	14.91	18.76	19.17	28.47	33.89	50.07	46.35	26.75	26.05
M.2	19.74	13.58	20.46	25.81	29.80	31.98	42.96	57.50	50.15	21.92	29.32
M.26	28.21	20.25	17.65	22.50	25.09	40.47	60.33	71.22	60.81	37.89	44.21

Figure 3. Seasonal starch levels of four Malus rootstock clones.

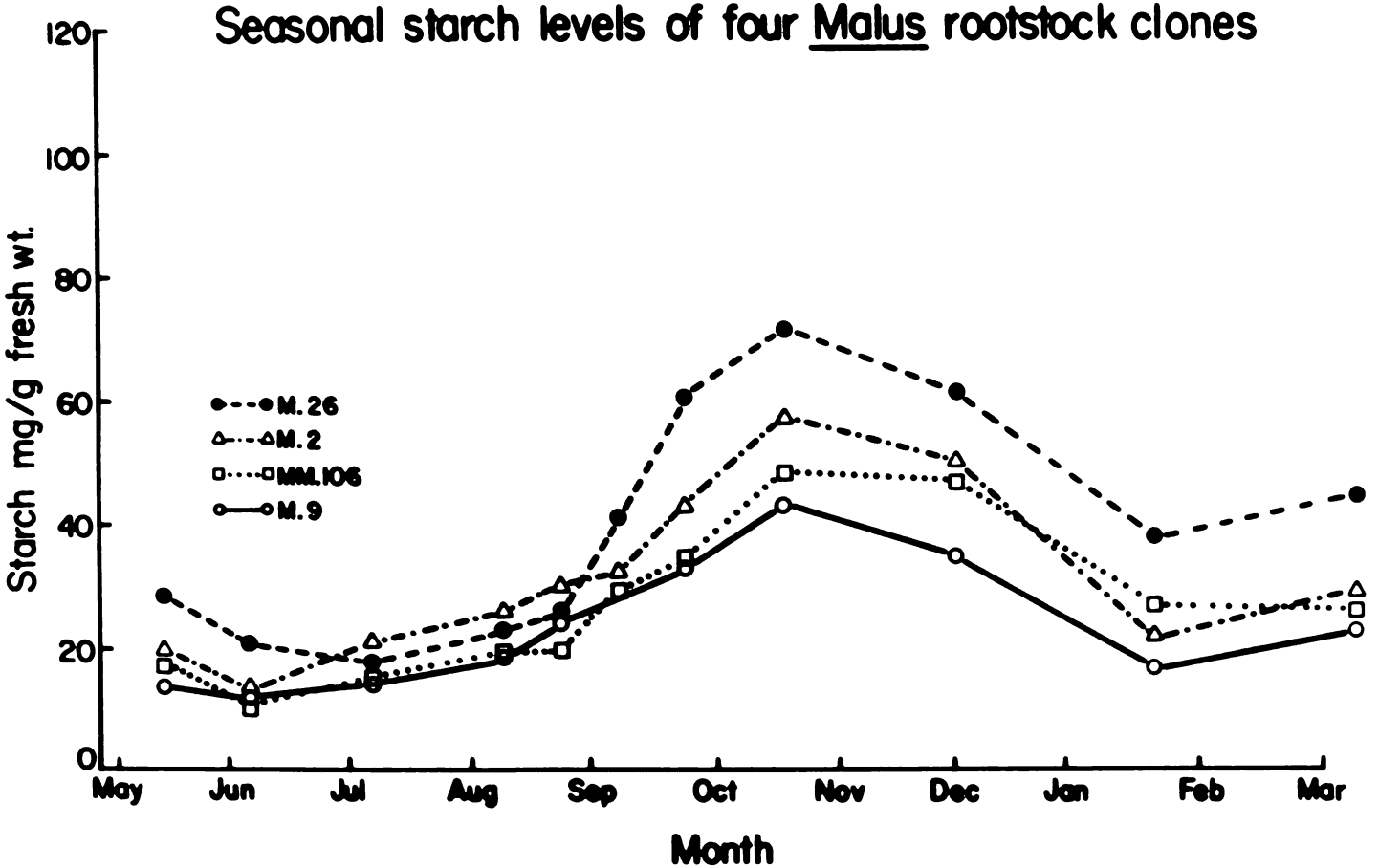
Seasonal starch levels of four Malus rootstock clones
Starch mg/g fresh wt.
120
100
80
60
40
20
0
M.26
M.2
MM.106
M.9
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Month

Figure 3

The relationship between clonal starch levels, rooting response, and seasonal variation illustrates some interesting points. Clonal trends are generally very consistent, with the exception of the large increase in starch in M.26 during the autumn maturation period, in relation to the other clones. M.26 starch level is not exceptionally high throughout the growing season, but this clone appears to have the capacity to accumulate large amounts of starch as dormancy approaches. This trend was also evident in the layered rootstocks.

The late autumn and early spring maxima in starch content, coupled with the winter minimum, provide an interesting trend when considered in relation to hardwood cutting propagation. It appears that physiological changes taking place in the stem during this period which affect starch level may also affect rooting capacity. A seasonal trend of greatest rooting success in hardwood cuttings taken in late autumn and early spring, with a mid-winter decrease, has been reported by Garner and Hatcher (32) and Doud and Carlson (24). The stem starch level during dormancy does not necessarily provide a causal relationship to rooting performance, but the similarities are evident. If stored food reserves play a major role in rooting success, the greatest ease of rooting should occur during periods of high starch content at late autumn maturity and early spring translocation. These results do not isolate starch effects from other possible biochemical changes taking place during dormancy.

Cutting rooting data in Table 1 from covered versus non-covered mother plants also indicate an influence of starch in root production. A 39% decrease in stem starch content was accompanied by a 27% decrease in rooting success in the cuttings from plants which had been kept in continuous darkness for a 10 day period. These results indicate that depletion of food reserves is associated with lowered rooting success.

A number of different sites of root emergence were noted in the clonal cuttings. (Figures 4 through 12). No quantitative data were gathered concerning site of root emergence, but greater diversity than in the rooted layers was quite evident. This is not surprising in view of the previously-noted differences inherent in the development of "morphological" versus "wound" roots. Roots in cuttings were observed to emerge from the nodal bud area, basal callus proliferation, lenticels, internodes, and wounded areas. In all cases, a natural or derived discontinuity in the superficial tissues which allowed meristematic callus proliferation seemed to favor root development.

Figures 4 and 5 show roots emerging from the bud gap area similar to those occurring in layered shoots. Cuttings did not exhibit this type of root emergence as extensively as layers did. Extensive callus formation occurred at the lenticels and leaf scars.

Root emergence from the basal cut and callus proliferation was the most commonly noted type of root development in cuttings, although it is very difficult to make a visual distinction

Figure 4. Root emergence from bud area in MM 106.
(Legend: B=bud, R=root.)

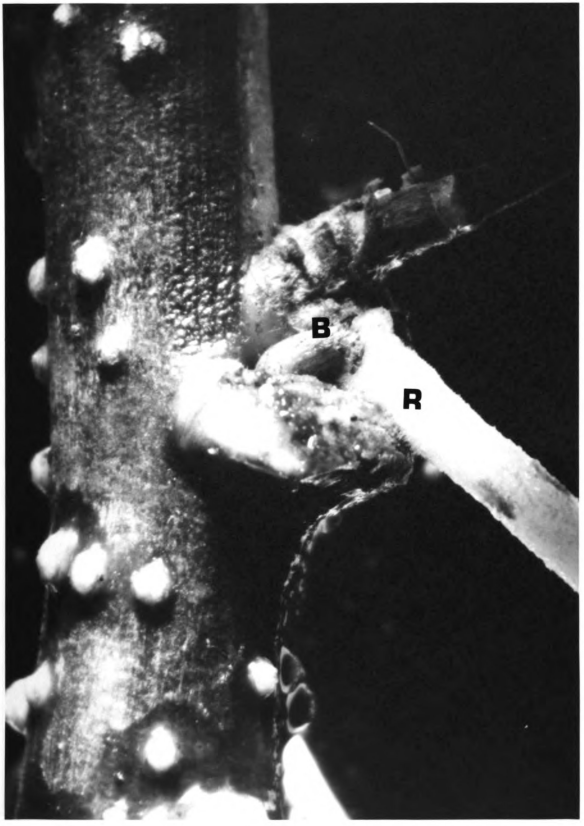

Figure 4

Figure 5. Root emergence from bud area in M.26.
(legend: B=bud, R=root.)

Figure 5

between roots growing out from internal stem tissues and those initiated in the more superficial callus tissues (Figures 6, 7, 8).

Nodular callus development often is prolific at the base of cuttings (Figure 6). This condition was common in certain clones and perhaps favored by certain environmental conditions in the propagation bed. In some Pyrus and Prunus clones used in a preliminary study it became essentially impossible to distinguish between small developing root tips and elongated callus nodules. Auxin application at the cutting base may favor root initiation and callus proliferation at this site.

Roots may emerge from a lenticel above the basal cut (Figure 9). Extensive callus development enlarged the lenticel from which the roots developed. This position for root emergence has been commonly noted in both softwood and hardwood cuttings.

Figure 10 shows roots in MM 106 emerging in an internodal position above the bud. A post facto determination of wounding at this point is not possible. The easy-rooting clones seem to have the capacity for developing many roots from each initiation site.

Roots also developed as a result of the deliberate wounding trial (Figures 11 and 12). These emerged through an extensive callus development at the wound. Some researchers have speculated that over-development of callus tissue may be a barrier to root emergence. These studies gave no indication that this theory is true.

Figure 6. Root emergence from basal callus in M.2.
(Legend: C=callus, R=root.)

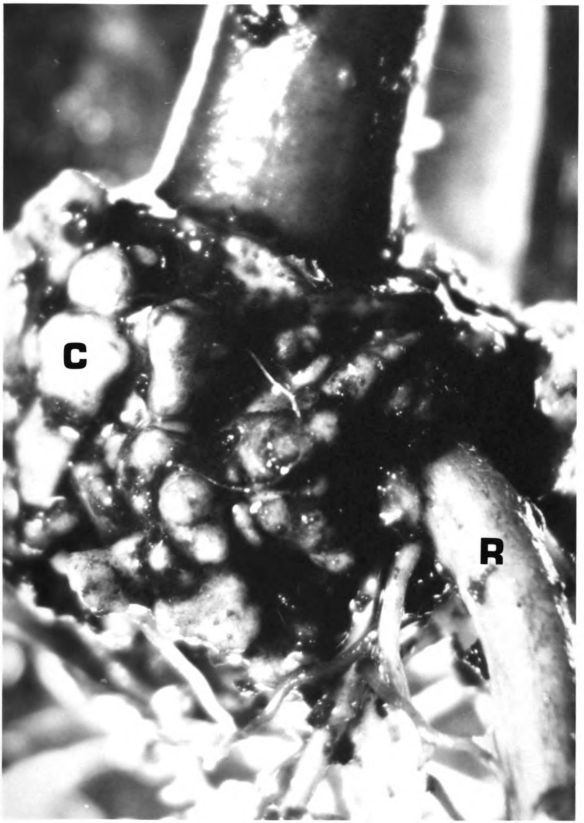

Figure 6

Figure 7. Root emergence from basal callus in M.26.
(Legend: C=callus, R=root.)

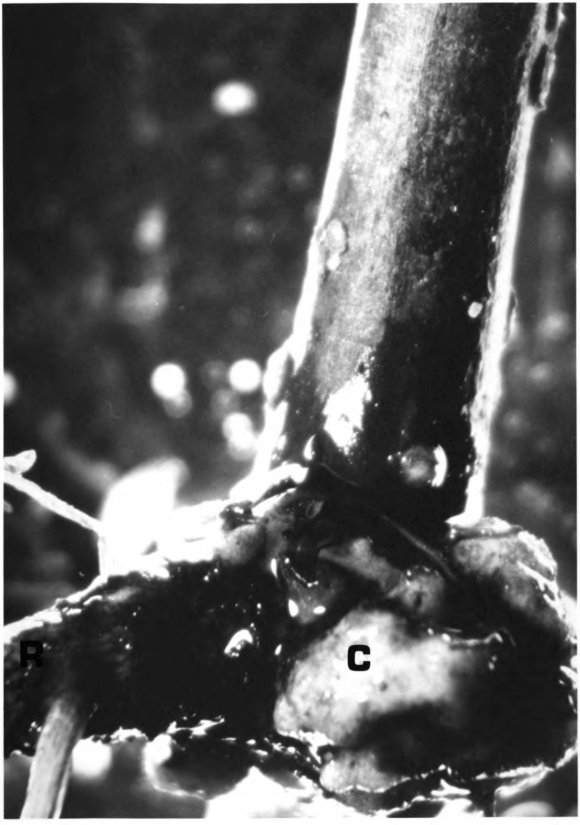

Figure 7

Figure 8. Root emergence from basal callus in M.9.

Figure 8

Figure 9. Root emergence from lenticel in M.2.

(Legend: L=lenticel.)

Figure 9

Figure 10. Root emergence from internode in MM 106.

(Legend: B=bud)

Figure 10

Figure 11. Root emergence from wounded area in M.2.
(Legend: W=wound.)

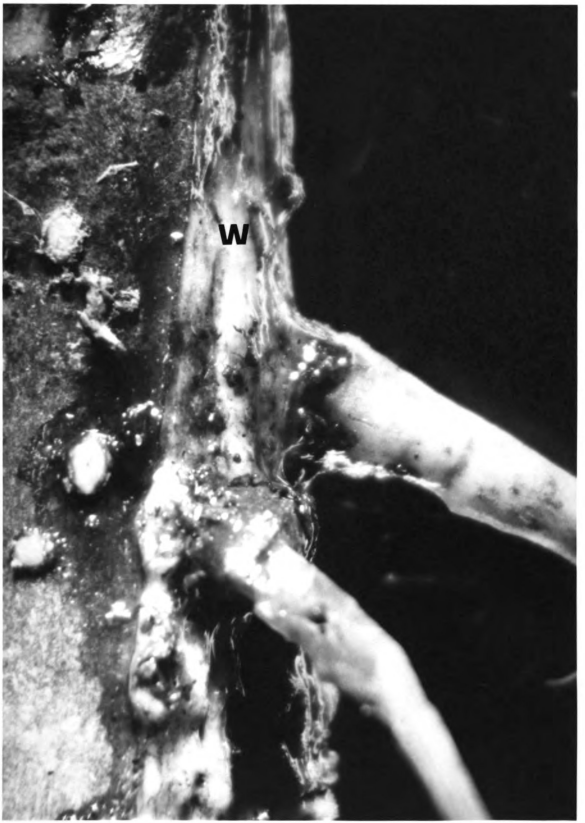

Figure 11

Figure 12. Root emergence from wounded area in MM 106.
(Legend: C=callus, R=root.)

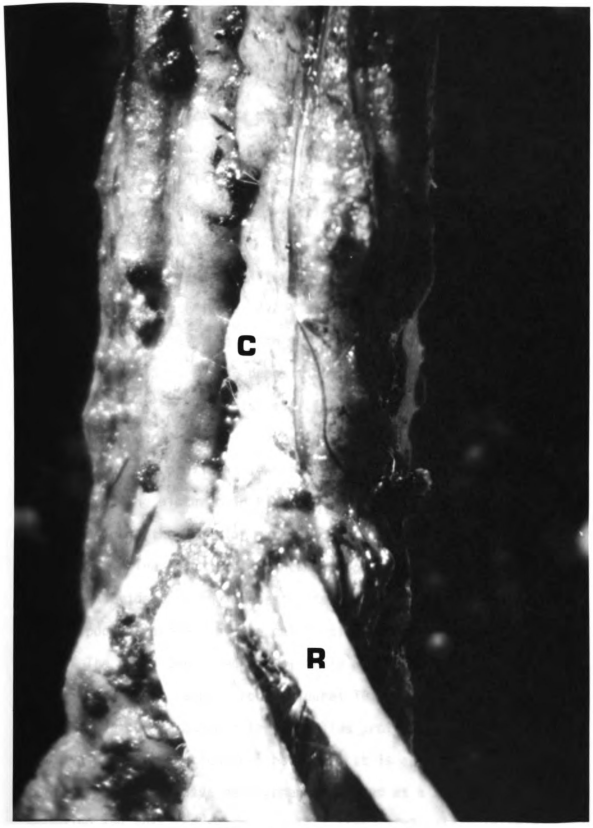

Figure 12

Microscopic studies provided further insights into root origin and initiation. Figure 13 shows sequential sections of root emergence from the wounded cutting pictured in Figure 11. Starch staining indicated a much lower reserve in the rooted cuttings than was the case for rooted layers. The lateral extent of the wound in Figure 13 is visible, with the root emerging near the middle. The root was initiated in the vicinity of the vascular cambium.

Extensive tissue proliferation was found as a result of the wounding procedure (Figure 14). Both xylem and phloem tissues were expanded toward the wound, with the root apparently initiated during this process. Some starch deposition occurred where the root connected with the xylem tissues. The extended phellogen layer was present near where the fleshy root joined the stem tissue.

Figure 15 provides views of root emergence from the wounded cutting pictured in Figure 12. Two roots are shown to be initiated near the vascular cambium. The wound extended to the xylem and failed to completely heal at one point. A phellogen layer was evident at the deepest extent of the wound, causing rather poor attachment of the callus tissue.

The roots developed one slightly above the other, from the same locus at the cambial zone (Figures 16 and 17). Extensive phloem development outward to the callus proliferation occurred. Root vascular connections can be seen. It is apparent that considerable new tissue development occurred as a result of wounding and that the roots were initiated fairly early in this process.

Figure 13. Sequential transverse sections of root emergence from a wounded cutting of M.2.

A) Root emergence from wounded area.
B) Stem section above roots, starch stain, x 16.
C) Extent of wounded area.
D) Tissue proliferation at wound.
E) Root position in relation to wounded area.
F) Phellogen formation at root base.
G) Root continuity with stem tissues, x 16.
H) Root continuity with stem tissues, x 25.

(Legend: CZ=cambial zone, P=phloem, PH=phellogen, PI=pith, R=root, W=wound, X=xylem.)

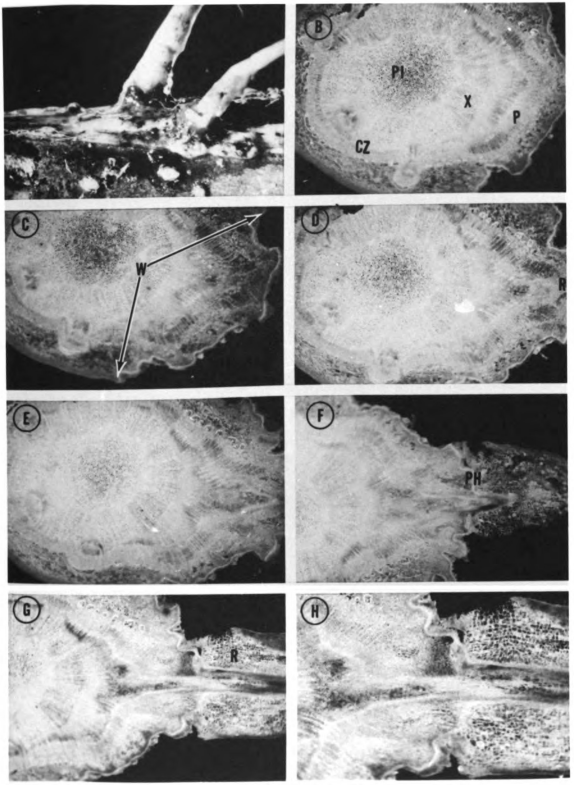

Figure 13

Figure 14. Root development from wounded area in M.2, with vascular and callus tissue proliferation, starch stain, x 16.

(Legend: CZ=cambial zone, P=phloem, R=root, X=xylem.)

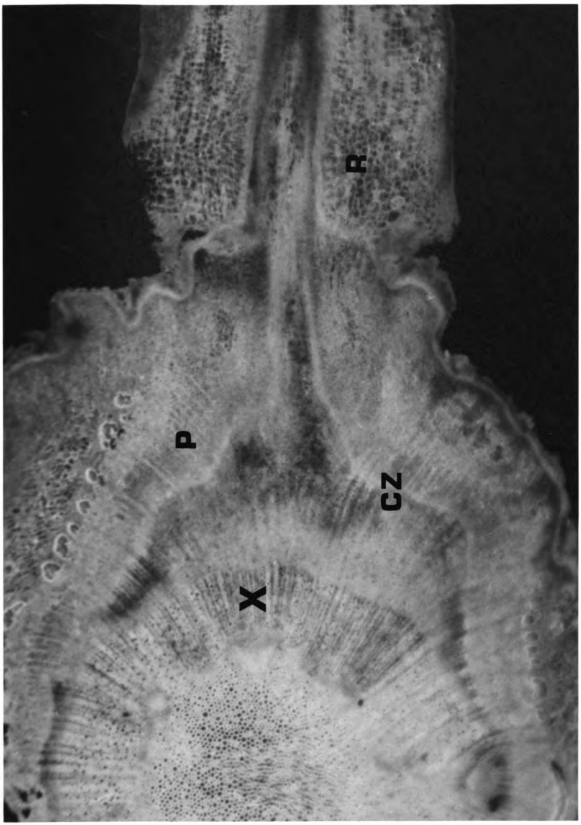

Figure 14

Figure 15. Sequential transverse sections of root emergence from a wounded cutting of MM 106.

A) Root emergence from wounded area.
B) Stem section near roots, starch stain, x 16.
C) Tissue proliferation at wound.
D) Root initiation near cambium.
E) Callus formation caused by wounding.
F) First root entirely visible.
G) Area between the two roots.
H) Second root entirely visible.

(Legend: C=callus, P=phloem, PH-phellogen, PI-pith, R=root, X=xylem.)

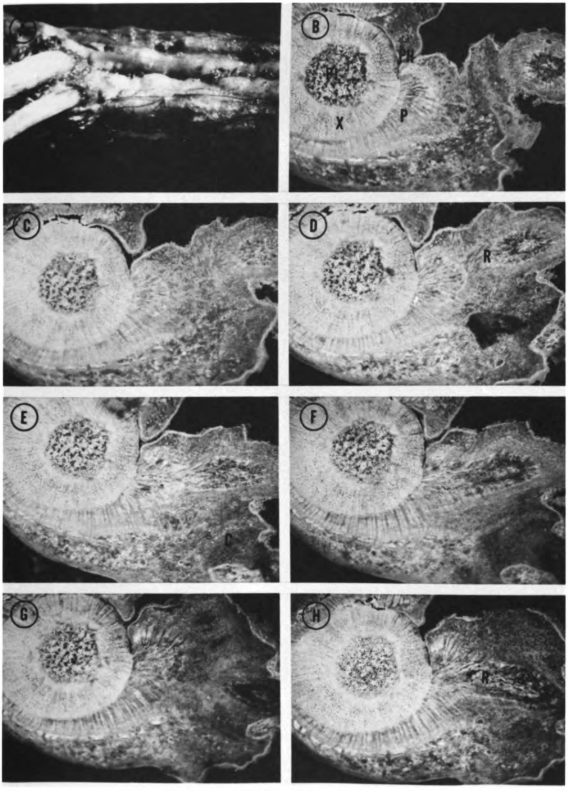

Figure 15

Figure 16. Root development from wounded area in MM 106, with vascular and callus tissue proliferation, starch stain, x 16.

(Legend: C=callus, P=phloem, PH=phellogen, R=root, X=xylem.)

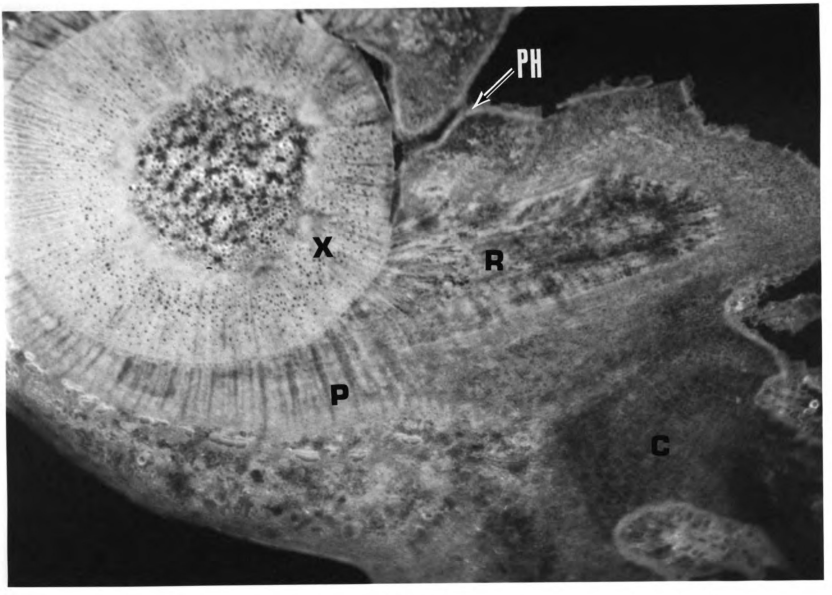

Figure 16

Figure 17. Root development from wounded area in MM 106, with vascular and callus tissue proliferation, starch stain, x 16.

(Legend: C=callus, R=root.)

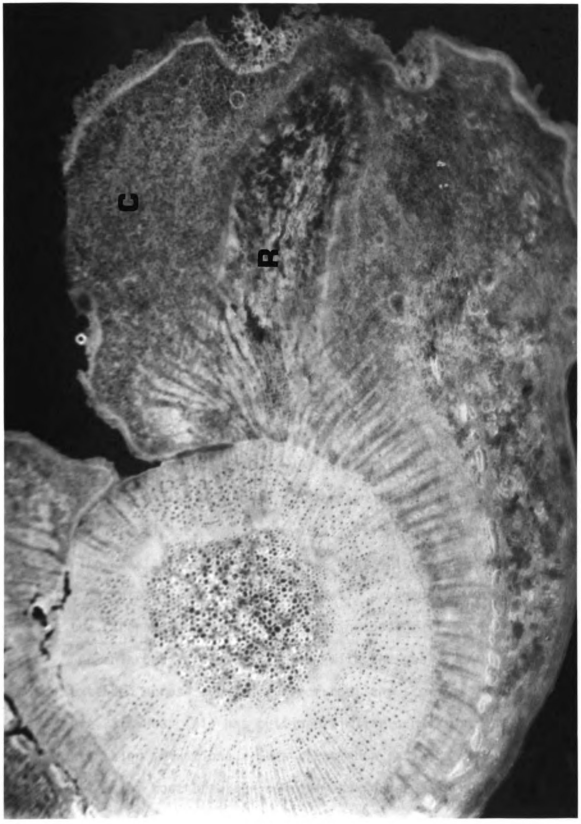

Figure 17

Sequential sections of root development from a lenticel of a cutting are pictured in Figure 18. An interesting point to be seen is the association of the root with a lateral leaf trace and gap, which extended upward to a bud above the lenticel. This indicates that the apparently-superficial root was in fact connected to tissues much deeper in the stem interior. The point of initiation appeared to be near phloem ray tissue.

An enlarged view of the lenticel area indicated a mass of vacular cells behind the lenticel (Figure 19). A phellogen layer covered the lenticel opening. The lateral leaf trace and gap can be seen internal to the vascular cells.

The root base was associated with proliferated phloem tissue and the gap area extended into the pith (Figure 20). Starch deposition was again evident at the base of the root. Cortical fibers were moved outward by tissue proliferation.

Figures 21 and 22 show, in polarized light, sections of cortical fiber tissue, which is typically thick walled and lignified. The abaxial ends of phloem rays abut this fiber ring. Rooting in cuttings was found to generally proceed from areas where the fibers were absent or discontinuous.

An enlarged view of Figure 14, with the phellogen layer which extended across the juncture of root and stem is pictured in Figure 23. Head (41) has outlined similar conditions in root abscission situations. This phenomenon explains some of the problems of root breakage commonly encountered in cutting

Figure 18. Sequential transverse sections of root emergence from a lenticel in M.2.

A) Root emergence from lenticel.
B) Stem section at lenticel, starch stain, x 25.
C) Gap area internal to lenticel.
D) Position of root emergence.
E) Starch deposition at root base.
F) Area between the two roots.
G) Root initiation near cambium, x 25.
H) Root initiation near cambium, x 40.

(Legend: G=gap, L=lenticel, P=phloem, PH=phellogen, PI=pith, R=root, X=xylem.)

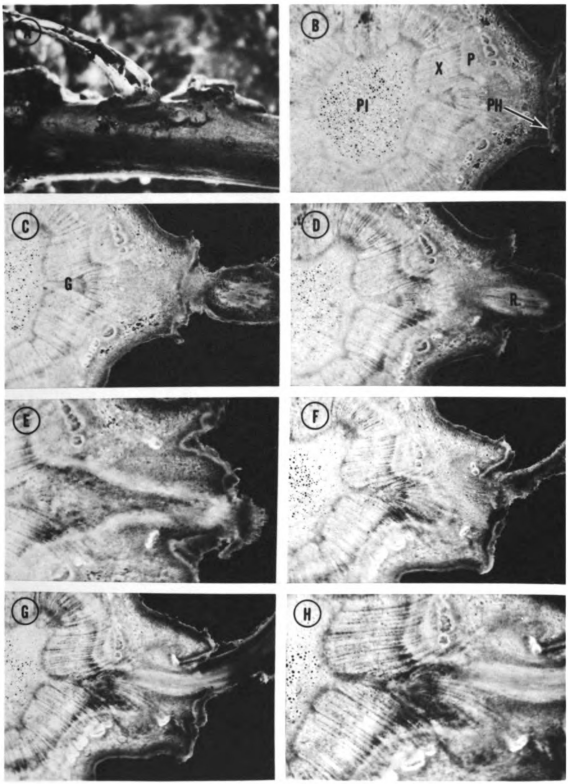

Figure 18

Figure 19. Lenticel area in M.2, starch stain, x 25.

(Legend: G=gap, L=lenticel, X=xylem.)

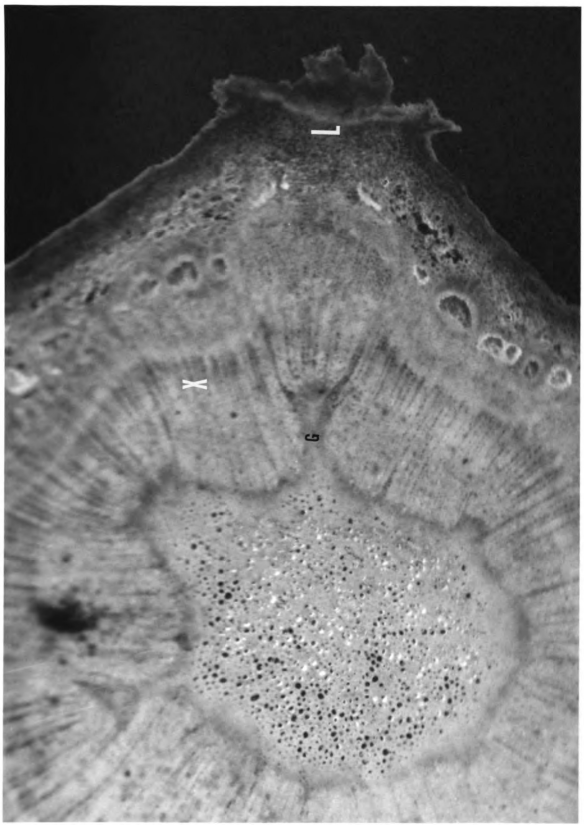

Figure 19

Figure 20. Root development from lenticel area in M.2, in association with the leaf gap area, starch stain, x 40.

(Legend: F=fibers, G=gap, R=root.)

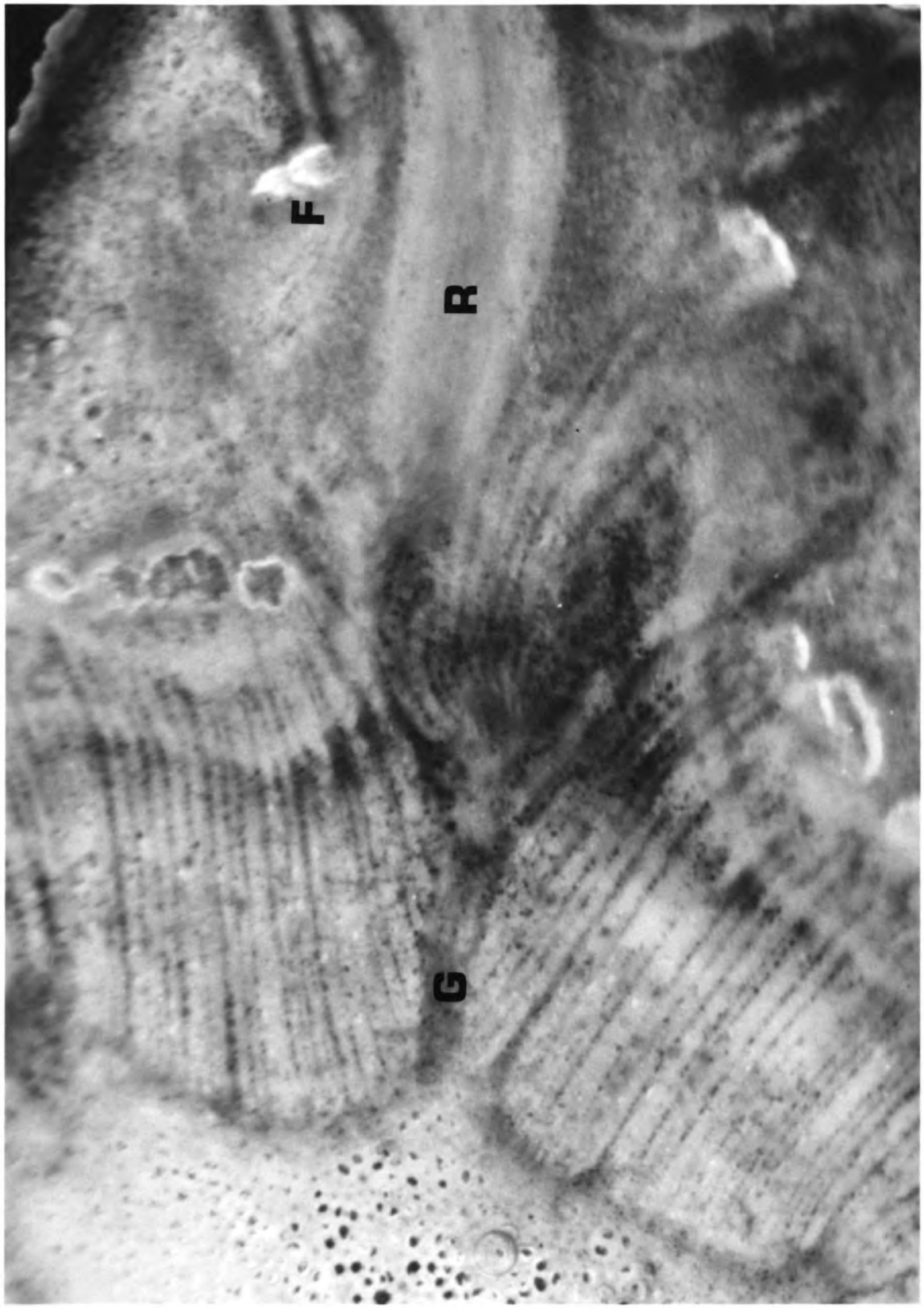

Figure 20

Figure 21. Cortical fiber tissue in M.9 transverse stem section, polarized light, x 125.

(Legend: C=cortex, F=fibers, P=phloem.)

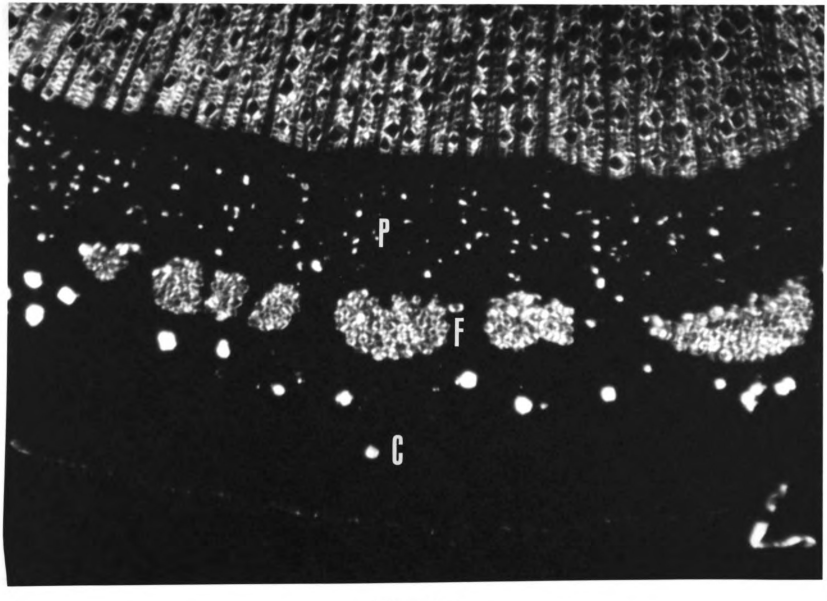

Figure 21

Figure 22. Thick-walled fiber cells, in M.9 transverse stem section, polarized light, x 500.

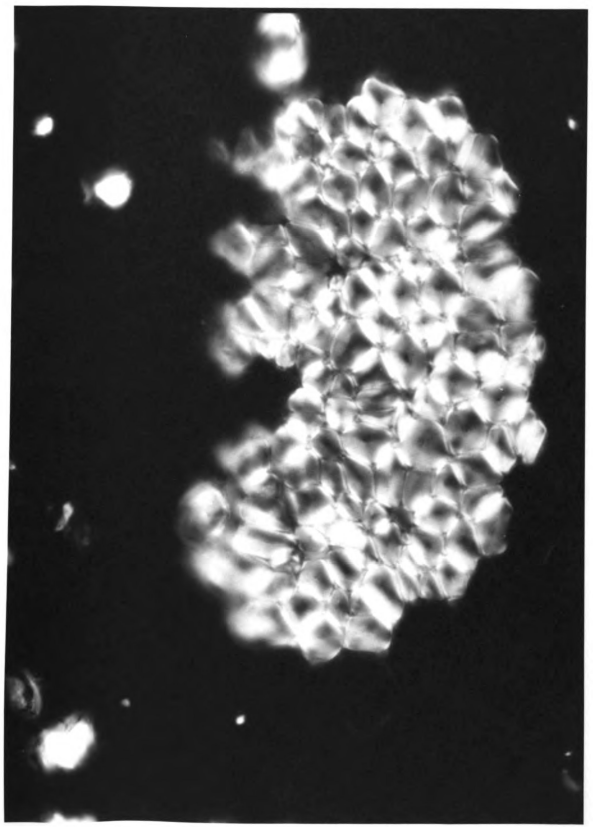

Figure 22

Figure 23. Abscission zone development at root base in M.2 cutting, starch stain, x 40.

(Legend: P=phloem, PH=phellogen, R=root.)

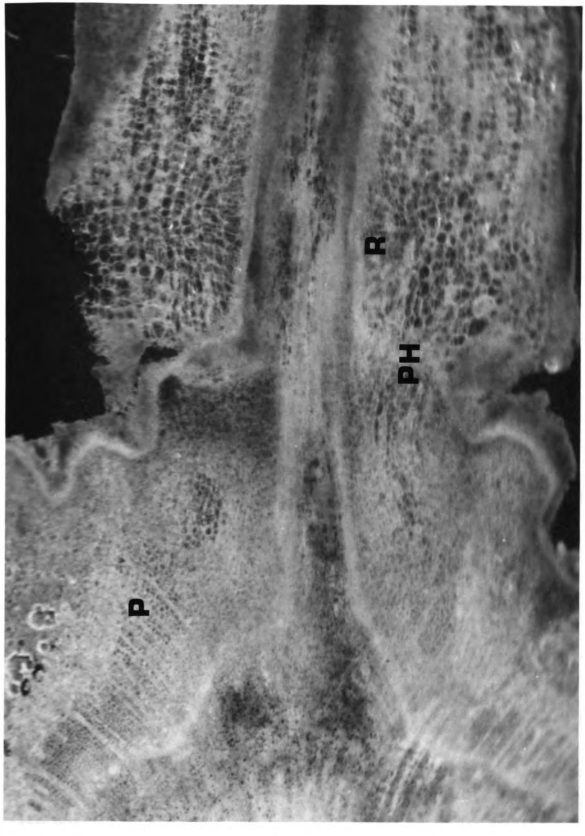

Figure 23

propagation. The presence of an abscission zone at the base of long fleshy roots provides conditions favoring root breakage during handling. This problem seems to be particularly acute in the case of solitary large-caliber roots typically produced by some clones. Considerable clonal diversity in type of root produced was noted in this study (Figures 2, 6, 7). M.2 in particular seemed to produce thinner and more flexible roots, with more lateral branching, than did M.9 and M.26. Solitary roots from a cutting are often thick, fleshy, and brittle, with no lateral root development. Many of these roots can be broken or lost during transplanting.

SUMMARY

This research was conducted to study adventitious root formation in softwood cuttings as influenced by stem anatomy, wounding, and level of starch reserves.

Root development in cuttings was shown to involve certain differences from that in layered shoots. A diversity of root emergence sites was noted, including bud and leaf gaps, phloem rays, lenticels, and callus proliferation. Root development was promoted by the exogenous application of hormone to the basal cut area. This treatment increased meristematic activity and tissue proliferation. Similarly, deliberate lateral wounding of stems also provided sites for root initiation and improved rooting percentage in shy-rooting clones.

Seasonal stem starch levels in cutting hedge plants provided information about the frequently-noted seasonal trends in rooting success. Rooting performance in cuttings can generally be

influenced by a combination of internal and external factors. Internal factors such as nutritional levels, food reserves, tissue differentiation, hormone supplies, and water relations can be partially controlled by the propagator through seasonal timing and cultural care of the mother plants. External factors such as hormone application, handling of cuttings and rooting environment are totally dependent on the propagator's skills.

The seasonal starch maxima and minima shown in this study indicated the importance of considering the internal plant factors when propagating different plant materials. This was particularly true in the case of hardwood cuttings, where starch levels coincided with reported winter rooting trends. Stored food reserves are especially important in dormant cuttings, when no redeposition is possible and depletion may occur due to excessive root elongation. These results may be of practical value in providing further information on balancing the interacting internal and external factors for optimum rooting performance. Biochemical studies may elucidate inherent differences between easy- and shy-rooting clones.

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Professional Thesis Preparation
Cliff and Paula Haughey
144 Maplewood Drive
East Lansing, Michigan 48823
Telephone (517) 337-1527