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LA THÈSE A ÉTÉ  
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Correlative effects of stock and scion organs and of graft partners  
on the developmental anatomy of the graft union in Coleus.

A thesis submitted to the School of Graduate Studies and Research  
of the University of Ottawa, in partial fulfilment of the requirements  
for the degree of Master of Science in Biology by

Frederick L. Stoddard

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## CONTENTS

Acknowledgments	ii
List of tables	iv
Abstract	v
Résumé	viii
General Introduction	1
Part 1. Effects of excision of stock and scion organs on the development of the graft union in <u>Coleus</u> : a histological study.	3
Introduction	4
Materials and Methods	6
Results	11
Figures and figure legends	20
Discussion	26
Literature cited	34
Part 2. Histological comparison of the development of grafts and wounds in <u>Coleus</u> .	38
Introduction	39
Materials and Methods	40
Results	46
Figures and figure legends	63
Discussion	83
Literature cited	107
Conclusion	117

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## LIST OF TABLES

## Part 1.

Table 1. Percentage of cohesion of stock and scion following removal of various organs.	12
Table 2. Numbers of xylem strands bridging the graft union following organ removal.	14
Table 3. Numbers of phloem strands bridging the graft union following organ removal	17
Table 4. Numbers of xylem strands bridging the graft following organ removal, as percentage of control.	29a

## Part 2.

Table 1. The characteristics for which cortex, vascular parenchyma and pith were examined in every scion, cutting, stock and stump.	45
Table 2. Comparison of grafts and wounds, 2 days after cutting.	50
Table 3. Comparison of grafts and wounds, 4 days after cutting.	52
Table 4. Comparison of grafts and wounds, 6 days after cutting.	55
Table 5. Comparison of grafts and wounds, 10 days after cutting.	58
Table 6. Nuclear location, by percentage of total, for scions and cuttings; or stocks and stumps.	61

v

ABSTRACT

In the first part of this study, effects of removal of leaves, shoots and roots on the formation of the graft union in Coleus stems were investigated. On the basis of counts of xylem strands bridging the grafts following organ removal, estimates of contributions of xylem-inducing stimuli were made: acropetal flow from stock to scion, 8 % of the xylem in grafts of otherwise intact plants; basipetal flow from immature stem tissue, 10 %; shoot tips and immature leaves on scion, 10 %; scion leaves that are at least half mature, 36 %. The remaining 36 % of xylem-inducing stimuli may be from synergistic effects of the scion organs. The scion buds and leaves nearest the graft contribute ca. 40 % of their respective organ-specific xylem-inducing stimuli although they represent only 30 % of their respective organs on the scion. Leaves and shoot tips on the stock have no effect on the graft. Severing the stock below the graft resulted in a reduction of graft-bridging xylem to 22 % of that in otherwise intact plants. The ratio of graft-bridging strands of phloem and xylem is not significantly changed by any treatment and averages 0.23:1, compared with 2.2:1 in intact internodes. Independence of control is shown for the following components of the grafting process: callus formation on the cut surfaces, necrotic layer removal, cohesion of the graft partners; and bridging of the graft by xylem, phloem and cambium. Grafts of isolated internodes cohere without apparent participation of the other components.

The second part of the study comprised a detailed microscopical examination of grafts of intact Coleus plants, grafts of isolated internodes, and the cut surfaces of plants severed at the grafting internode

and left open to the air or covered with Parafilm. The early stages of graft formation and wound healing are similar, as cytoplasm and nuclei accumulate at the wound-facing ends of the cells. The number of nuclei at the cell ends near the cut is greater than that at the distant ends of the cells, significantly so in the stocks and stumps. Either graft partner or covering allows hypertrophy of the underlying cells, many of which die when left unprotected. This hypertrophy is accompanied by a small amount of cell division in isolated internode grafts, and by a larger amount in control grafts and covered wounds. New surfaces produced by such cell expansion are indistinguishable from ordinary parenchymatous cell walls in appearance and histochemistry. Stock-scion contact may occur at sites of newly exposed surfaces or at those still covered with necrotic material, but there is no way of determining at which sites there is cohesion. Some scions and cuttings produce a possibly hemicellulosic material that is frequently found at the graft interface. Dead cell materials are largely dispersed, and there is little evidence for their dissolution. Wound gum may appear as dense plugs in severed xylem vessels, and is distinct from the necrotic layer which has erroneously been given the same name. A thin refractile layer, of nature uncertain beyond its high phenolic content, covers the vascular parenchyma of many control stocks and uncovered stumps and a few control scions, but is not found in isolated internode grafts or covered wounds. By day 6 clear differences emerge in patterns of cell division. Grafts form callus masses between the partners, with random planes of division, while wounds form wound periderms with divisions largely parallel to the cut, regardless of the presence or absence of covering. A plant part must be in contact with a living graft partner

to prevent the suberization found in all older wounds. Some xylem differentiates in the wound periderm of protected wounds. It is concluded that graft partners provide each other considerably more than simple protection from the external environment, including some specific inhibition of suberization.

## RESUME

Dans la première partie de cette étude, les effets de l'excision des feuilles, des pousses et des racines sur le développement de l'union entre tiges greffées étaient examinés chez Coleus. En se basant sur le compte des torons de xylème enjambant les greffes après l'excision des organes, on a fait des évaluations des contributions des stimuli provoquant la formation de xylème: coulement acropétale de la porte-greffe au greffon, 8 % du xylème différencié dans les greffes des plantes autrement intactes; coulement basipétale du tissu des tiges qui n'est pas mûr, 10 %; des pointes des pousses et feuilles pas mûres sur le greffon, 10 %; des feuilles du greffon qui sont au moins demis-mûres, 36 %. Le 36 % de reste des stimuli provoquant la formation du xylème peuvent être des effets synergistiques des organes du greffon. Les bourgeons et feuilles du greffon auprès de la greffe contribuent à 40 % de leurs stimuli spécifiques de l'organe, quoiqu'ils représentent seulement 30 % de leurs organes spécifiques sur le greffon. Les feuilles et les pointes des pousses sur la porte-greffe n'ont pas d'effet sur la greffe. Séparation de la porte-greffe au-dessous de la greffe a résulté dans une réduction de xylème enjambant la greffe à 22 % de ça dans les plantes autrement intactes. La proportion des torons de phloème et de xylème n'est pas changée d'une manière significative par aucun traitement et elle donne en moyenne 0.23:1 en comparaison de 2.2:1 dans les internoeuds intacts. On démontre l'indépendance de contrôle pour les parties constituantes du processus de greffage suivantes: formation du cal sur les surfaces coupées, enlèvement de la couche nécrosée, cohésion du greffon et de la porte-greffe, et enjambement de la greffe par le xylème, le phloème et le cambium. Les greffes des internoeuds iso-

lés adhèrent sans la participation apparente des autres parties constituantes.

La deuxième partie de cette étude a compris un examen détaillé au microscope des greffes de plantes de Coléus autrement intactes, des greffes des internoeuds isolés, et des surfaces coupées des plantes désunies à l'internoeud greffé et laissées exposées à l'air ou couvertes avec du Parafilm. Les premiers étages de la formation de la greffe et de la cicatrisation sont semblables, parce que le cytoplasme et les noyaux s'accumulent aux bouts des cellules faisant face à la blessure. Le nombre des noyaux aux bouts des cellules près de la blessure est plus grand que cela aux bouts éloignés, d'une manière significative dans les portes-greffes et les souches. Soit l'associé de la greffe, soit couverture, permette l'hypertrophie des cellules sous-jacentes, beaucoup desquelles meurent quand laissées exposées. Quelques divisions cellulaires accompagnent cette hypertrophie dans les internoeuds isolés, et un plus grand nombre de divisions dans le cas des greffes contrôlées et des blessures couvertes. Les surfaces nouvelles produites par cette dilatation cellulaire sont indistinguibles des murs ordinaires des cellules parenchymateuses en aspect et en histochimie. Le contact entre le greffon et la porte-greffe peut se rencontrer aux lieux des surfaces nouvelles, ou à celles encore couvertes de matières nécrotiques, mais il n'y a pas moyen de déterminer à quels lieux il y a cohésion. Quelques greffons et boutures produisent une matière, peut-être hemicellulosique, qui se trouve fréquemment au dièdre de la greffe. Les matières nécrotiques sont dispersées, et il y a peu d'évidence pour leur dissolution. Une "gomme de blessure" peut se manifester en formant des bouchons épais dans les vaisseaux de xylème

coupé, et elle est distincte de la couche nécrosée qui a reçu le même nom par erreur. Une couche mince et réfractive, de nature indéterminée sauf qu'elle contient beaucoup de substance phénolique, couvre la parenchyme vasculaire de beaucoup de porte-greffes témoins et de souches découvertes et de quelques greffons témoins, mais elle ne se trouve pas dans les greffes des internoeuds isolés ou des blessures couvertes. Avant le sixième jour, des différences claires émergent dans les types de division cellulaire. Les greffes font des masses de cal entre les associés, avec des plans inégaux de division, tandis que les blessures font des péridermes cicatriciels avec des divisions en grande partie parallèles à la coupe, peu soigneux de la présence ou absence de couverture. Une partie de la plante doit être en contact avec un associé vivant de la greffe pour prévenir la subérisation trouvée dans les blessures plus vieilles. Certain xylème différencie dans le périderme cicatriciel des blessures couvertes. On conclut que les associés de la greffe fournissent l'un à l'autre considérablement plus que de la défense contre le milieu externe, y inclus quelque inhibition spécifique de subérisation.

## GENERAL INTRODUCTION

Grafting was probably practiced as long ago as 1560 B.C., when a Chinese text discussed varieties of peach, propagation of which would have been unlikely without grafting (Roberts 1949). In the fourth century B.C., Theophrastus gave the first known written account of using rootstocks, in that case for apples. - By 50 B.C. there were over 20 varieties of apple in Rome, many of which may have been dwarfed by rootstocks for use in topiary gardens. In Spain, the Arab, Ibn el-Awam (flourished 1150 to 1170) wrote on the propagation of apples by grafting (McKenzie 1956).

The importance of grafting to horticulture in Renaissance times is shown by the fact that the first English book on gardening (according to Henrey 1975), is an anonymous publication from 1520 on "The Crafte of Graffynge and Plantynge of Trees". In 1561, Cordus Valerius, a German, described apple growing and grafting in "Historia Stirpium lib." (McKenzie 1956). Parkinson (1629) described virtually all common grafting methods, from inarching to budding.

Today, increasing numbers of crops are grown on grafted plants - e.g., most tree fruits, grapes, blueberries - wherever the native root is undesirable for some reason, such as poor growth in the local soil, susceptibility to rootborne diseases, or the ability of another rootstock to change plant size or hasten fruit production.

In Indonesia, grafting has found an unusual application in the so-called Mukibat system. There, a cassava variety able to produce especially large tubers has a shoot system unable to fully exploit this potential, so a more efficient shoot system is grafted on, thus doubling yield (De Bruijn and Dharmaputra 1974).

The earliest known scientific examination into grafting was published in 1758 by Duhamel du Monceau. In this study of graft anatomy he noted the proliferation of parenchyma-like cells in the area of contact between graft partners (Brabec 1965). Chronologically, the next important works, both on aspects of the developmental anatomy of the graft union, were by Vöchting (1892) and Wright (1893). For a long time, a few extremely bad electron micrographs of incompatible woody grafts (Buchloh 1960, 1962) represented the only electron microscopy of the graft line. Very few have appeared since (Yeoman and Brown 1976, Moore 1978b, Yeoman et al. 1978).

With the use of grafting so widespread, it seems remarkable that there has been no investigation of the effect of various plant organs on the developmental anatomy of the graft union since Kabus did his preliminary tests in 1912. The first part of this research was undertaken to correct this omission; the second, to investigate certain aspects of the grafting process, and to compare them to similar wounds, at the maximum resolution possible with the optical microscope.

Note. - References for this introduction listed with Literature Cited for part 2 of this thesis.

## Part 1.

Effects of excision of stock and scion organs  
on the development of the graft union  
in Coleus: a histological study.

## INTRODUCTION

When plants are grafted, the stock and scion always bear different numbers and kinds of organs. Yet, experiments on the correlative effects of these organs on the grafting process have been reported only once, and in a limited context. Kabus (1912), using potato tubers, showed that, for successful grafts, buds had to be present on both graft partners if the tubers were cut in air, but not if the tubers were cut under water. Brabec (1965) reviewed the literature on the physiology of grafting and made no mention of other experiments on the effects of removal of stock and scion organs on the grafting process.

The effect of stock leaves on incompatibility in grafts of Cucumis melo L. on Cucurbita ficifolia Bouch. was investigated by De Stigter (1971a, 1971b, 1971c). In these grafts, phloem connections do not function for some time after xylem connections are operative, so the roots receive no scion photosynthate. The greater the number of rootstock leaves, then, the longer the roots survive, but the more delayed is the process of recovery from the early incompatibility. In any case, recovery takes too long for field-grown plants to survive, and thus can be observed only in hydroponically grown plants.

Evidence from other experiments indicates the possibility that grafting might itself consist of a number of processes controlled independently. For instance, Muzik (1958) showed that stems of Vanilla orchids grafted to each other without the subsequent formation of vascular tissue connecting stock and scion, and we showed that, in grafts of pea roots, vascular tissue formation on the side of the graft toward the shoot tip is independent of eventual graft cohesion (Stoddard and McCully 1979).

The present study was designed to determine the effect of various plant organs - roots, shoot tips, leaves - on the grafting process. It also demonstrates the separability of the grafting process into a number of components. We have chosen to use autografts of Coleus blumei Benth. because the hormonal and correlative influences on wound healing and the production of vascular tissue have been well studied in this plant, principally by Jacobs and coworkers (Jacobs 1952; LaMotte and Jacobs 1963; Thompson and Jacobs 1966) and Roberts and coworkers (Fosket and Roberts 1964; Roberts and Fosket 1966). Since Coleus has little apical dominance, one may manipulate numbers of shoot tips and leaves in both stock and scion. Finally, the use of autografts eliminates incompatibility as a cause of graft failure.

## MATERIALS AND METHODS

Plants. - Cuttings of the red clone of Coleus blumei grown at Carleton University were rooted and grown in 10-cm plastic pots filled with soil in a greenhouse for 3 wk. The plants were then placed in a west-facing laboratory window for 1 wk, at the end of which each plant had four to six pairs of mature leaves (lamina ca. 8 cm long). Plants were thoroughly watered the day before grafting.

Grafting procedure. - The technique used for grafting pea roots (Stoddard and McCully 1979) was adapted in the following manner. Both ends of a wooden support stick, 2 mm in diameter and 10 to 15 cm long, were bound with Parafilm to the intact stem so the middle of the internode below the third pair of mature leaves was near the middle of the stick. Parafilm was then wound 2 or 3 times around this internode and the stick to bind the grafting area firmly to the splint. Using a new surgical steel razor blade, we made a diagonal cut through the Parafilm and internode such that the third pair of mature leaves was above the top and bottom ends of the cut (fig. 1). More Parafilm was wound over the cut to hold the graft partners tightly together.

Each grafted plant was treated immediately by one of the following procedures (fig. 1): A, control (no further operations); B, all leaves with lamina longer than 4 cm were removed from the scion; C, the apical bud was excised together with all leaves shorter than 4 cm, and all axillary buds were cut from the scion; D, all leaves and buds were removed from the scion; E, the scion was severed just below the third pair of mature leaves; F, all leaves and axillary buds were removed from the stock; G, the scion was severed above the third pair of mature leaves; H, same as G, but axillary buds were also removed; and I, the

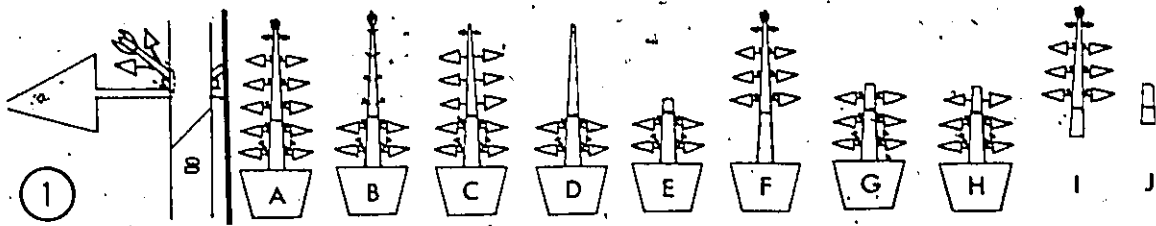


Fig. 1. Diagram on the left shows the orientation of the graft line in relation to the nodes on either side of it. The dotted line shows the cut to remove the axillary buds and/or branches. A, Grafted control. B, All leaves with lamina longer than 4 cm removed from the scion. C, Apical bud excised together with all leaves shorter than 4 cm, and all axillary buds cut from the scion. D, All leaves and buds removed from the scion. E, Scion severed just below the third pair of mature leaves. F, All leaves and axillary buds removed from the stock. G, The scion severed above the third pair of mature leaves. H, Same as G, but axillary buds also removed. I, Stock severed just above the fourth pair of mature leaves. J, Isolated internode. Graft line (horizontal line in A to J) is at the same level in all treatments.

stock was severed just above the fourth pair of mature leaves. These cuttings were kept with their bases in a few mm of water and enclosed in a transparent Plexiglas container in a growth chamber at 25°C, 16 h light, 2500 lx.

The final treatment consisted of grafting excised internodes (fig. 1 J). The internode below the third pair of mature leaves was excised; the top was identified with a diagonal cut. This internode was bound to a stick of similar length, cut and held together with Parafilm as described above, and maintained on moistened filter paper in a closed petri dish in the growth chamber.

Plants with intact root systems (i.e., those from treatments A to H) were maintained in loosely covered transparent Plexiglas boxes in a west-facing window. The plants were checked daily and water added as necessary to ensure that humidity was high enough to maintain scion turgor but low enough to prevent water of condensation from inhibiting graft cohesion. The boxes were turned 90° clockwise on alternate days.

Experiments were conducted during spring and summer of 1979 with batches of 40 plants, 15 each for any two treatments B to J plus 10 controls, and were repeated once.

Excision, sectioning and clearing of the graft region. - Four, 6, 8, 10, and 12 days after grafting, the grafts were excised with a cut parallel to the graft and 3 to 5 mm above it, and with a transverse cut 2 to 3 mm below the lowest point of the graft. The segments were held in 0.05 M potassium phosphate buffer solution, pH 7, for up to 1 h. They were then hand sectioned longitudinally so each section showed the diagonal aspect of the graft. The sections were cleared by heating to the boiling point in 60 per cent lactic acid and were left in the acid

as it cooled overnight. Cleared sections were soaked at least 15 min in distilled water, then for a minimum of 15 min in 0.067 M  $K_2HPO_4$ , and mounted in 0.05 per cent aniline blue (Lot # 1523, Polysciences Inc., Warrington PA) in 0.067 M  $K_2HPO_4$ . Coverslips were sealed to the slides with nail polish. If kept in the dark, sections remain in good condition for many months.

Sections were cut thick (up to 200 micrometers) to reduce the chances of increasing the counts of graft-bridging xylem or phloem strands by splitting them longitudinally, or of decreasing counts by splitting the strands transversely. Even such thick sections are considerably softened by the clearing and must be handled carefully to avoid damage.

Some old grafts and ungrafted plants were sectioned, stained in 0.5 per cent aqueous basic fuchsin (C.I. 42500, Fisher Scientific Co., Fairlawn NJ) for 1 min, destained in 0.5 per cent sodium metabisulphite in 0.1 N HCl until only xylem elements and phloem fibres were stained, and mounted in the same medium.

Observation of graft region and counting of xylem and phloem strands bridging the graft. - Sections must be allowed to remain in the aniline blue solution for at least 2 days before examination. Sections of grafts that are cleared, washed and freshly mounted in aniline blue, when viewed with bright field optics, are almost colourless. With epifluorescence optics this material is also unusable for counting because of excessive glare from xylem autofluorescence. After 2 days in the dark, however, the xylem elements and necrotic layer have become stained by the blue, non-fluorescent components of the commercial dye mixture (see Smith and McCully 1978), making them visible in bright field and markedly reducing their autofluorescence while the fluorochrome-induced callose fluores-

cence in the sieve tubes is not noticeably altered. These changes are permanent over several months. With contrast thus enhanced, observations of the necrotic layer and callus, and counts of xylem strands, were made using bright field optics. Further observations of callus and counts of phloem strands were made using epifluorescence optics.

From thick hand sections containing a large number of xylem elements, fairly repeatable counts were obtained by focusing through the section.

Counts do not represent bundles of elements; individual xylem elements crossing the line of the graft were counted. Thus, a bundle of 16 xylem strands was counted as 16, not as 1. The callus was soft and easily distorted so no actual measurements of thickness were made. Where part of the graft line in the region where counts were being made was obscured, its location could be inferred from adjacent visible parts.

Counts were tested for significant differences using the Student-Newman-Keuls test (Zar 1974).

Microscopy and photomicrography. - Slides were examined with a Carl Zeiss Universal microscope, equipped with bright field and epifluorescence optics (exciter filter BG 12, beamsplitter Fl 480, and barrier filter 50). Specimens viewed in bright field were recorded on Kodak Ektapan 4" x 5" sheet film, rated ASA 100, and developed in Kodak DK-50. Fluorescence was recorded on Kodak Panatomic-X 35 mm roll film, rated ASA 64 and developed in Ilford Microphen.

## RESULTS

Anatomy of the uncut stem at the graft site. - The anatomy of Coleus has been described in detail by Sloover (1958). Figure 2 shows a cross section of the stem in the grafting region to provide orientation for subsequent figures. Between the large corner vascular bundles, and joined to them by a young interfascicular cambium, lie variable numbers of much smaller bundles, some of which contain no xylem (Aloni and Jacobs 1977b). The walls of phloem fibre initials have not started to thicken. The stem has a large pith area, chlorophyll-rich cortical parenchyma, and no cork cambium has yet been initiated. Pith cells are large and roughly isodiametric, in contrast to the smaller, somewhat axially elongated cortical parenchyma cells.

Developmental sequence of the normal graft. - In most of the grafts, the lips of stock and scion curl away from the axis of the plant. The remaining contact zone stretches from side to side across the graft, representing about half to three-quarters of the available surface (inset, fig. 2). The gaps under the lips are gradually filled (see below).

The necrotic layer, formed soon after cutting from the walls and contents of killed cells, is strongly birefringent (fig. 4) owing to the stacking of the collapsed walls on the cut surface.

By day 4 callus growth, generalized over the cortical parenchyma but especially evident over the corner vascular bundles, has already disrupted parts of the necrotic layer (fig. 3). The pith parenchyma, however, has not yet proliferated (fig. 3), and necrotic layers overlying it are not affected (fig. 4). About half of the grafts cohere in the middle region (inset, fig. 2; fig. 3; table 1), and while new xylem

Table 1.

Percentage of cohesion of stock and scion following removal of various organs.

Treatment	Daily Sample Size	Age (Days)				
		4	6	8	10	12
A. Control	13	54	85	85	100	100
B. Scion defoliated	6	33	83	100	100	100
C. Scion disbudded	3	0	67	100	100	100
D. Scion denuded	6	67	100	100	100	100
E. Scion severed	5	80	100	100	100	100
F. Stock denuded	3	100	100	100	100	100
G. Scion severed 1 node above graft	5	20	40	80	100	100
H. As above, axillary buds removed	5	20	80	100	100	100
I. Stock severed	4	50	100	100	100	100
J. Isolated internode 4-8		50	75	38	57	43

Note. - Grafts were said to "cohere" if the stock and scion remained together during sectioning, and not to "cohere" if the partners fell apart during unwrapping or sectioning. In practice, very few grafts that remained together during unwrapping failed to do so during sectioning.

and phloem strands have differentiated in both the stock and the scion, connections across the graft are lacking (figs. 3, 5).

Almost all grafts cohere by day 6 (table 1), and many have a few graft-bridging xylem and phloem elements (figs. 6, 7; tables 2, 3). Callus formation has progressed, especially on the scion, filling the gaps between the graft partners. Most of the pith cells at the graft line remain undivided (fig. 7). The necrotic layer is noticeably thinner, even over the pith (fig. 7).

In subsequent days more xylem and phloem strands bridge the union (tables 2, 3). The pith cells start to proliferate at the graft line by day 8, laying down walls roughly parallel to the graft line. In many places the necrotic layer is obliterated (fig. 8). Often the phloem (less often the xylem) strands tend to congregate at a particular crossing point (figs. 9, 10), fanning out to a greater or lesser degree in either graft partner.

The callus continues to proliferate and usually fills all gaps by day 10. By day 12 there are almost continuous ribbons of xylem along the slanted sides of the cut (fig. 11), and the phloem is similarly multiplied (fig. 10). The divisions in the pith continue, forming walls parallel to the cut surface (figs. 12, 13). The necrotic layer is largely eliminated, as shown by its lack of birefringence and of distinguishable compressed walls over large areas (fig. 12). Eventually the graft line is obliterated between the xylem and the epidermis (fig. 14) and the necrotic layer is seen only in the pith region as a thick wall retaining its birefringence (fig. 13).

The secondary walls of the xylem elements differentiating during the grafting process have reticulate pitting and are lignified, as

Table 2.

Numbers of xylem strands bridging the graft union following organ removal.

Treatment	Daily Sample Size	Age (Days)				
		6	8	10	12	
A. Control	13	52 ± 26 (9)	175 ± 59 (8)	150 ± 63 (9)	195 ± 64 (9)	
B. Scion defoliated	6	24 ± 29	29 ± 26	48 ± 51	60 ± 32	
C. Scion disbudded	3	11 ± 10	84 ± 57	62 ± 14	152 ± 31	
D. Scion denuded	6	7 ± 8	28 ± 24	28 ± 17	39 ± 24	
E. Scion severed	5	0	7 ± 5	15 ± 10	26 ± 8	
F. Stock denuded	3	71 ± 15	107 ± 67	178 ± 12	224 ± 46	
G. Scion severed 1 node above graft	5	5 ± 9 (4)	9 ± 7 (4)	67 ± 51	73 ± 79 (3)	
H. As above, axillary buds removed	5	18 ± 27	40 ± 33	38 ± 35	36 ± 29	
I. Stock severed	4	0	40 ± 28	49 ± 45	36 ± 38	
J. Isolated internode	4-8	0 (4)	0 (8)	0 (7)	0 (7)	

Note. - Figures are means ± S. D., with sample size in parenthesis where it differs from the corresponding figure at the left of the table.

indicated by autofluorescence. The elements themselves vary in shape; some, especially those differentiated early in grafting and in the outermost pith near the original vascular bundles, are rectangular in longitudinal section, like the parenchyma cells near the cambium, but not fusiform like the original secondary xylem (fig. 3). Elements formed somewhat later are much less regular in shape (fig. 7), taking on rounded or irregular forms like the cells, largely pith, amongst which they differentiate. Eventually, xylem elements form with shapes typical of those derived from a cambium (fig. 11).

The short, narrow sieve elements differentiating during grafting form strands which meander through the inner cortex (figs. 9, 10). In form, though not in dimension, these sieve elements resemble those normally seen in both primary and secondary phloem of Coleus.

Near the graft the cross-sectional area of the xylem is large and gradually decreases until a few millimeters from the graft where it is comparable to that in the equivalent internode of ungrafted plants (figs. 14, 15). The extra strength conferred by this thickening was illustrated when a 12-day-old graft was broken. The line of fracture went through the vascular tissue of the stock below the zone of extra xylem and crossed the pith jaggedly, variously at, below, and above the graft line.

From day 8, most scions have numerous (up to 12) short adventitious roots, emerging 1 to 3 mm above the graft. These roots interfere directly with the graft only rarely, when they are caught in the gap where a stock or scion lip has curled away from the axis. There is no visible preponderance of these roots on any side of the stem.

Effect of treatments on rate of cohesion and anatomy of the graft.

- Speed of cohesion (table 1) and time of appearance of the first graft-bridging xylem (table 2) and phloem (table 3) are not significantly affected by any of the experimental treatments. Ultimate percentage of cohesion, which depends so much on grafting technique, was depressed only in the isolated internodes (table 1).

Visual estimation of callus thickness showed that the amount of callus was reduced at the base of the denuded scion (treatment D) and at the base of the severed scion (treatment E). Callus was absent in the pith of isolated internode grafts (figs. 16, 17) and occurred only in a very restricted area of cortex near the vascular bundles (fig. 18). Other treatments did not visibly affect callus formation.

The necrotic layer was also apparently unaffected by most treatments, being virtually obliterated by day 12, albeit somewhat more slowly in the grafts with denuded or severed scions (treatments D and E). In the isolated internodes, however, there was no apparent degradation of the necrotic layer (figs. 16, 17), in spite of which, 15 of the 30 grafts firmly cohered, and only three of them had any vascular connections. One at day 6 had a single connecting xylem strand (fig. 18); one at day 8 had two connecting phloem strands (figs. 19, 20); and one at day 12 had one strand each of xylem and phloem (fig. 21).

The numbers of xylem and phloem strands which bridge the graft are strongly influenced by the various treatments (tables 2, 3). From day 6 to day 10 the number of xylem strands in the control grafts was significantly greater (at the 5 % level) than all treatments except the denuded stock, treatment F, which was indistinguishable from control. The numbers of xylem strands in disbudded scions (treatment C) approach those of the control until day 12, when the two numbers do not signifi-

Table 3.

Numbers of phloem strands bridging the graft union following organ removal.

Treatment	Daily Sample Size	Age (Days)			
		6	8	10	12
A. Control	13	3 ± 4 (9)	23 ± 26 (8)	26 ± 27 (11)	52 ± 40 (8)
B. Scion defoliated	6	3 ± 6 (5)	10 ± 16 (5)	22 ± 22	24 ± 20
E. Scion severed	5	0	0	1 ± 2	2 ± 1
I. Stock severed	4	9 ± 18	8 ± 6	13 ± 17	8 ± 7 (3)
J. Isolated internode	4-8	0 (4)	0 (8)	0 (7)	0 (7)

Note. - Figures are means ± S. D., with sample size in parenthesis where it differs from the corresponding figure at the left of the table.

cantly differ from each other, and are significantly different from the remaining treatments (B, D, E, G, H) which in their turn show no significant differences among themselves. Figures 22 to 27 show the effects of some of these treatments on the anatomy of the graft union.

The ratio of xylem to phloem strands bridging the graft was not significantly changed from the control by either of the most severe treatments (stock cut below graft, treatment I, and scion cut above graft, treatment E), or by a less severe treatment (scion without mature leaves, treatment B). Therefore, it was deemed unnecessary to count phloem for all the test groups. In some cases, however, phloem connections without xylem (stock severed, treatment I, day 6) and xylem without phloem (scion severed, treatment E, day 8) were observed.

While the differences in numbers of xylem strands between treatments might not be significant, on days 10 and 12 a trend of decline as more organs are removed from the scion has been clearly established (table 2), which is often visible in individual sections by day 12 (figs. 11, 22, 23, 27). Differences in amount of callus formed after the different treatments are also apparent in these sections.

The numbers of phloem files showed no significant differences until day 12, when the number of strands in the control was significantly greater at the 2.5 % level than the number in the grafts with scion severed just above the graft (treatment E) and significantly greater at the 10 % level than the numbers in grafts severed just below the graft (treatment I) and in those with defoliated scions (treatment B). These three treatments were not significantly different from each other even at the 10 % level (i.e.  $A > E$  but  $A = I = B$  and  $I = B = E$  at 2.5 %;  $A > I = B = E$  at 10 %). These differences are visible in the micro-

graphs; controls (fig. 11), defoliated scion (fig. 24), severed scion (fig. 25) and severed stock (fig. 26) are shown.

From day 8, visual estimates of numbers of adventitious roots on scions are lower than in control plants only in the denuded scion, treatment D, and the scion severed above the graft, treatment E. The isolated internodes show no adventitious roots. Where the stock is severed just below the graft (treatment I) there are a few adventitious roots at the base of the stock by day 4 and many by day 6. In these grafts the scion has fewer roots than the stock but obviously more than the control scion. Most of these scion roots wither by day 12 or soon after removal of the Parafilm, while the stock roots continue to grow.

Effectiveness of axillary bud removal. - Old grafts with denuded scions remain weak. By day 10 only two of the experimental plants had grown shoots on the disbudded and defoliated scion, presumably from axillary buds which were missed by the disbudding. Since there were extra plants in this experiment, the two with shoots, one without, and two controls were kept for an additional 3 wk. When the shootless scion was flexed, the graft broke very easily, and there was evidence of only a few connecting xylem elements. The two scions with one shoot each did not break when flexed to a slightly greater degree. They showed visibly less shoot growth when compared with the controls. The older controls were used to demonstrate the localized thickening near the graft (fig. 14). In plants from which the buds were removed, only these two grew other buds.




Fig. 2. Freehand cross section of an ungrafted internode subtending the third pair of fully expanded leaves. Two primary phloem bundles (P), the corner bundle (CB) and the vascular bundle in the middle of the side of the stem (not shown but just below field of view) are connected by a newly differentiated interfascicular cambium (C). Toluidine blue O staining, x 83. Inset shows the curling of the lips of stock and scion away from the stem axis; ca. life size.

Figs. 3-12, Freehand longitudinal sections of Coleus stem in the grafted regions. Cleared in lactic acid. Oriented so the scion is above the stock.

Fig. 3. Day 4 graft. The scion has formed callus from the xylem parenchyma to the inner cortex, while the stock has formed very little callus. The stock and scion cohere in the pith (arrow). Aniline blue staining, x 40.

Fig. 4. Day 4 graft. The many walls of the killed cells, lying parallel, show strong birefringence. Polarization microscopy, x 160.

Fig. 5. Day 4 graft. No new phloem has differentiated in the callus. Aniline blue induced fluorescence, x 80.

Fig. 6. Day 6 graft. A sieve tube (arrowheads) traverses the graft line (opposed arrows). Aniline blue induced fluorescence, x 125.

Fig. 7. Day 6 graft. Xylem (X) has differentiated in the outermost pith, just inside the young cambium (plane of section), and has bridged the graft line, on which the necrotic layer (arrowhead) is still present and is moderately stained. Near the vascular bundle in the stem corner, callus formation (\*) has continued. Aniline blue staining, x 40.

Fig. 8. Day 8 graft. More xylem traverses the graft line

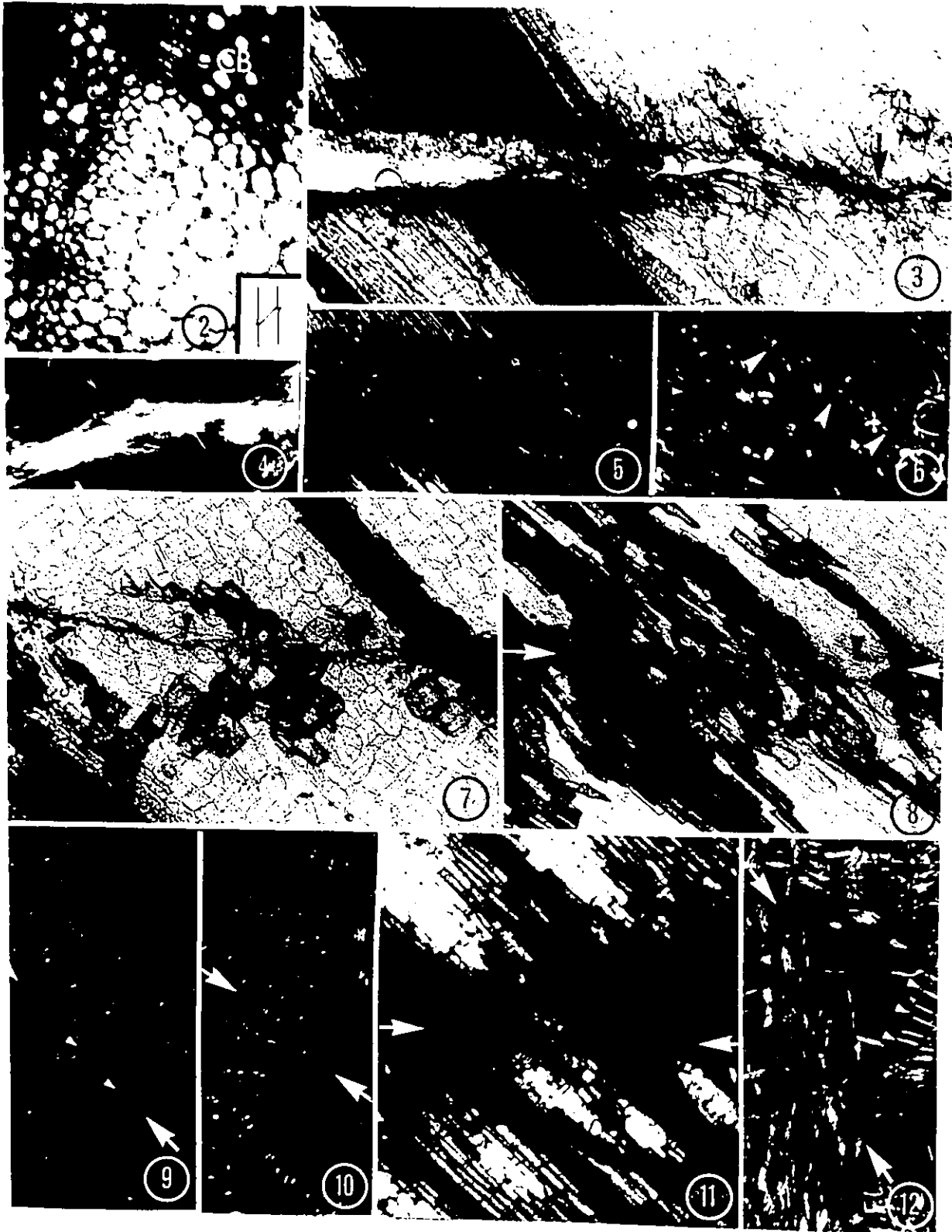
(opposed arrows), and the necrotic layer (arrowhead) is no longer strongly stained. Aniline blue staining, x 40.

Fig. 9. Day 8 graft. Several sieve tubes (arrowheads) traverse the graft line (opposed arrows). Aniline blue induced fluorescence, x 80.

Fig. 10. Day 10 graft. A "bundle" of sieve tubes traverses the graft line (opposed arrows) and fans out in stock and scion. Aniline blue induced fluorescence, x 80.

Fig. 11. Day 12 graft. A large amount of xylem traverses the graft line (opposed arrows), still faintly visible as a slightly darker line. Many xylem elements in both stock and scion have the appearance of being derived from a cambium. Aniline blue staining, x 40.

Fig. 12. Day 12 graft. The birefringence of the necrotic layer has virtually disappeared (cf. fig. 4) from the graft line (opposed arrows). Some pith cells on either side of the graft line have divided several times forming new walls (arrowheads) approximately parallel to the graft line. Polarization microscopy, x 65.



Figs. 13-27. Freehand longitudinal sections of Coleus stem in the grafted regions. Oriented so the scion is above the stock. All except fig. 15 have been cleared in lactic acid.

Fig. 13. Day 30 graft. The necrotic layer is still evident as a thick dark line in some places in the pith. New cell walls in the pith cells, parallel to the graft line, are clearly at a sharp angle to the original axis of the stem (double-headed arrow). Basic fuchsin staining, x 65.

Fig. 14. Day 30 graft, same section as in fig. 13. Near the graft line there is much more xylem than farther away (though not visible at this magnification, 10 to 15 layers as opposed to 4 to 6). Basic fuchsin staining, x 8.

Fig. 15. Fresh (uncleared) section of an internode from an ungrafted plant in the region comparable to the day 30 graft. There are only four layers of secondary xylem. Basic fuchsin staining, x 80.

Fig. 16. Isolated internode graft, day 12. The necrotic layer is not noticeably changed from day 4, maintaining its appearance of a stack of crushed walls. Pith cells on either side of the graft line show no signs of having divided. Aniline blue staining, x 105.

Fig. 17. Identical to fig. 16, but polarization microscopy.

Fig. 18. Isolated internode graft, day 6. Callus has filled in the small gap between stock and scion near the corner vascular bundles. The cut lines of stock and scion are still clearly visible (opposed arrows). A single xylem file traverses the graft. Aniline blue staining, x 140.

Fig. 19. Isolated internode graft, day 8. One sieve tube traverses the graft line (opposed arrows) at the arrowhead. Aniline blue induced

fluorescence, x 140.

Fig. 20. Another field of view of the same section with the same labeling as in fig. 19. Near the sieve tube which traverses the graft line, some new xylem has differentiated in the stock. x 140.

Fig. 21. Isolated internode graft, day 12. Xylem traverses the graft line (opposed arrows). Aniline blue staining, x 90.

Fig. 22. Graft with defoliated scion (treatment B), day 12. Both stock and scion have formed callus, and a moderate amount of xylem traverses the graft line (opposed arrows). Aniline blue staining, x 40.

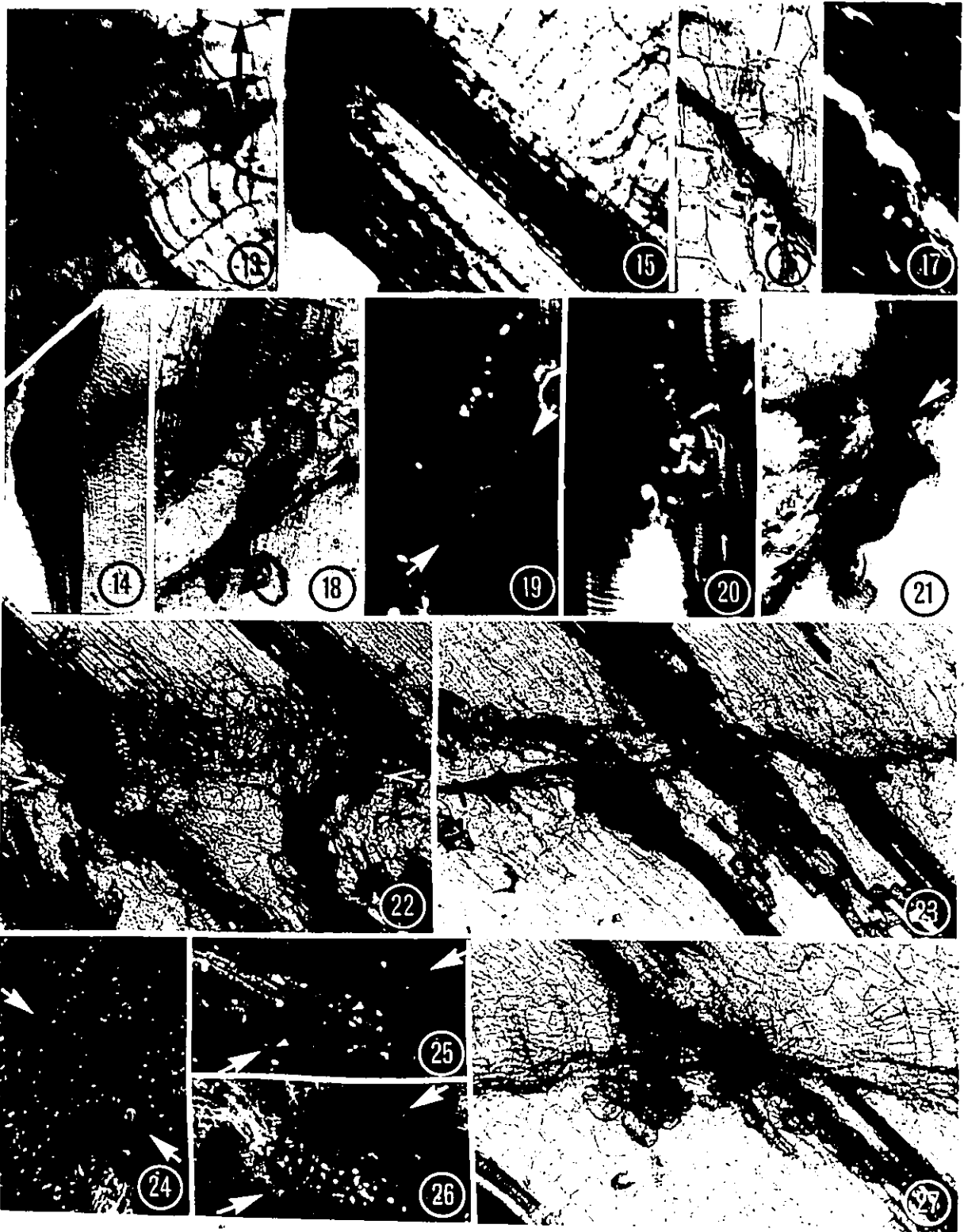
Fig. 23. Graft with scion severed just above the graft line (treatment E), day 12. There is less callus formation than in fig. 22, and less xylem traverses the graft line. Aniline blue staining, x 40.

Fig. 24. Same graft as in fig. 22. Many sieve tubes traverse the graft line (opposed arrows). Aniline blue induced fluorescence, x 50.

Fig. 25. Same graft as in fig. 23. Few sieve tubes traverse the graft line (opposed arrows); only two (arrowheads) are shown here. Aniline blue induced fluorescence, x 75.

Fig. 26. Graft with stock severed just below the graft line (treatment I), day 12. A few sieve tubes traverse the graft line (opposed arrows). Aniline blue induced fluorescence, x 50.

Fig. 27. Same graft as in fig. 26. The scion has formed extensive callus with many new walls parallel to the cut surfaces. Such divisions are absent from the stock. A small amount of xylem traverses the graft line. Aniline blue staining, x 40.



## DISCUSSION

The Coleus stem graft is comparable in its development to other herbaceous dicotyledonous grafts (Wright 1893; Crafts 1934; Lindsay et al. 1974; Stoddard and McCully 1979 and references therein). In each plant, grafting includes the formation of a necrotic layer and its subsequent reduction or elimination, growth of callus, cohesion of stock and scion, and the differentiation of graft-bridging vascular tissue and cambium. We show that several of these processes are independent events and not essential to the cohesion of stock and scion per se. While necrotic layer formation and cohesion are common to grafts in all the treatments, one or more of the processes of callus formation, necrotic layer removal, and vascular differentiation can be absent from a graft, depending on the type and amount of stock and scion tissue remaining.

Cohesion and removal of necrotic layer. - The isolated internode grafts show the independence of necrotic layer formation and subsequent graft cohesion from the other grafting events, which simply did not occur in the isolated internodes. These two processes were not affected by the treatments to stock and scion. It is not clear how these isolated internodes cohere without removal of the necrotic layer. In other descriptions of graft anatomy, the point is made that graft continuity is first established when callus bridges form where the necrotic layer is broken (Wright 1893; Crafts 1934; Mendel 1936; Buck 1954; Fletcher 1964; Copes 1969). Removal of the necrotic layer may depend on cell division, for not only does the layer remain where callus formation is absent or minimal but it is removed where callus formation is profuse.

The actual nature of the cohesion has never been adequately clari-

fied by anatomical, physiological or biochemical studies. Obviously some sort of "cement" is produced by the cells on one side, or both, of the graft. Results with the isolated Coleus internodes show that these cells do not need to divide before producing the cement, part of which has been speculated to be pectin (Lindsay 1972). It is clear that, in the isolated Coleus internode grafts, some mechanism must exist whereby the cement can bond stock to scion regardless of the presence or absence of a necrotic layer. Whatever cell-to-cell recognition is involved in the grafting process must take place as part of cohesion, and the system wherein cohesion occurs, unobscured by other grafting components, should be useful for investigating these recognition phenomena.

Callus formation at cut surfaces. - In most dicotyledonous grafts, it is the production of callus on stock and scion that leads to the establishment of stock-scion contact and has therefore been taken to be the first step toward cohesion (Wright 1893; Crafts 1934; Mendel 1936; Buck 1954; Fletcher 1964; Copes 1969; Stoddard and McCully 1979), though we have now shown the independence of cohesion from callusing. Upon removal of stock and scion parts, however, the amount of callus formed (visual estimates) declines in parallel with counts of xylem and phloem strands bridging the graft (tables 2, 3). This suggests a common stimulus. The exception is the severed stock, in which case there is copious callus formation in the scion, almost none in the stock, and few xylem or phloem strands bridge the graft.

The scion forms much more callus than does the stock, as has been observed in other plants (Wright 1893; Shipley 1930; Sharples and Gunnery 1933; Copes 1969; Stoddard and McCully 1979) where the graft partners are of comparable size. This result is explained by natural polarity,

for the root-tip-facing end of any segment of root or shoot forms more callus than the shoot-tip-facing end (Shippy 1930; Bloch 1943). In bud grafting the sizes of the cut surfaces are so different as to overwhelm any such innate difference (Mendel 1936; Buck 1954; Fletcher 1964).

Graft-bridging xylem and phloem. - In the grafts where the scion is severed and in isolated internodes, scion and stock are connected by vascular tissue, although the scion forms very little callus, which is restricted to the inner cortex. Vascular connections are also formed without apparent disruption of the necrotic layer in isolated internodes. In most grafts there was some vascular differentiation before cohesion. In failed grafts of pea roots, vascular differentiation occurred in the scion without any cohesion (Stoddard and McCully 1979). Graft-bridging strands of xylem and phloem develop initially from cells in contact with the respective severed bundles and may connect with the vascular tissue newly produced by the cambia in stock and scion where that production is heavy (Benayoun et al. 1975). In other grafts, such as those in isolated internodes (fig. 19), the new vascular tissue connects directly to the old.

Comparisons of counts of strands of xylem and phloem bridging the graft. - The amount of graft-bridging xylem is strongly influenced by the presence of leaves and branches on the scion, not at all by their presence on the stock. Comparisons of the numbers of xylem strands, in treatments with various amounts of scion tissue removed (table 2) allow estimation of the relative contributions of organs to the total xylem-inducing stimuli, under the assumption that there is a linear relationship between the concentration of the stimuli and the amount of xylem induced. Since all ages show the same general correlations (table 2),

the average of numbers of xylem strands bridging the graft on days 6, 8, 10 and 12 (Table 4) is used as the basis for comparison with the control, which is taken as 100 %, though this technique obscures time-course data.

Stem tissue of the internode used has virtually no xylem-inducing stimuli, since the isolated internodes form virtually no graft-bridging xylem; therefore the severed scion must be similarly without stimuli. On this basis, 8 % of the stimulus flows acropetally into the severed scion from the intact stock. In comparison, Jacobs (1952) and Aloni and Jacobs (1977a) showed that acropetal flow of xylem-inducing stimuli around stem wounds in this same species may vary from 0 to 25 % of total, depending on growth conditions. A further 10 % of stimuli are either pulled acropetally into the denuded scion by the increased transpiration of the larger surface, or flow basipetally from the maturing tissue in the stem, or some combination of these processes. Another 10 % of stimuli come from the scion shoot tips, and 36 % from the leaves. The pair of leaves and buds nearest the graft contributes ca. 40 % of their stimuli although each pair represents only ca. 30 % of the leaf or bud tissue present in the intact scion. This last result may be due to a lack of additivity of stimulus from one type of source; or it may be due to the proximity of the organs to the graft, since Thompson (1966) showed a logarithmic decline in radioactivity from  $^{14}\text{C}$ -labeled indoleacetic acid applied to Coleus stems as the compound moved basipetally.

These estimates total only 64 % of the xylem produced in control plants, a highly significant difference ( $p < 0.001$ ) possibly due to a synergistic effect of all three organs and/or the effects of the wounding inherent in the treatments.

When the stock was denuded of leaves and shoots but left as a con-

Table 4.

Numbers of xylem strands bridging the graft union following organ removal, as percentage of control.

Treatment	Average number of xylem strands, days 6, 8, 10 and 12	Average number of xylem strands, as percentage of control
A. Control	142	100
B. Scion defoliated	40	28
C. Scion disbudded	77	54
D. Scion denuded	25	18
E. Scion severed	12	8
F. Stock denuded	145	102
G. Scion severed 1 node above graft	38	27
H. As above, axillary buds removed	33	23
I. Stock severed	31	22
J. Isolated internode	0	0

nection between scion and roots (treatment F), there was no significant difference in numbers of graft-bridging xylem from the control. This result agrees well with the data of Kaan Albest (1934), Jacobs (1952) and LaMotte and Jacobs (1963), that removal of leaves and shoots below a wound in a stem has no effect on regeneration of xylem or phloem around that wound.

In contrast, in the grafts whose stocks were cut off below the graft (treatment I) the graft-bridging xylem averaged 22 % of that in controls. If this reduction were due to the absence of xylem-inducing stimuli coming from the roots, it would surely have been restored rapidly since adventitious roots were initiated at the base of the stock at the same time as the graft was taking. Perhaps the severing of the stock reduces the basipetal flow of xylem-inducing stimuli elaborated in the scion by removing a sink, which when subsequently restored is divided between the adventitious roots of stock and those of the scion. That there is an accumulation of auxin at the base of the scion is shown by the increase in number of adventitious roots emerging above this graft as compared with the control.

Since removal of plant parts causes changes in numbers of graft-bridging phloem strands that become significant at the 5 % level only on day 12, we conclude that phloem induction is less strongly influenced by scion organs than is xylem induction. The phloem/xylem ratio, though not significantly changed by any treatment (isolated internodes, treatment J, not included because of insignificant production of xylem and phloem), varies from 0.071:1 where the scion is severed just above the graft (treatment E), through 0.18:1 in the control and 0.31:1 where the stock is severed below the graft (treatment I) to a high of 0.37:1

for the leafless scion (treatment B), averaging 0.23:1. In the intact stem, the ratio of strands is 2.2:1. These results contrast strongly with those of Aloni and Jacobs (1977b), where the ratio of phloem to xylem strands regenerating around a stem wound (2.24) in Coleus was almost the same as the ratio of bundles severed in wounding (2.14). There is no apparent explanation as to why, in grafting, phloem should regenerate less than xylem, when there is more phloem than xylem in the intact stem, unless some of that phloem in the intact stem is not functional.

Graft-bridging cambium. - A graft-bridging cambium is formed sometime between day 12 (fig. 11) and day 30 (fig. 14). The differentiation of the vascular cambium is independent of xylem and phloem differentiation in certain situations. In some grafts a cambium forms without pre-existent wound-bridging xylem and phloem (references in Mendel 1936; Buck 1954; Warren Wilson 1978 and references therein). In others, however, the cambium differentiates between the wound-bridging xylem and phloem (Crafts 1934; Stoddard and McCully 1979) after these tissues have bridged the graft. In wounded stems and roots as well, the bridging cambium may be preceded by differentiation of xylem and phloem (Robbertse and McCully 1979) or may be independent of it (Warren Wilson 1978).

Phytohormones and graft formation. - Our experiments make it clear which organs contribute to the formation of the graft union, and it remains to correlate them with knowledge of the sources and effects of the various phytohormones.

Since stocks form very little callus until cohesion, hormones originating in the stocks and wound hormones can be eliminated as possible exciters of cell division at the early stages of the grafting process.

The delayed cell division and vascular differentiation in the stock must be due to hormones originating in the scion and making their way across the graft union.

An auxin gradient is necessary for vascular tissue regeneration (Jacobs 1952; Sachs 1968) and callus formation (Jacobs 1979). Buds inserted into callus induce the differentiation of xylem and phloem (Camus 1949). The organs producing most auxin, the leaves (Jacobs 1952) and shoot tips (Scott and Briggs 1960; Thimann et al. 1971) correspond to the most effective organs inducing formation of callus and of vascular tissue. The scion is the only effective source of auxin, which must be continuously supplied, because auxin-destroying enzymes accumulate at cut surfaces within 6 h (Iversen and Aasheim 1970). Statements are contradictory on the effects of cytokinins and gibberellins on differentiation of xylem and phloem in parenchyma around wounds or in explants (Fosket and Roberts 1964; Roberts and Fosket 1966; Waisel et al. 1966; Earle 1968; Harrison and Klein 1979).

Clear evidence for the production of a wound hormone, traumatin, at cut surfaces has recently been provided (Zimmerman and Coudron 1979) and linked to induction of cell division. If indeed a wound hormone is produced in Coleus grafting, its only role is in the induction of cohesion, which takes place in isolated internode grafts in the apparent absence of other processes.

Graft incompatibility. - Where stock and scion are incompatible, the graft partners may reject each other at any of the steps of graft formation, as shown by the following examples selected from the literature on incompatibility.

The process of cohesion itself may fail, as in tomato on Pelargonium

(Wright 1893). Vascular tissues may fail to connect the graft partners, as in Vanilla orchid grafts, which survive until put under transpirational stress, the parenchymatous cells at the union evidently providing enough flow in ordinary circumstances (Muzik 1958). Cambia of stock and scion may fail to unite or to remain united, leading to interruptions of vascular continuity and scion death as in pear grafted on apple (Proebsting 1928; Bradford and Sitton 1929). Callus formation in one partner may be excessive, due to faulty technique leading to pressure which separates the graft partners (Bradford and Sitton 1929). No data are available which imply continued presence of necrotic layer as cause for graft failure although in some incompatible interspecific Prunus grafts "the xylem at the line of union is degenerated into a gummy mass" (Proebsting 1928, p. 80). Thus the literature on incompatibility corroborates our evidence for the separability of the components of the grafting process and the independence of their control.

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Part 2.

Histological comparison of the development  
of grafts and wounds in stems of Coleus

## INTRODUCTION

The results of the previous part of this investigation (Stoddard and McCully 1980) showed that there are differences in the ways in which control grafts and isolated internode grafts develop. To investigate these differences more closely, grafts of isolated internodes, and of otherwise intact plants, were prepared and studied by high-resolution optical microscopy at various stages of development. As well, the question was posed, how do these extreme grafting situations differ from open wounds or from wounds covered with an abiotic "graft partner"? Therefore, plants were cut in the internode that would have been grafted, the two parts separated, and either left bare or covered with a layer of Parafilm, and studied along with the grafts mentioned above.

## MATERIALS AND METHODS

Grafts of otherwise intact plants (treatment A) and of isolated internodes (treatment J) were prepared as described previously (Stoddard and McCully 1980) in late winter of 1980. As well, some plants were cut apart in the same internode as would have been grafted, and the stumps placed in the humid chamber with the grafts of whole plants. The cuttings were placed upright in the growth chamber with the isolated internodes, in a transparent Plexiglas box with the tips of the cut surfaces just touching the moist paper towelling lining the bottom of the container. Similar cuttings and stumps were prepared and the cut surfaces covered with Parafilm.

Two, four, six and ten days after cutting, three of each type of graft, cutting or stump were excised as described previously (Stoddard and McCully 1980), cut longitudinally into six to nine pieces, fixed in 3 % (w/v) glutaraldehyde (J. B. EM Services Reg'd, Pointe-Claire, P. Q.) in 0.025 M potassium phosphate buffer, pH 6.8, overnight at 0°C, dehydrated in a methoxyethanol, ethanol, n-propanol, n-butanol series, and embedded in glycol methacrylate (Hartung Associates, Camden, NJ) (Feder and O'Brien 1968). Sections were cut an indicated one micrometer thick on a Sorvall Porter-Blum MT-1 microtome (Ivan Sorvall, Newtown, Conn.) and mounted on glass microscope slides (Clay Adams Gold Seal, Becton Dickinson and Co., Parsippany, NJ).

At every age and for every graft or wound type, three plants were prepared, with a few minor exceptions such as at day four for covered wounds, where only two plants were available. For both sides of each graft or wound, six to ten microscope slides were prepared with 5 to 8 serial or near-serial sections each, and each slide subjected to

a different histochemical procedure. Generally the best section on each slide was examined in detail. Thus each observation of a side of a graft or wound is based on about six sections of each of three (rarely two) individuals.

Sections were stained according to one of the following methods, at room temperature unless otherwise noted.

1. Periodic acid - Schiff reaction (Feder and O'Brien 1968)(PAS). Aldehydes were blocked with 2,4-dinitrophenylhydrazine (Eastman Kodak Co., Rochester, NY) saturated in 15 % (v/v) aq. acetic acid, for 10 min. Slides were washed 20 min. in running water, dried, and immersed in 1 % (w/v) aq. periodic acid (J. T. Baker Chemical Co., Philipsburg, NJ) for 10 min., rinsed quickly with water and immersed in Schiff reagent (Lots 793767-12 and 701581-12, Fisher Scientific Co., Fairlawn, NJ) for 15 min. Sections were washed with three quick changes of 0.5 % (w/v) aq. sodium metabisulphite, then with distilled water for 3 min.

1a. Some of these slides were counterstained with Sudan black B (Edward Gurr Ltd., London, U.K.) saturated in 70 % (v/v) aq. ethanol, for 1 to 5 hours, washed twice quickly with 70 % (v/v) aq. ethanol and once with distilled water, and dried.

1b. Other slides were counterstained with aniline blue (Lot 1523, Polysciences In., Warrington, PA) 0.05 % (w/v) in 0.067 M aq.  $K_2HPO_4$ , 5 min., rinsed quickly with distilled water, and dried. Sections were sparingly rehydrated by breathing on them just before mounting the coverslip (Smith and McCully 1978).

1c. As partial control for the PAS reaction, a few sections were stained with Schiff reagent without any pretreatment.

2. Toluidine blue O (O'Brien et al. 1964). Sections were stained

with toluidine blue O (Lot 119962, C. I. 52040, BDH Chemicals, Poole, U.K.) 0.05% (w/v) in benzoate buffer, pH 4.4, for about 1 min., rinsed 3 min. in running water and dried. Sections were sparingly rehydrated as in step 1b above.

3. Alcian blue - acid fuchsin. Slides were immersed in a solution of alcian blue 8GS (Lot 71123, J. B. EM Services Reg'd) 0.3 % (w/v) aq., for 45 min., washed with water 10 min., and dried (method of K. J. Clarke, pers. comm. 1980). Slides were then immersed in acid fuchsin (C. I. 42685, Allied Chemical Corp., New York, NY) 0.5 % (w/v) aq., 43°C, for 4 min., rinsed quickly with two changes of distilled water at 43°C, and dried.

4. Calcofluor White M2R New (Hughes and McCully 1975). Slides were immersed in Calcofluor White M2R New (American Cyanamid Co., Montreal, P.Q.) 0.01% (w/v) aq., for 5 min., rinsed quickly in distilled water and dried.

5. Autofluorescence. Slides received no treatment. These slides were also examined with phase contrast optics and with polarization microscopy.

In treatments 1 to 5, coverslips were mounted with a drop of immersion oil (Carl Zeiss, Oberkochen, Federal Republic of Germany).

6. To test the effect of pH on autofluorescence, acetate buffers pH 4 and 5, and phosphate buffers pH 6.8 and 8.3, were prepared (Barka and Anderson 1963). A single slide with 5 good serial sections was selected, and a coverslip mounted in the most acid buffer. After 3 min. for equilibration, the slide was examined and the first section photomicrographed. The coverslip was removed and the sections washed about 2 min. with distilled water and dried. The procedure was repeated with

the second buffer, examining the second section, and so on, similar areas being chosen for micrographs. Finally the slide with the next serial section was tested with the most alkaline buffer to check on cumulative effects of buffer on the sections. It was impossible to use the same part of the same section every time because loss of autofluorescence was detectable after the 3 minute fluorescence micrograph exposure.

In late autumn 1980, another experiment was conducted in which four isolated internodes were prepared, with three control grafts, three parafilm covered wounds, and four grafts with parafilm inserted between stock and scion. After 10 days, the ends of the isolated internodes and the grafted or wounded surfaces of the other plants were longitudinally hand-sectioned and stained in Sudan black B, saturated in 70 % (v/v) aq. ethanol, for 5 hours, soaked in 70 % aq. ethanol until parenchyma cells were destained, rehydrated and mounted in water.

Slides were examined with a Carl Zeiss Universal microscope, equipped with bright field and epifluorescence optics. For autofluorescence and aniline blue induced fluorescence, exciter filter BG 12, beamsplitter Fl 480, and barrier filter 50 were used. For Calcofluor induced fluorescence, exciter filter UG 1, beamsplitter Fl 480, and barrier filter 44 were used. Specimens viewed in bright field were recorded on Kodak Ektapan 4" x 5" sheet film, rated ASA 100 and developed in Kodak DK-50. Fluorescence was recorded on Kodak Panatomic-X 35 mm roll film, rated ASA 64 and developed in Ilford Microphen.

Comparative data were prepared for all grafts during microscopic examination by filling in all relevant parts of a table listing 39 possible characteristics for each side of each graft or wound, and for each of cortex, vascular parenchyma and pith (table 1).

Data for nuclear location were compared among types of graft and wound and different ages with two-factor analyses of variance, nuclear placement being one of the factors. Sources of interaction between nuclear location and plant type were identified with Student-Newman-Keuls tests (Zar 1974).

Table 1.

The characteristics for which cortex, vascular parenchyma and pith were examined in every scion, cutting, stock and stump.

Live cells

Nuclei: location  
morphology  
Plastids: starch content (range of 0 to 2)  
colour with toluidine blue  
Cytoplasm: amount (range of 0 to 3)  
distribution  
Walls: staining  
Hypertrophied cells: amount and location of swelling  
nuclear location  
amount of cytoplasm  
Divisions: numbers  
orientation (axial, transverse, etc.)  
location  
Binucleate cells  
Suberization

Necrotic materials

Cut cell walls: staining  
appearance  
Cut cell contents: nuclei: staining  
morphology  
plastids: staining  
appearance  
cytoplasm: staining  
appearance  
Covering of dead cells: staining  
appearance  
Thin refractile layer: location  
appearance  
Foam in cut xylem  
Dense plugs in cut xylem  
Cell death incidental to cutting: location  
appearance of cell contents  
collapse of dead cells

New surfaces and contact zone

New surface: staining  
appearance  
Contact zone: location  
new surfaces or not  
appearance  
Intercellular slimy-looking material: amount (range of 0 to 2)  
staining

## RESULTS

Anatomy of the uncut stem at the graft or wound site. - In the uncut stem, the cells are highly vacuolate, and the cytoplasmic ground substance is rarely directly visible (figs. 1, 2) although demonstrable by the presence of apparently healthy organelles. Cells of the collenchyma and cortical parenchyma have numerous plastids (fig. 1), each with prominent starch grains (fig. 2), closely appressed to the perimeter of the cell. Cells of the vascular parenchyma and pith have fewer and rather smaller plastids. In all cells, the plastids stain blue to green with toluidine blue, red with acid fuchsin, and their starch grains are PAS-positive. Nuclei are usually found along the longitudinal walls of the cells, although a small percentage (ca. 15 %) may lie at either of the cell poles. The nuclei stain blue-purple with toluidine blue, red with acid fuchsin, and exhibit strong glutaraldehyde-induced fluorescence.

The walls of non-lignified cells stain pink-purple with toluidine blue, are weakly autofluorescent, and PAS-, alcian blue- and Calcofluor-positive. The outer surface of the epidermis is bounded by a thin, Sudan black positive cuticle. Aniline blue induced fluorescence is shown by sieve plates in phloem, and by the pit fields that are infrequently found in such thin sections. Xylem consists entirely of vessel elements, the secondarily thickened walls of which are strongly autofluorescent and birefringent, weakly acid fuchsin-, Calcofluor- and Schiff reagent-positive, and bright green with toluidine blue. Vessels are spaced apart with at least one and usually three files of vascular parenchyma. Walls, nuclei and plastids in intact cells retain their original staining properties throughout the grafting or wound healing

process.

Day 2 grafts. - In grafts of otherwise intact plants (hereafter, "control grafts"), there have been few changes in the appearance of the intact cells. Some starch has been lost from plastids in the stock near the cut. The graft-facing walls of a few cells in the vascular parenchyma have become noticeably convex (fig. 3). Other cells bulge into the sides of the cut vessels, through the perforations in the secondary walls. Some of the cortical cells in two scions had also swollen at the ends facing the grafts (fig. 7). There has been a detectable increase in the amount of cytoplasm at the ends of some of the expanded cells (fig. 3). No cells have yet divided. As in all these swollen cells in subsequent ages and different graft or wound types, aniline blue positivity, where present, is not consistently associated with the visibly bulging parts of the walls. The mutual independence of nuclear location, aniline blue positivity, and the outward face of expanding cells is shown in fig. 4. Some walls of dead parenchyma cells show patchy aniline blue positivity (fig. 5).

The dead cell walls retain their original staining properties, and in the cortex and some of the vascular parenchyma (fig. 6), their configuration as well; in the rest of the vascular parenchyma, especially in the scion, and in the pith of all grafts and wounds, the cut walls have collapsed (fig. 3). Necrotic nuclei are characteristically circular or extensively flattened in sectional view, with a dense ring around a pale granular core, staining very dark violet with toluidine blue (figs. 3, 7). These nuclei may be attached to a wall, or suspended by a strand of dead cytoplasm. Necrotic plastids are similar in appearance to those in intact cells, although some loss of staining

intensity is observed (fig. 11), and starch is retained throughout the grafting or wound healing process. The necrotic cytoplasm is usually green to blue with toluidine blue, acid fuchsin positive, moderately autofluorescent, and negative to the other histochemical reactions employed. In appearance it may be granular or sheetlike; it may cling to the cut walls (figs. 3, 11), bridge the cut end of a cell (fig. 6), or form a central strand in the cell cavity (fig. 15).

Many cut vessels of the stocks are blocked with a bubbly appearing or foamlike substance (hereafter, "foam") (fig. 8) that is moderately autofluorescent (fig. 9), green with toluidine blue, slightly acid fuchsin positive, and negative by the other staining reactions used. This foam merges with a thin layer (figs. 7, 8, 10) over the cut vascular parenchyma, which has identical but much more intense staining properties. In addition, this layer is highly refractile, as shown by its glistening appearance, and intense blackness when viewed with phase-contrast optics (fig. 12). It is also moderately birefringent, such that it is bright with the same position of polarizer and analyzer as makes walls parallel to it bright (fig. 13). Its intense autofluorescence is independent of pH in the range 4.0 to 8.3. The thin refractile layer may be attached to the ends of cut cell walls, capping the cells, folding over and coating the insides of the cut xylem elements as far as the foam, or it may be apparently free over much of its surface. Sometimes two thin refractile layers cover the surface, one of each type described (fig. 10). Thin refractile layers were found on all stocks of day two control grafts (fig. 7; table 2) but on only one scion; they are also found on other graft and wound types at other ages (tables 2 to 5), but never on cuttings.

Some vessels, usually in scions, are plugged with a dense material instead of the foam, with different staining properties (fig. 14). The dense plugs are brilliantly autofluorescent, and stain deep violet blue, with a few small green spots, with toluidine blue; parts of the plugs are positive to acid fuchsin, alcian blue and the PAS reaction.

Cut sieve tubes, on the other hand, are plugged with a somewhat diaphanous acidophilic material that forms a lump on the sieve plates and long, loose-appearing sheets from the graft- or wound-facing side (figs. 3, 15).

The other graft and wound types show several differences from the control grafts (table 2). In the isolated internodes, somewhat fewer cut cell walls in the vascular parenchyma have collapsed (fig. 15) and few cut cell ends are covered with dead cytoplasm (fig. 15). The major differences here are the nearly complete loss of starch, and absence of the thin refractile layer.

The uncovered wounds are distinguished by the extensive loss of starch, and the death of some cells not caused directly by cutting. Often these dead cells are apparently intact, but have the cytoplasmic and nuclear morphologies of necrotic cells. Many cells have large accumulations of cytoplasm at the wound-facing ends (figs. 16, 17). As in the control grafts, the stumps have some of the refractile material over the vascular region (fig. 17).

Covered wounds at this age are remarkable for accumulating cytoplasm at the wound-facing ends of many cells (figs. 18 to 23), retention of starch by live cells, and for the amount of cell expansion (fig. 18). Some cells already extend past the cut line (fig. 18). In any graft or wound, the new surface thus exposed is typical of parenchymatous cell

Table 2.

Comparison of grafts and wounds, 2 days after cutting.

Characteristic	Control graft	Isolated internode	Uncovered wound	Covered wound
Divisions	0	0	0	0
Hypertrophy of cells of vascular parenchyma	few, slight toward gap	few, slight toward gap	several, slight to moderate, toward gap	several, moderate into gap
Accumulation of cytoplasm at wound-facing ends of cells	0	0	0	large amount
Starch content	some loss	almost all gone	most lost	little loss
Presence of thin refractile layer (maximum 3)	1 scion 3 stocks	0 0	0 cuttings 3 stumps	0 cuttings 0 stumps
Inclusions in xylem	foam, 1 stock plug, 1 scion	0 foam plug, 1 stock	foam, 1 stump 0 plug	foam, 1 cutting 0 plug
Cell death not caused directly by cutting	0	0	up to 3 layers, collapsed	0
Tylosis formation	incipient	incipient	incipient	incipient
Suberization	0	0	0	0

walls in appearance and in histochemical properties, and generally is devoid of necrotic materials. Dead cytoplasm caps many cut cells in the vascular parenchyma (figs. 18, 21).

Day 4 grafts. - By the fourth day the first few divisions have appeared in the scions of the control grafts, parallel to the cut surface or transverse to the plant axis. There is some accumulation of cytoplasm in cells of the scion (fig. 24), rather less in those of the stock (fig. 25), usually at the graft-facing ends of the cells of the vascular parenchyma. The nucleus is not always in the midst of the principal aggregation of the cytoplasm (figs. 24, 25). More cells are swollen in all tissues of the scion, though in the stock such hypertrophy is still largely restricted to the vascular parenchyma; no cells have yet expanded past the original cut line (table 3). There has been further collapse of cut walls in these grafts. Only a few caps of dead cytoplasm remain covering cells of the vascular parenchyma of some graft partners. The thin refractile layer is still present over the vascular parenchyma of all control stocks (fig. 25), while fragments are found on the scions. The associated foam is found in some of the xylem of both graft partners, but none of the dense plugs are seen.

The isolated internode grafts distinguish themselves (table 3) by the large hypertrophied cells in the vascular parenchyma, often protruding well into the graft gap, rather more in the scion (fig. 26) than in the stock (fig. 27). Though easily damaged in the fixation process, these cells show healthy nuclei and a certain accumulation of cytoplasm at an early stage (fig. 26), though less than in the control grafts. By the time the bulbous protuberances reach the same size as their confined bases, they appear as low in cytoplasm as any highly vacuolate cell before

Table 3.

Comparison of grafts and wounds, 4 days after cutting.

Characteristic	Control graft	Isolated internode	Uncovered wound	Covered wound
Divisions	few, parallel to cut or transverse to stem axis	very few, parallel to cut	few, parallel or transverse	numerous, parallel
Hypertrophy of cells of vascular parenchyma	several, moderate toward gap	numerous, large, well into gap	0	numerous, moderate, into gap
Accumulation of cytoplasm at wound-facing ends of cells	some	0 to little	large amount	0
Starch content	some loss, scions all gone, stocks	almost all gone	little loss	little loss, cuttings; moderate loss, stumps
Presence of thin refractile layer (maximum 3)	2 scions 3 stocks	0	0 cuttings 1 stump	0
Inclusions in xylem	foam, 1 scion and 1 stock	plugs, 2 scions	plugs, 1 stump and 1 cutting	foam, 1 stump
Cell death not caused directly by cutting	0	very few	up to 4 layers, collapsed	very few
Tylosis formation	slight	moderate	moderate	many blockages
Suberization	0	0	few to many intact cells nearest cut, cuttings only, incipient	0

grafting (figs. 26, 27). The cut cell walls have not collapsed further, and some have been filled by the swelling cells. Some capping of the cut cells by dead cytoplasm remains (fig. 27). A few cells in the cortex of both stock and scion of one graft had died incidental to grafting. Whether this cell death occurred after grafting or as a side effect to it is not apparent; see Discussion.

The surfaces of the uncovered wounds (table 3) are still characterized by dieback and collapse of several cell layers (figs. 28, 29). Virtually all the cut walls have collapsed. The cytoplasm of most of the intact cells in the layer nearest the cut (fig. 28) is highly acidophilic and autofluorescent, and stains dark green with toluidine blue. At later ages these reactions are associated with Sudan black positivity. Both stump and cutting have started to accumulate starch in the live plastids, rather closer to the cut in the stump than in the cutting. No swollen cells remain other than those forming tyloses. No cut cells are capped by dead cytoplasm (fig. 28).

The parafilm covered cuttings have produced many more division walls in all tissues (fig. 30) than any other treatment at this age, and the stumps have only slightly fewer (table 3). At the same time the cuttings have produced an almost continuous new surface over the vascular parenchyma by hypertrophy of the live cells nearest the graft (fig. 31). The stumps have some well expanded cells, but not to such an extent as the cuttings. The accumulation of cytoplasm mentioned previously has disappeared. Some of the cut cortical walls in the cuttings and stumps have collapsed, while a very few cut cells in the vascular parenchyma of a stump are capped by dead cytoplasm (fig. 32).

Day 6 grafts. - Unlike the grafts described previously (Stoddard and

McCully 1980), these control grafts have not cohered by day 6. Divisions have become more numerous in all tissues of the control scions, equal numbers transverse to the plant axis and parallel to the graft line (fig. 33; table 4), except in the starch sheath where all longitudinal divisions are found. In sections of the stock, slightly fewer new walls are found. Cortical cells (fig. 34) show further accumulation of cytoplasm to levels comparable to those in the adjacent vascular parenchyma (figs. 35, 36), which have not changed since day 4. Cells of the scion generally have more cytoplasm than those of the stock. The number and distribution of swollen cells have not changed appreciably since day 4 (fig. 35), while some cells have protruded past the cut line. The cut cell walls in the cortex still retain their original configuration, but those in the vascular parenchyma that have not been supported by cellular hypertrophy have collapsed (fig. 35). No caps of dead cytoplasm remain over the cut cells. The thin refractile layer was present on all three scions with foam in one, and both foam and the layer were present in one stock (fig. 35). No dense plugs are present.

Swelling of cells past the cut line continues to characterize the isolated internode grafts, especially the scions (figs. 37, 38). The surfaces between the hypertrophied cells are covered with necrotic material (fig. 37) while the new surfaces facing the graft partners are typical of ordinary parenchymatous cell walls (figs. 37, 39, inset in 39). Where these new surfaces come together in a graft (fig. 40), the new contact wall is only sometimes distinguishable by thickness from adjacent walls (fig. 42). In some gaps between graft partners is a slimy-looking material (fig. 43) with unusual staining properties, purplish-pink with toluidine blue, very weak positivity to the PAS reaction or to alcian

Table 4.

Comparison of grafts and wounds, 6 days after cutting.

Characteristic	Control grafts	Isolated internode	Uncovered wound	Covered wound
Divisions	numerous, all planes	few to several, all planes	numerous in cuttings, few in scions, along stem axis or parallel to cut	numerous, axial or parallel
Hypertrophy of cells of vascular parenchyma	several, large into gap	numerous, very large, into gap	0	numerous, large, divided, into gap
Accumulation of cytoplasm at wound-facing ends of cells	large amount	0	greatest amount	0
Starch content	some loss, scions almost all gone, stocks	almost all gone	fully restored, scions; most lost, stumps.	moderate loss
Presence of thin refractile layer (maximum 3)	3 scions 1 stock	0 scions 1 stock?	0 cuttings 1 stump	0
Inclusions in xylem	foam, 1 scion and 1 stock	0	foam, 1 stump	foam, 2 cuttings
Cell death not caused directly by cutting	0	a few	up to 4 layers	few
Tylosis formation	Many blockages	many blockages	many blockages	blockage and dismemberment
Suberization	0	0	few to many intact cells nearest cut	few to many intact cells nearest cut

blue, spotty calcofluor positivity (fig. 43, inset), and absence of autofluorescence. While best shown here, this material was also found in four isolated internode scions, one control scion, two parafilm covered cuttings and four covered stumps, at various ages. Material with a somewhat similar appearance and more intense PAS positivity is associated with abundant dark rod-shaped bacteria (fig. 28) which are not detected in the grafts and wounds producing the substance described here. Divisions are virtually absent except in one covered graft, where there are equal numbers of transverse and graft-parallel new walls, though some in the starch sheath were again longitudinal. Cut walls have not collapsed further, many being supported by the expanded cells, and are no longer capped by dead cytoplasm.

The uncovered cuttings show the first indications of suberization. Sudan black positivity (figs. 41, 44), autofluorescence (fig. 45) and deep green staining with toluidine blue are well developed in the layer of intact cells nearest the cut. The next layer is very rich in cytoplasm (fig. 46). The stumps as yet show no suberization (figs. 47, 48).

The parafilm covered wounds are characterized by the combination of extensive cell expansion and division (figs. 49, 53) with some suberization of a few intact cells nearest the cut in the cuttings (fig. 50) and of extensive parts of the cell layer nearest the cut in the stumps (figs. 51, 52). Often there have been numerous parallel divisions within an original mother cell wall (figs. 49, 51). The cells in the stumps that have invaded the cut xylem vessels have expanded and pulled them apart (fig. 53). No caps of dead cytoplasm remain over cut cells. Some cut cortical walls are collapsed, the rest retaining normal orientation, often because of support from expanding cells (fig. 49).

Day 10 grafts. - By the tenth day all control grafts in this sample had cohered. Many cells have expanded past the cut line, especially from the scion, and have divided many times (fig. 54; table 5). Many cells retain visible amounts of cytoplasm (figs. 55, 56) although considerably less than previously. Some division walls are parallel to the graft, especially in areas where there is yet no contact, but where there is contact it is hard to define any common plane of division. The walls of those few cut cells not plugged by hypertrophy of subjacent cells have collapsed, but their staining properties are unchanged. Where they can be found, nuclei and plastids from the dead cells have not noticeably changed since day two. The thin refractile layer was found on only one stock in areas that had not cohered, foam was in the xylem of two stocks, and dense plugs were absent. As always, the new surfaces exposed by cell expansion in the cortex and vascular parenchyma resemble ordinary parenchyma walls, and where they meet, the join is detectable only by geometry (fig. 55). In the pith the swollen cells are still covered by some necrotic material, so the line of join is thicker than adjacent walls, and has areas of different staining properties resulting from the dead cell contents (fig. 56). Some of the slimy-looking material mentioned for the day six isolated internodes is present between the graft partners (fig. 56). Suberization is absent from the graft interface and from the cut surfaces which have not yet cohered.

Cells of the isolated internodes have continued expanding toward and into the graft gap (fig. 4) and the cut xylem (table 5). Most cut cell walls outside of the pith have been filled by expanded cells which present the usual new surface. Demonstrable contact zones (fig. 57) are found only with necrotic materials intervening, either in the

Table 5.

Comparison of grafts and wounds, 10 days after cutting.

Characteristic	Control grafts	Isolated internodes	Uncovered wounds	Covered wounds
Divisions	numerous, all planes	few to several, all planes	numerous, parallel	numerous, parallel
Hypertrophy of cells of vascular parenchyma	numerous, large, divided, into gap	numerous, large into gap	numerous in cuttings, toward gap, divided; 0 in stumps	numerous, large divided, into gap
Accumulation of cytoplasm at wound-facing ends of cells	small amount	0	0	very small amount cuttings; 0 in stumps
Starch content	fully restored, scions; most lost, stocks	some loss	fully restored	fully restored
Presence of thin refractile layer (maximum 3)	1 stock	0	0	0
Inclusions in xylem	foam, 2 stocks	foam, 1 scion	0	0
Cell death not caused directly by cutting	0	very few	many layers	few
Tylosis formation	many blockages	many blockages	blockage with dismemberment	blockage with dismemberment
Suberization	0	0	few to many intact cells nearest cut, cutting; layer nearest cut, stump	layer of intact cells nearest cut

central pith (fig. 58) or on the boundary between vascular parenchyma and pith (fig. 59). Dead walls and contents remain between the graft partners in most areas of cohesion.

In one of these stocks, a vessel element (fig. 60), already matured to the point of having small secondary thickenings (figs. 61, 62) appears to have ceased that line of differentiation and reverted to parenchymatous status following cutting. New walls have been laid down (figs. 60, 62) and the wall facing the cut bulges toward it (fig. 61).

The lower ends of the isolated internodes form a complete wound periderm with profuse suberization (fig. 63) while divisions are few and suberization at best spotty at the apical ends (fig. 64).

The uncovered cuttings have renewed cell expansion and cell division by the tenth day (table 5) producing a new surface that is only partly clean of necrotic materials (fig. 66). The outermost layer of cells is largely suberized (fig. 65). There has been no cleanup of necrotic material where it is surrounded by live cells, as shown by the unchanged plastids with their starch grains intact (fig. 67). As usual, some dead walls are collapsed, others supported by invading cells. There has been little cell death beyond the first one or two exposed layers. Very few cortical and vascular parenchyma cells have suberized in the cuttings. In the stumps the situation is quite different. Extensive dieback has been followed by division of cells parallel to the inner boundary of the dead area (fig. 68). The outermost layer or two of these cells has suberized (fig. 69) and the cells that have invaded the cut xylem have expanded and pulled it apart (fig. 68).

The parafilm covered cuttings (fig. 70) and stumps (fig. 71) have

both produced a large number of new cells protruding from the vascular parenchyma, all new walls being parallel to the wound line (table 5). Rather less expansion and division, still mostly parallel to the surface, have taken place in the cortex. A very few cut walls remain collapsed, most having been invaded by swelling cells. Diffuse slime of the sort noted for day six isolated internode grafts is found in two stumps and one cutting. Many cells in the outermost layer in cuttings and stumps have suberized (figs. 71, 72, 73). The only new xylem cells to have differentiated in the pads of new tissue covering the wounds were seen over the vascular parenchyma in a number of cuttings (fig. 74).

In the grafts separated by parafilm, the scions have numerous new walls parallel to the cut line, and the outermost layer of cells is suberized in places (fig. 75). The stocks have very few new walls, again parallel to the cut surface, but no Sudan black positivity was found (fig. 76)..

Nuclear location. - In one section of each stock, scion, cutting or stump, the location of each healthy-appearing nucleus was scored according to the adjacent wall (side wall, or transverse wall nearest to, or farthest from, the wound), or central if not appressed to any wall. An average of 16 nuclei per section were thus scored, for a total of 2037. The original axis was always used as the reference, though the axis of cell polarity often changes in the process of expansion and division.

Nuclear location was not significantly affected by age or type of graft or wound. There was, however, a difference between stocks and stumps on the one hand, and scions and cuttings on the other (table 6). Nuclear distribution is also unaffected by swelling of cells, but after

Table 6.

Nuclear location, by percentage of total, for scions and cuttings, or stocks and stumps.

Side of graft	Total nuclei	Cell sides	Nuclear location within cell		
			Wound-proximate end	Wound-distant end	Central
Scion/cutting	1217	<u>65</u>	<u>15</u>	<u>9</u>	<u>11</u>
Stock/stump	820	<u>69</u>	<u>16</u>	<u>8</u>	<u>7</u>
Total	2037	<u>66</u>	<u>15</u>	<u>9</u>	<u>9</u>

Note. - Results within a line with common underlining are not significantly different at the 5 % level.

cell division the four categories of location become more nearly equal. These data were not statistically tested because this randomization of the distribution of nuclear location is gradual, and because of the small sample sizes necessarily involved and of the difficulty encountered in some sections of stating whether or not a given cell has divided or is merely curving out of the plane of section.

## FIGURE LEGENDS

All photomicrographs show longitudinal sections. Unless otherwise indicated, Glycol Methacrylate-embedded material was used, cut an indicated one micrometer thick, taken through part of the corner vascular bundles so all tissue types could be examined.

Fig. 1. Ungrafted stem, epidermis (E) and cortex, showing numerous parietal plastids and a nucleus (arrowhead). Toluidine blue staining, x 270.

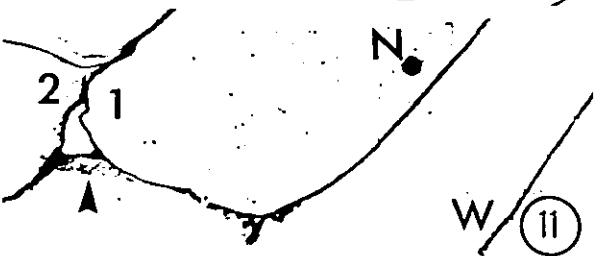
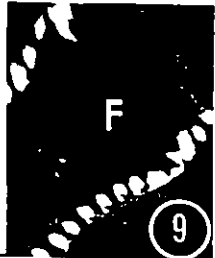
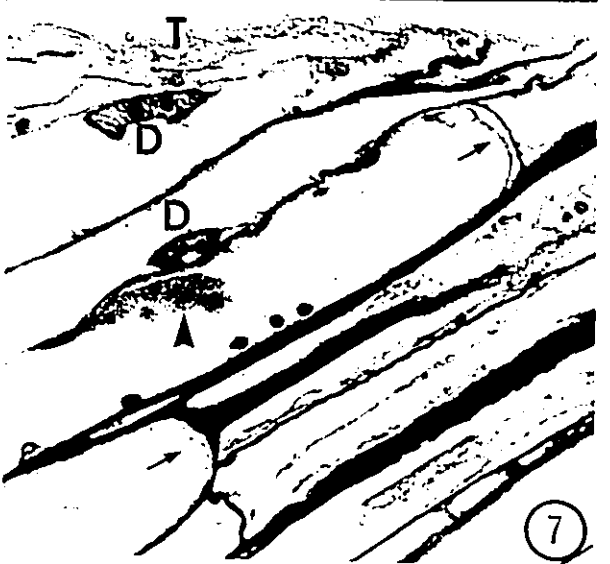
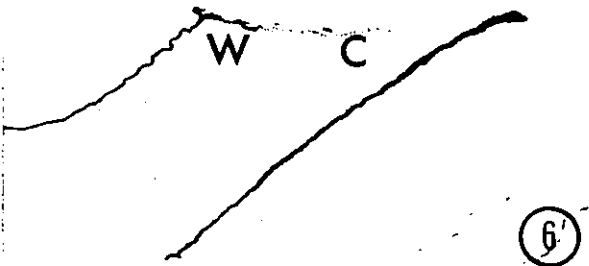
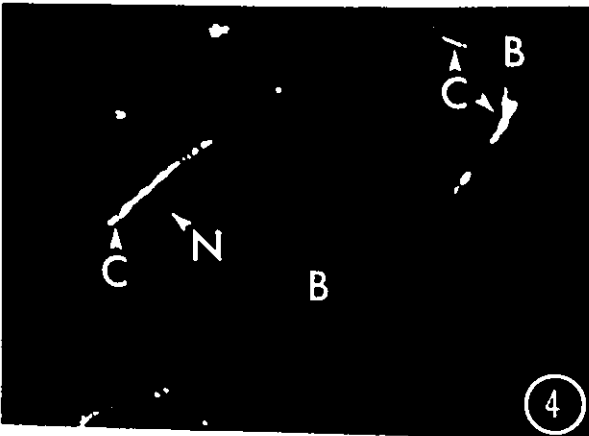
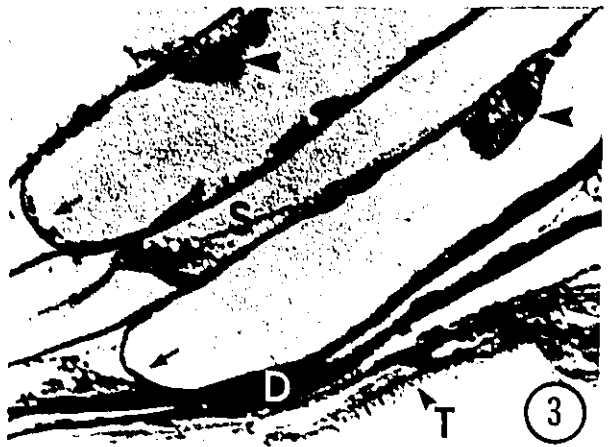
Fig. 2. Ungrafted stem, cortical cells showing starch grains in chloroplasts. Periodic acid-Schiff (PAS) reaction, x 1000.

Fig. 3. Day 2 control scion, phloem region. The swelling ends of the cells often have visible cytoplasm associated with them (arrows). Intact nuclei (arrowheads) show the health of these cells, while a necrotic nucleus (D) stains quite differently. A thin refractile layer (T) appears as a light grey boundary. A cut sieve tube (S) shows the characteristic slime leading from the plugged sieve plate. Toluidine blue staining, x 1300.

Fig. 4. Day 10 isolated internode stock, xylem parenchyma. Nucleus (N), areas of aniline blue positivity (callose, C) and areas of prominent cell bulging (B) lie in different parts of the cell. PAS reaction, aniline blue induced fluorescence, x 610.

Fig. 5. Day 2 uncovered stump, cortex. A number of cell layers have collapsed, one of them being distinguished by abundant aniline blue positivity (bright spots). I, outermost intact cell. PAS reaction, aniline blue induced fluorescence, x 1540.

Fig. 6. Day 2 control stock, cortex. Necrotic cytoplasm (C), forming a cap over the cut cell, is paler than the adjacent fragment of



wall (W). Toluidine blue staining, x 910.

Fig. 7. Day 2 control stock, vascular region. Healthy nuclei (arrowhead) and necrotic (D) are easily distinguished. Cytoplasm is especially prominent at the swelling end of the healthy cell (arrow). A thin refractile layer (T) is present. Toluidine blue staining, x 1100.

Fig. 8. Day 2 control stock, xylem region. Foam (F) blocks the cut end of a xylem vessel and merges with a fragment of thin refractile layer (T). Toluidine blue staining, x 720.

Fig. 9. Day 2 control stock, xylem. The foam (F) is autofluorescent. Autofluorescence, x 880.

Fig. 10. Histochemistry of thin refractile layer. The layer (T) coating the inside of the xylem vessel and forming a cap across it is highly autofluorescent. Autofluorescence, x 660.

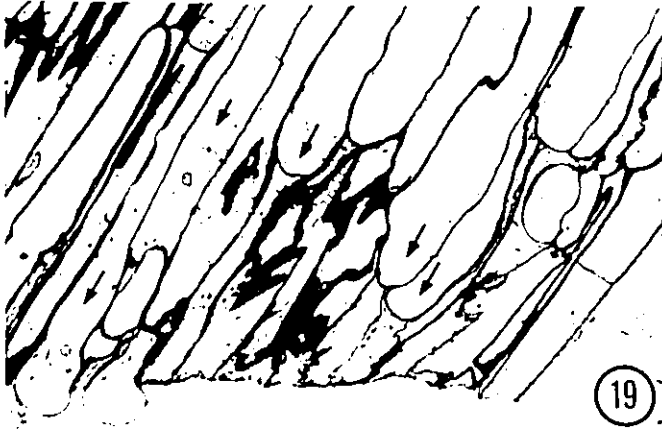
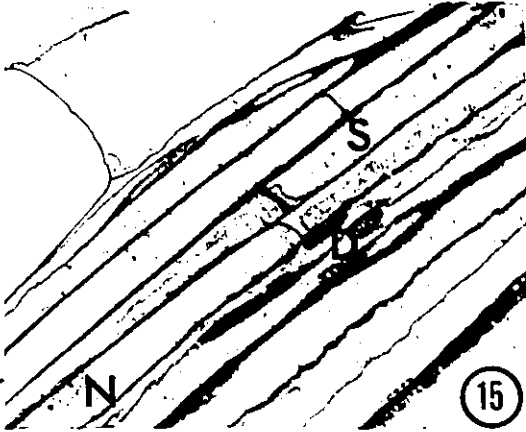
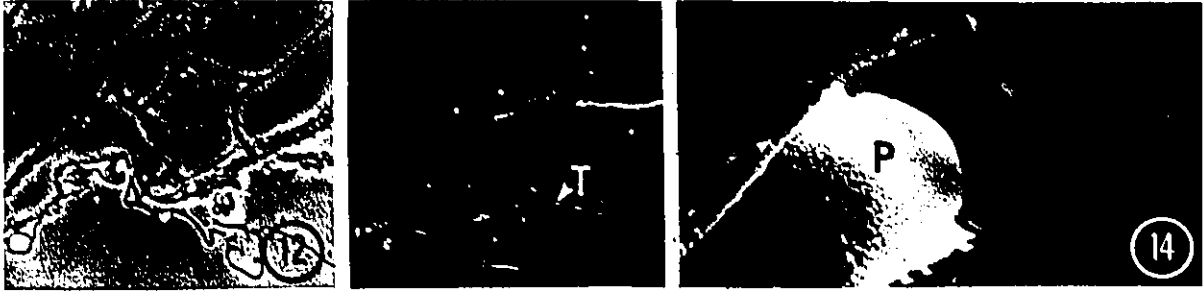
Fig. 11. Day 2 isolated internode scion, cortex. Cut walls (W) retain their original orientation, and are lined with necrotic cytoplasm (arrowhead). Necrotic plastids (2) are indistinguishable from healthy ones (1) in a cell with a healthy nucleus (N). Toluidine blue staining, x 960.

Fig. 12. Histochemistry of thin refractile layer. The layer (T) is extremely dark when viewed with phase contrast optics. Phase contrast microscopy, x 440.

Fig. 13. Histochemistry of thin refractile layer. The layer (T) is also birefringent. Polarization microscopy, x 520.

Fig. 14. Day 4 uncovered stump, xylem region. The dense plug (P) in this xylem element is brightly autofluorescent. Autofluorescence, x 530.

Fig. 15. Day 2 isolated internode stock, cortex and vascular



region. Cut cells are not capped by necrotic cytoplasm or thin refractile layer. A sieve tube (S) is blocked by slime. Necrotic nuclei (D) contrast with the healthy one (N). Note absence of accumulation of cytoplasm. Toluidine blue staining, x 580.

Fig. 16. Day 2 uncovered cutting, vascular region. Many cells have a considerable amount of cytoplasm at their wound-facing ends, and have healthy nuclei. Some of the ends facing the wound bulge noticeably (arrows). Necrotic cytoplasm (C) and crushed walls (W) cover the surface. Toluidine blue staining, x 360.

Fig. 17. Day 2 uncovered stump, vascular region. A good deal of rather foamy refractile layer (T) covers much of the surface. The end of a cell with a healthy nucleus (N) is close to the cut surface. Toluidine blue staining, x 750.

Fig. 18. Day 2 parafilm-covered cutting, vascular region. Two cells exceedingly rich in cytoplasm at their ends have swollen past the cut surface. Necrotic cytoplasm (C) clings to parts of their surfaces. Toluidine blue staining, x 900.

Fig. 19. Day 2 covered cutting, vascular region. Many cells have accumulated cytoplasm at their cut-facing ends (arrows). A portion is shown enlarged in fig. 18. Toluidine blue staining, x 270.

Fig. 20. Day 2 covered cutting, vascular region. Two cells have expanded past their original confines (opposed arrowheads), and have abundant cytoplasm in the expanding ends. Toluidine blue staining, x 800.

Fig. 21. Day 2 covered cutting, vascular region and inner cortex. Foam (F) and a dense plug (P) are present in the same xylem vessel. Necrotic cytoplasm covers much of the surface. Healthy cells have much

cytoplasm at their wound-facing ends (arrows). Toluidine blue staining, x 380.

Fig. 22. Day 2 covered stump, vascular region. Slight accumulation of cytoplasm surrounds the healthy nucleus of a swelling cell that is covered with necrotic cytoplasm (C) and broken walls (W).

Toluidine blue staining, x 1010.

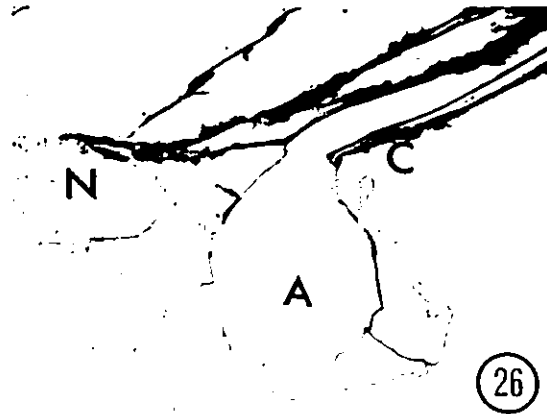
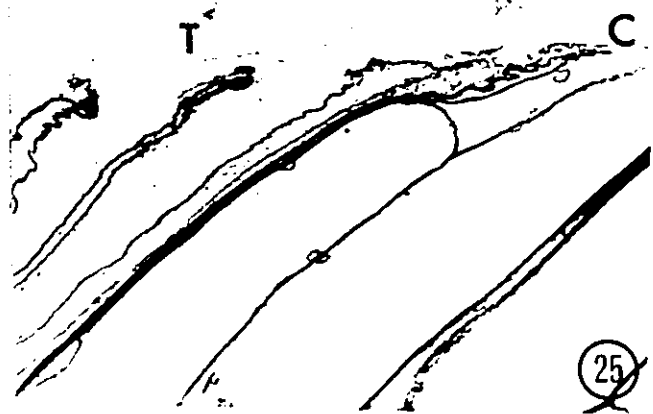
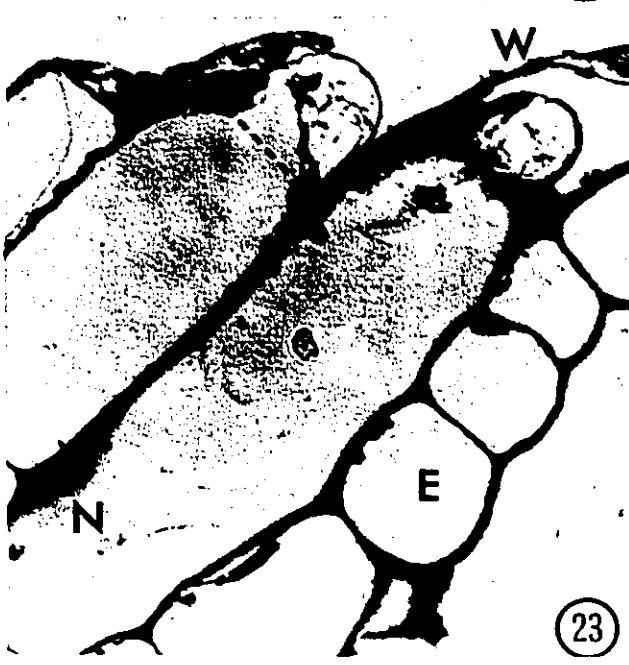
Fig. 23. Day 2 covered stump, collenchyma. Healthy cells (note nucleus [N]) have bulged out past their original ends (broken lines) toward the cut line. E, epidermis; W, cut walls. Cytoplasm shows signs of cell damage during fixation. Toluidine blue staining, x 700.

Fig. 24. Day 4 control scion, cortex. The expanding end of the cell has a small amount of cytoplasm. The original location of the transverse wall is clearly visible where the wall doubles in thickness (arrowheads). N, healthy nucleus. Toluidine blue staining, x 960.

Fig. 25. Day 4 control stock, vascular region. Necrotic cytoplasm (C) caps some cut cells, and a loose thin refractile layer (T) covers this part of the section. The graft-facing end of the healthy cell bulges toward the graft gap. Toluidine blue staining, x 840.

Fig. 26. Day 4 isolated internode scion, phloem region. Several cells protrude well into the graft gap. One (A) has been damaged, while another has a healthy nucleus (N). Necrotic cytoplasm (C) covers adjacent cells. Note uniform staining of new surfaces of protuberances. Toluidine blue staining, x 480.

Fig. 27. Day 4 isolated internode stock, phloem region. One cell protrudes well into the graft gap. Adjacent healthy cells are also swelling (arrows). The cut surface is largely covered with necrotic cytoplasm (C). N, part of healthy nucleus. Toluidine blue staining,



x 590.

Fig. 28. Day 4 uncovered cutting, vascular region. In some cells (e.g., at A) the cytoplasm stains dark green and has pulled away from the wall, while adjacent cells (L) show no such phenomenon. Many bacteria (B) are found in some cell cavities. Toluidine blue staining, x 470.

Fig. 29. Day 4 uncovered stump, vascular region. Dieback has left a thick collection of walls (W) on some surfaces. A piece of thin refractile layer (T) remains. Bacteria (B) are present in a cell cavity. Toluidine blue staining, x 920.

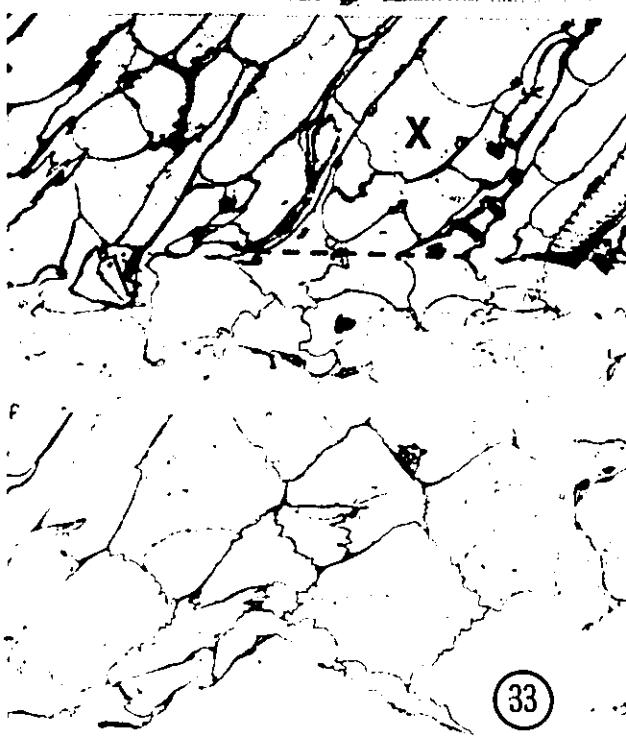
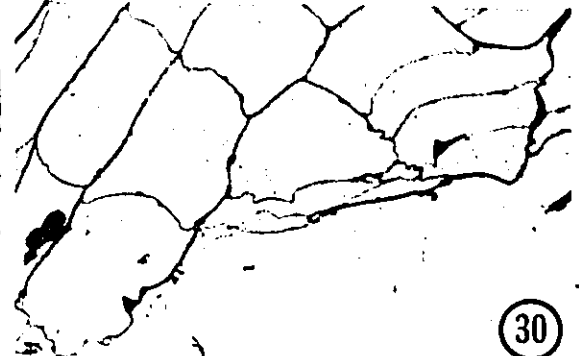
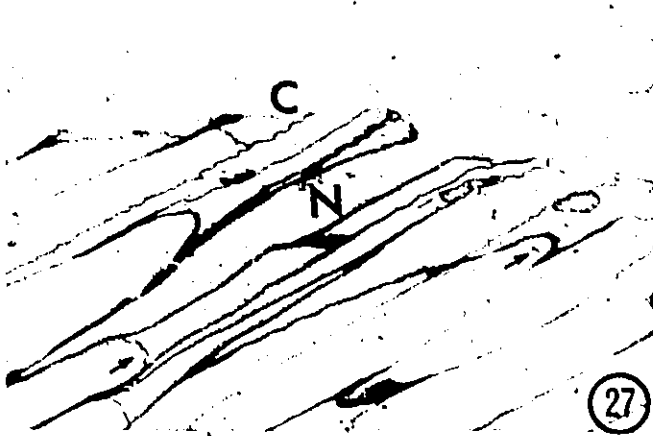
Fig. 30. Day 4 covered cutting, cortex. Several divisions have taken place roughly parallel to the cut line. Toluidine blue staining, x 270.

Fig. 31. Day 4 covered cutting, vascular region. Cell expansion has blocked xylem vessels (X) and created a new surface about 50 micrometers from the cut surface (partly marked by broken line). Toluidine blue staining, x 270.

Fig. 32. Day 4 covered stump, vascular region. The cut surface is here unmarked by necrotic cytoplasm and unbroken by expanded cells. The formation of cellular blockage of vessels is shown where one parenchyma cell has expanded into the xylem through at least three perforations (arrowheads) in the lignified wall. Toluidine blue staining, x 240.

Fig. 33. Day 6 control scion, pith. Several new walls have formed roughly parallel to the cut line. Toluidine blue staining, x 180.

Fig. 34. Day 6 control scion, cortex. The principal cell shown has some cytoplasm at the end that is bulging toward the cut surface from the original cell boundary (broken line). Toluidine blue stain-



ing, x 340.

Fig. 35. Day 6 control stock, vascular region. Two cells (1, 2) show accumulation of cytoplasm and nuclei at the wound facing ends, and a third (3) has expanded in an unusual fashion. Toluidine blue staining, x 380.

Fig. 36. Detail from fig. 35. A bubbly thin refractile layer (T) covers the cut surface. The expanded cell shows only the bulbous part, at the head of which is the nucleus. Toluidine blue staining, x 1000.

Fig. 37. Day 6 isolated internode scion, vascular region. Necrotic materials (M) lie between the expanded cells, while the exposed surfaces (arrowhead) show only new wall material. Toluidine blue staining, x 1050.

Fig. 38. Same section as fig. 37. Numerous well-expanded cells have formed a nearly continuous new surface over the vascular parenchyma, and have blocked xylem vessels (X). Toluidine blue staining, x 240.

Fig. 39. Day 6 isolated internode stock, vascular region. Two cells (A, B) shown here have swollen past the cut line; many cells bulge into cut xylem elements. Some necrotic cytoplasm (C) remains capping cut cells. Toluidine blue staining, x 400. Inset: the new surface (arrowhead) on cell B has no necrotic material on it. Toluidine blue staining, x 940.

Fig. 40. Day 6 isolated internode graft. Two contact zones (arrowheads) are spaced across the interface. Unidentified intercellular material (I) appears in the graft gap. Toluidine blue staining, x 105.

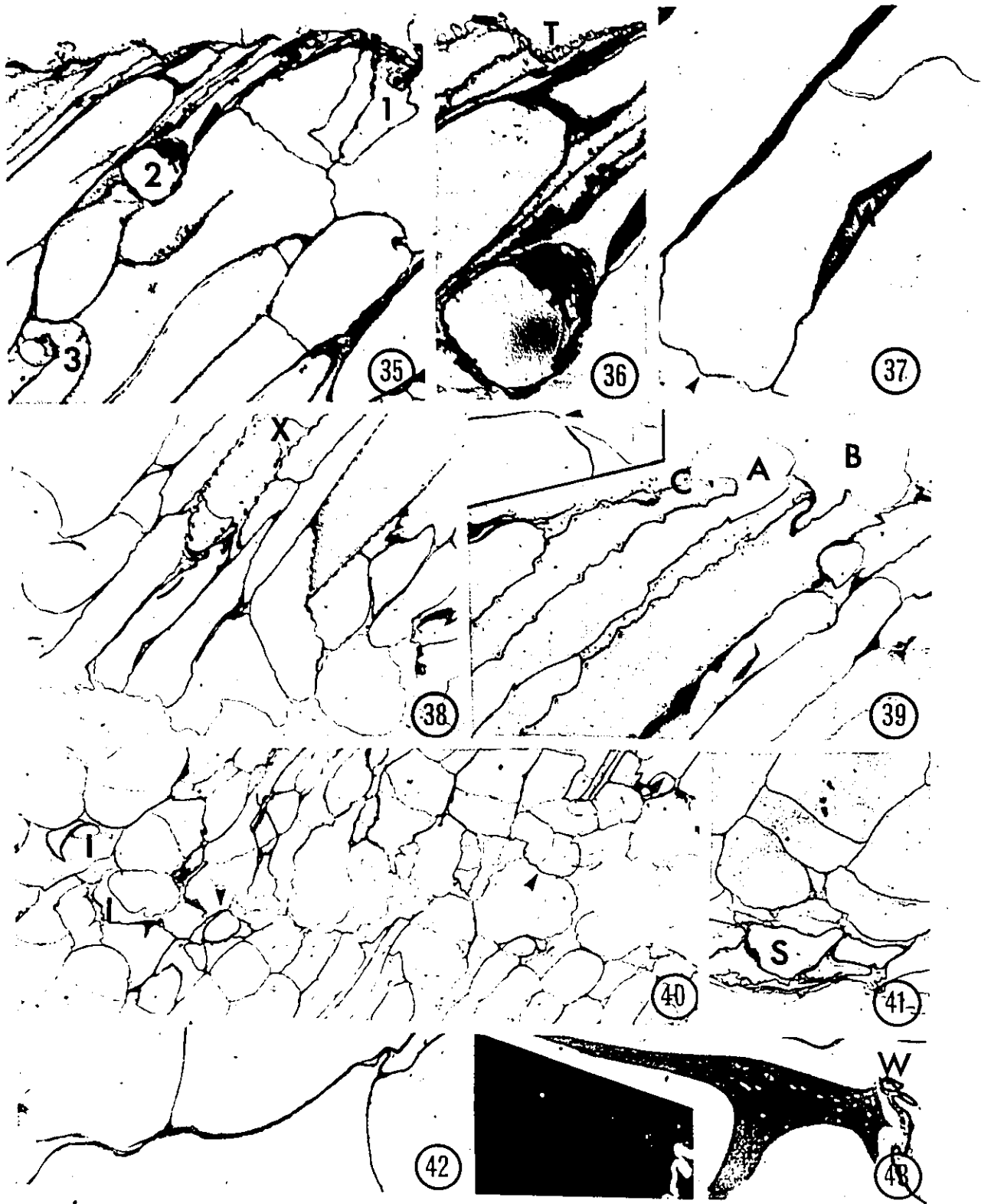


Fig. 41. Day 6 uncovered cutting, pith. The outermost intact cells are completely suberized and form a continuous boundary (S). PAS reaction followed by Sudan black B staining, x 240.

Fig. 42. The right-hand contact zone from fig. 40. No necrotic material intervenes between the contacting walls. Toluidine blue staining, x 1040.

Fig. 43. One of the patches of unidentified intercellular material from fig. 40, next to a wall (W) covered with necrotic cytoplasm. Note apparent holes, few dark spots. Toluidine blue staining, x 1020. Inset: Calcofluor-induced fluorescence. Note few bright spots. x 750.

Fig. 44. Day 6 uncovered cutting, cortex. Healthy, densely cytoplasmic cells (C) adjoin fully suberized cells. Cf. figs. 45, 46. PAS reaction followed by Sudan black B staining, x 880.

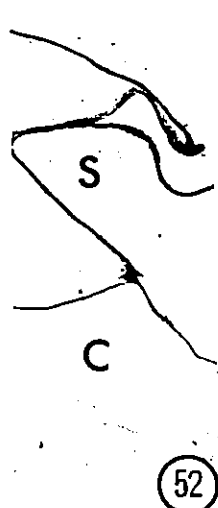
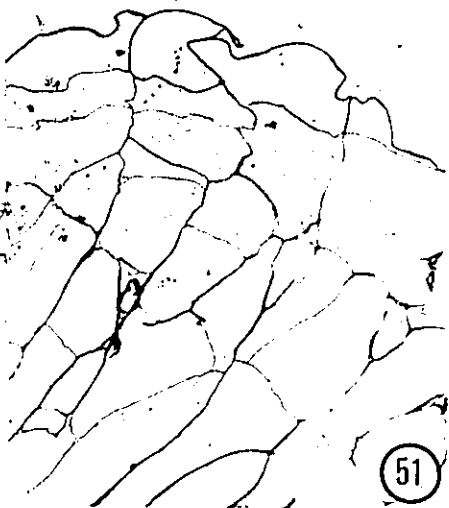
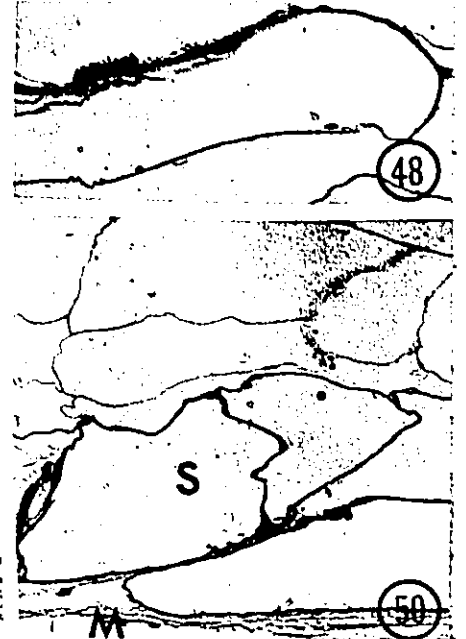
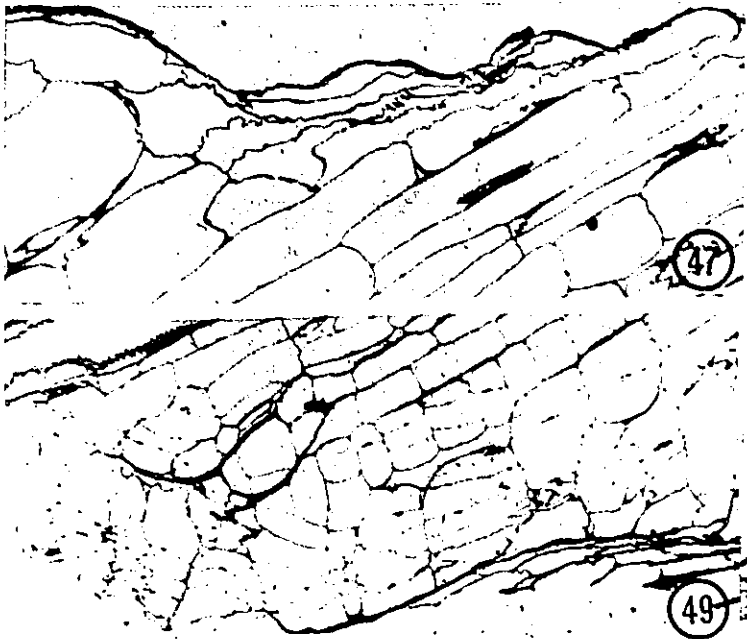
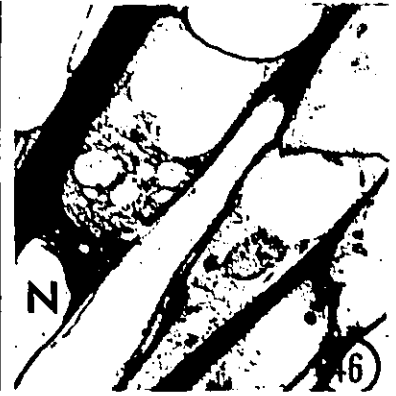
Fig. 45. Day 6 uncovered cutting, cortex. The Sudan black positive surface of the apparently empty cells (in figs. 44, 46) is highly autofluorescent. Note rich cytoplasm of adjacent healthy cell (L). Autofluorescence, x 1300.

Fig. 46. Day 6 uncovered cutting, cortex. Healthy cells immediately adjacent to necrotic ones (N) have much cytoplasm. Toluidine blue staining, x 850.

Fig. 47. Day 6 uncovered stump, pith and vascular region. Dieback and cell collapse have left an uneven surface, below which are no divisions or cell expansion. Toluidine blue staining, x 210.

Fig. 48. Day 6 uncovered stump, cortex. The outermost layer of cells shows no suberization. Here little dieback has followed wounding. PAS reaction followed by Sudan black B staining, x 700.

Fig. 49. Day 6 covered cutting, cortex and vascular region.



Divisions are parallel to the living surface, whether formed directly by the cut or subsequently by dieback and collapse of adjacent cells. Toluidine blue staining, x 135.

Fig. 50. Day 6 covered cutting, cortex. A thick necrotic layer (M) remains. The outermost two layers of intact cells are suberized (S). Many new walls are parallel to the cut surface. PAS reaction followed by Sudan black B staining, x 770.

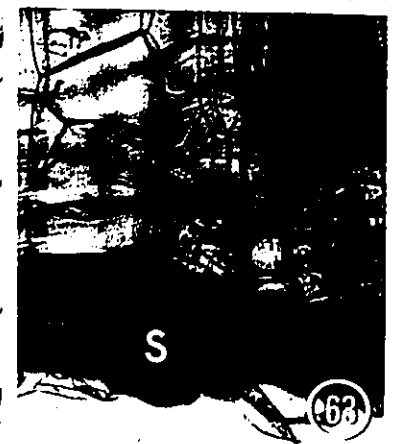
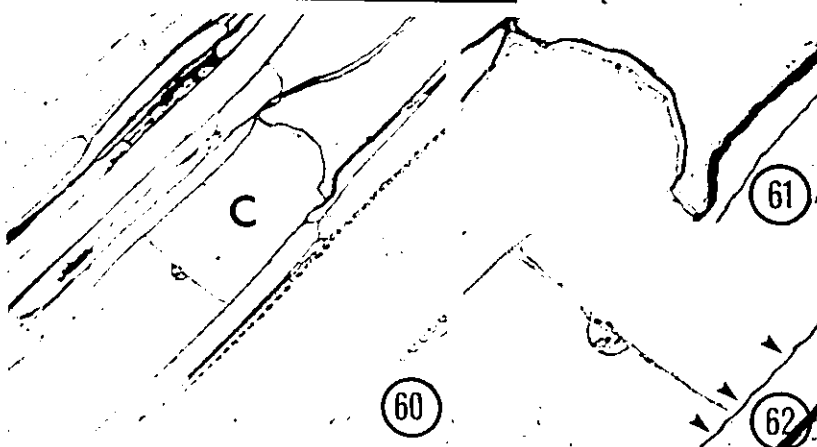
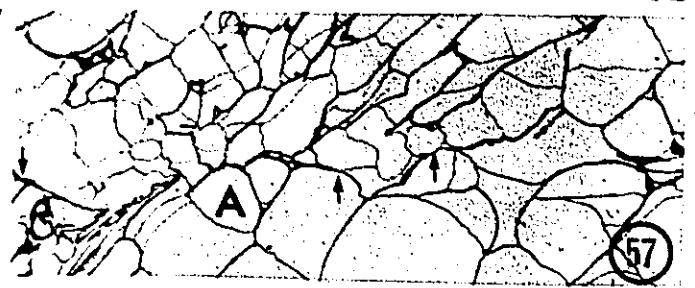
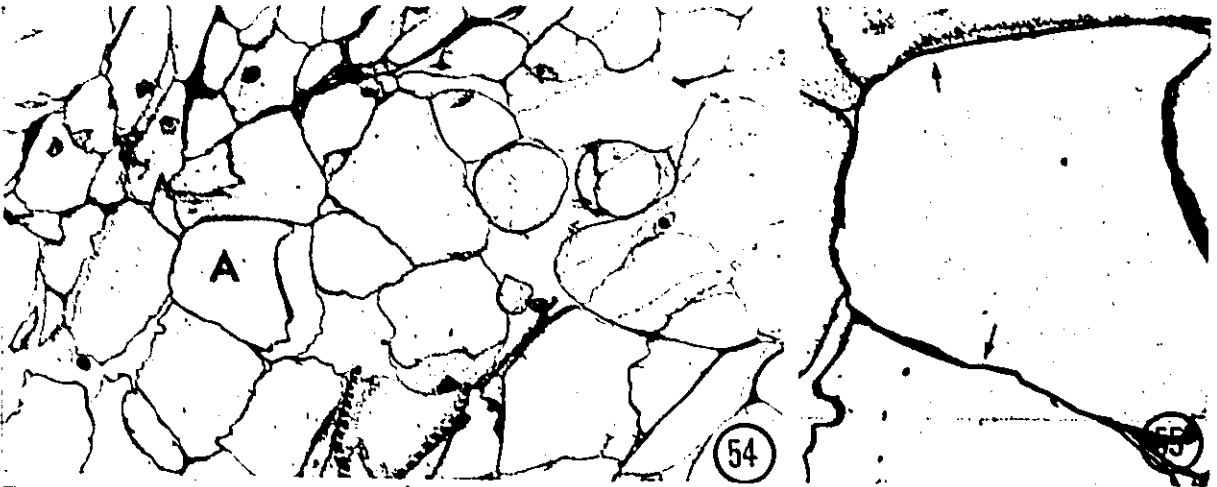
Fig. 51. Day 6 covered stump, vascular region. Two or three layers of cells have new walls parallel to the cut surface, and the outermost layer is suberized. PAS reaction followed by Sudan black B staining, x 230.

Fig. 52. Detail from fig. 51 showing the boundary between suberized cells (S) and non-suberized cells (C). PAS reaction followed by Sudan black B staining, x 880.

Fig. 53. Day 6 covered stump, xylem region. Behind the cut surface, many cells have filled the cut xylem, and pulled it apart by further expansion. There has been no expansion beyond the cut surface. Toluidine blue staining, x 240.

Fig. 54. Day 10 control graft. Expansion of cells from both stock and scion has obscured the graft line, which may be close to the cut line of the stock, or closer to the middle of the graft gap. The cell marked (A) is magnified in fig. 55. Toluidine blue staining, x 260.

Fig. 55. Detail from fig. 54. The central cell may be from either stock or scion, but it is clear from its location that one of the walls arrowed, probably the upper one, must be a contact wall. Neither wall has any necrotic material between it and the abutting wall. Toluidine blue staining, x 920.



60

62

63

Fig. 56. Day 10 control graft, pith. Some necrotic material remains between these two cells, the upper from the scion, the lower from the stock. Unidentified intercellular material (I; see fig. 43) fills part of the graft gap. Toluidine blue staining, x 1130.

Fig. 57. Day 10 isolated internode graft, vascular region and pith. Some of the contact zones are arrowed. Note frequent divisions, often parallel to the cut surface. Cell A is shown enlarged in fig. 59. Toluidine blue staining, x 100.

Fig. 58. Day 10 isolated internode graft, pith. Much necrotic material (M) lies on the graft interface. Autofluorescence, x 550.

Fig. 59. Detail of fig. 57. Contact zone between outer pith of stock and vascular parenchyma of scion. Intervening necrotic materials increase the thickness of the contact wall (opposed arrowheads). Toluidine blue staining, x 1000.

Fig. 60. Day 10 isolated internode stock, vascular region. A xylem vessel element has ceased differentiating and reverted to parenchyma-like cells (C). Toluidine blue staining, x 270.

Fig. 61. Detail from fig. 60. The bulging outermost cell wall of the reverted element. Toluidine blue staining, x 880.

Fig. 62. Detail from fig. 60. The nucleus lies at the cut-facing end of one of the new cells. Some wall thickenings lie at arrowheads. Toluidine blue staining, x 880.

Fig. 63. Day 10 isolated internode, basal end, hand section, pith. Numerous parallel divisions have formed a wound periderm, the outer two layers of which are suberized (S). Sudan black B staining, x 100.

Fig. 64. Day 10 isolated internode, apical end, hand section, pith. Few divisions have taken place, and only one or two cells have

suberized (S). Note especially unstained cell (C) at exposed surface, which shows that the surface staining is not artefactual. Sudan black B staining, x 100.

Fig. 65. Day 10 uncovered cutting, vascular region. The outermost layer of cells (S) is suberized. Necrotic cytoplasm (C) remains behind the new surface. PAS reaction followed by Sudan black B staining, x 880.

Fig. 66. Day 10 uncovered cutting, cortex and vascular region. Few cells have collapsed in the cortex, but a thick covering of necrotic material (M) lies over part of the vascular parenchyma. Numerous divisions parallel to the cut surface have taken place, but only in those cells nearest the cut. General expansion of the daughter cells instead of longitudinal expansion into cell cavities has meant that no surface is bare of necrotic materials. Toluidine blue staining, x 230.

Fig. 67. Day 10 uncovered cutting, cortex. Cells have expanded around the dead plastids (P) and other necrotic material. The plastids retain their starch. None of these exposed cells is suberized. PAS reaction followed by Sudan black B staining, x 720.

Fig. 68. Day 10 uncovered stump, xylem region and pith. Extensive dieback (D) in all tissues has produced a new surface of healthy cells, parallel to which numerous divisions have taken place. The outermost two or three layers of cells thus produced have suberized (S). Cut xylem elements have been invaded and pulled apart by expanding parenchyma cells, some of which have suberized. PAS reaction followed by Sudan black B staining, x 270.

Fig. 69. Detail from fig. 68 showing interface of dead cells (D), suberized cells (S) and live cells (L). PAS reaction followed by Sudan black B staining, x 880.

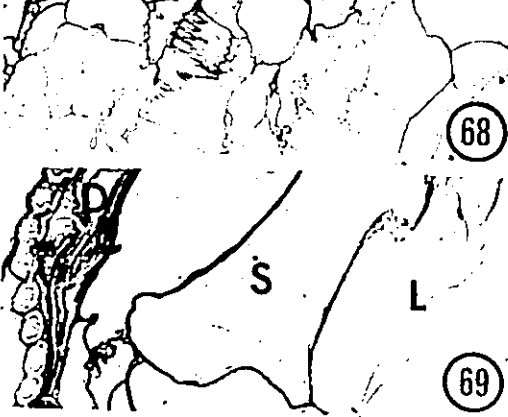
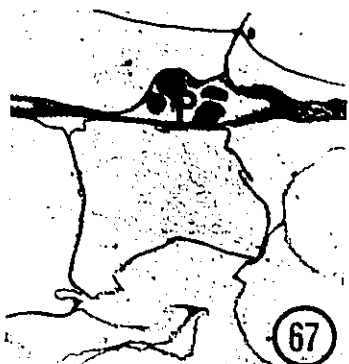
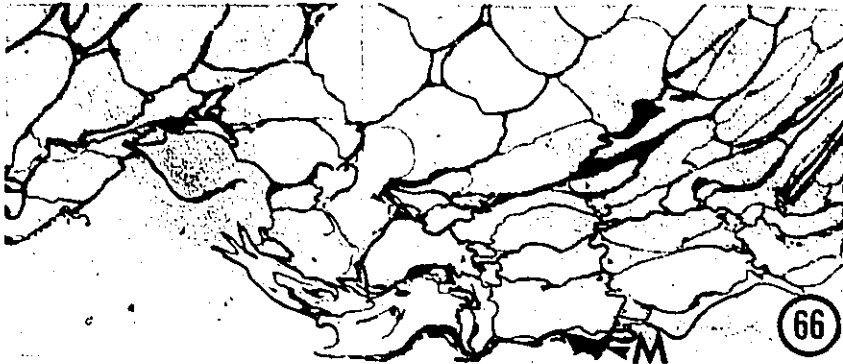


Fig. 70. Day 10 covered cutting, cortex and vascular region. Few cortical cells have divided, but there has been extensive cell division and expansion in the vascular parenchyma. Most new walls lie parallel to the surface. New xylem elements (x) have differentiated in the vascular parenchyma. Toluidine blue staining, x 130.

Fig. 71. Day 10 covered stump, vascular region. Several divisions parallel to the surface, and some cell expansion, have produced a wound periderm over the vascular parenchyma. The outermost layer of intact cells is suberized (S). PAS reaction followed by Sudan black B staining, x 230.

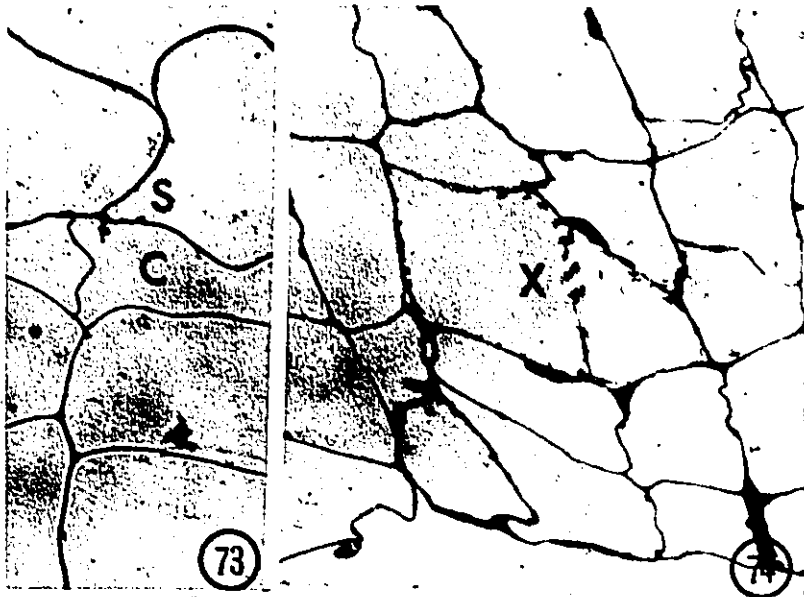
Fig. 72. Day 10 covered cutting, hand section, pith. An extensive wound periderm has formed, the outer undivided cells having suberized (S). Sudan black B staining, x 130.

Fig. 73. Day 10 covered stump, detail of fig. 71. The outermost layer of cells is suberized (S), inner cells are not (C). PAS reaction followed by Sudan black B staining, x 900.

Fig. 74. Day 10 covered cutting. New xylem cells (X) have differentiated in the callus over the vascular parenchyma. Toluidine blue staining, x 380.

Fig. 75. Day 10 graft with partners separated by Parafilm, hand section, scion cortex. Some wound periderm formation is seen with suberization (S) of the outlying cell layers. Sudan black B staining, x 270.

Fig. 76. Day 10 graft with partners separated by Parafilm, hand section, stock pith. Only two layers of new walls (W) are seen, parallel to the cut line. No suberization is found. Sudan black B staining, x 270.



## DISCUSSION

The processes of wound healing and graft formation in Coleus have some initial similarities but soon diverge, especially in the patterns of development of the cell masses formed at the cut surfaces. The early similarities and later differences are discussed separately below. A concluding section on graft hybridization, incompatibility and disease transmission is appended.

1. Early similarities. a. Necrotic materials. - Dead cells found in the grafts and wounds may be divided into three categories. There are those which have been split and obviously killed directly by cutting. Others, not at the cut line, nevertheless take on histochemical reactions suggesting necrosis, and may be said to have died incidental to cutting. Finally, some cells, especially the thin-walled hypertrophied ones, are damaged during the fixation process and show some disorganization of cytoplasm although their histochemical reactions are those of normal, not necrotic, cells. The sensitivity of these delicate cells to injury during preparation has frequently led to loss of information on their internal organization.

The necrotic layer, also known as isolation layer, insulation layer, contact layer, and incorrectly as wound gum, initially consists of the walls and contents of cells killed by the preparation of the graft partners (Vöchting 1892, Wright 1893, Kabus 1912, Mendel 1936, Juliano 1941, Buck 1954a, 1954b, Copes 1969, Stoddard and McCully 1979). The necrotic cytoplasm, plastids and nuclei take on a phenolic reaction, probably due to precipitation of aromatic compounds liberated from cut vacuoles (Matile 1978) onto the protein components. The walls remain unstained by the phenolic components for the entire 10 day exami-

nation period, possibly because of the low content of reactive protein groups (Preston 1974).

The necrotic materials show no visible changes before disappearance. Staining intensity does not gradually fade, structures do not fragment, but necrotic cytoplasm and organelles do become hard to find. Early in the development of all grafts and wounds, caps of dead cytoplasm which cover many cut cells, especially in the vascular parenchyma, are lost. These caps, being thin, might have collapsed into the cell cavities and merged with the dead cytoplasm there, or they might have been degraded and lost.

The loss of other dead components generally takes place after they have been contacted by live cell walls. Dead cell contents are obscured wherever there has been cell expansion to or beyond the cut line, but they may remain intact in cavities surrounded by live cells (fig. 67). The rapidity of the loss suggests either an efficient degradation process, or an efficient loosening mechanism allowing the components to be lost during fixation. Wall ingrowths, resembling those of transfer cells, which could account for such resorption, have been reported on the graft-facing walls of Sedum telephoides grafted incompatibly onto Solanum pennellii (Moore 1978b). No such ingrowths, however, were seen near the cuts in the Coleus stems, and their role must remain conjectural for now.

Some amorphous necrotic material often covers parts of the surfaces of the grafts even after contact (figs. 56, 58, 59). It is impossible to tell what effect these remnants of necrotic material have on cohesion, although it is generally held that cell contact, by definition, is first established where the necrotic layer is broken (e.g.,

Wright 1893, Mendel 1936, Buck 1954a, 1954b, Fletcher 1964, Stoddard and McCully 1979). This matter is discussed further below with regard to the production of new surfaces.

Unlike the cell contents, the cut walls always remain visible and distinct. In the pith they collapse and are pushed bodily outward by growth of the subjacent cells. Usually, in the cortex and vascular parenchyma, the dead cell cavities eventually become filled by expansion of subjacent cells, and the old walls cause an apparent addition to the thickness of parts of the longitudinal walls of these swollen cells. This intimate contact of new wall and old is not associated with any destruction of the old wall visible at the level of the optical microscope. This result is not unexpected, for it would seem difficult for a cell to selectively remove a wall that is caught tightly between its own wall and that of an adjacent cell without disrupting its own wall, a system not at all comparable to that of differentiating xylem wherein the cell digests only its own wall (Esau 1977). It does, however, provide a purely physical method for obscuring old walls, which could partly account for past reports of their disappearance along with the rest of the necrotic materials (Wright 1893, Bradford and Sitton 1929, Mendel 1936, Juliano 1941, Buck 1954a, 1954b, Fletcher 1964, Copes 1969, Stoddard and McCully 1979).

The subsequent death of cell layers behind unprotected wounds should come as no surprise. It is perhaps more unexpected that the covered wounds experienced no greater dieback than any graft. Thus the limiting of necrosis of the graft surfaces is due not to the presence of living tissue in the graft partner, but merely to the presence of protection from the environment.

1b. Nuclear placement. - The general distribution of nuclei, with a large constant proportion along the lengthy side walls and smaller proportions along the shorter end walls, may be explained on the basis of random placement. The chance of fixing the nucleus against a long side of a cell is enhanced by that length. This explanation allows for the randomization of distribution of nuclei following division, when the walls become more nearly equal in length. It does not, however, account for the tendency of more nuclei to be found at the wound-facing ends of the cells than at the wound-distant ends, regardless of expansion of those cell ends, a tendency that achieves very great statistical significance in the stocks and stumps (table 6). Nevertheless, the proportion of nuclei at the wound-facing ends of the cells is so small as to bring into question its biological significance. There is no need to invoke nuclear proximity to allow cell wall growth, since in various models of cell growth, more than one part of the wall is receiving additions (Wardrop 1962, Roelofsen 1963) and obviously the nucleus can not be in two or more places at once. Even in root hairs, with unipolar tip growth, the nucleus is well back from the growing tip (Eckdahl 1957).

Other nuclear and cytoplasmic reactions have been shown in the hours following wounding of onion epidermis, but delaying the first observations of the Coleus until two days had elapsed has prevented direct comparison to those results, which include loss of nuclear dry mass in the two cell layers nearest the wound (15 min., Kulfinski et al. 1978), increases in cellular respiration (maximum at 30 min.), nuclear volume (1 h) and nucleolar volume (3 h) and nuclear movement within 2 h (Ziegler 1954).

The uniformity of nuclear distribution among types and ages of grafts and wounds implies a uniformity in the stimuli displacing those nuclei under the different circumstances, followed either by an absence of a redistributive influence or by the presence of a factor maintaining the status quo. Nuclear location, especially when along the sides of the cell is often independent of the site of aggregation of the cytoplasm, particularly when that is at the wound-facing end of the cell (e.g., figs. 3, 7, 24). It has been recognized for at least a century that both nuclei and cytoplasm move toward a wound (Tangl 1884), probably under the same stimulus. Both Ritter (1911) and Ziegler (1954) concluded that when the nucleus is moved, it is merely carried along in the cytoplasmic flow.

1c. Cytoplasmic changes. - As in many other graft (Wright 1893, Lachaud 1975, Stoddard and McCully 1979) or wound (Tangl 1884, Ritter 1911, Ziegler 1954) systems, cytoplasm accumulates near the injured surfaces. This accumulation may be due to either or both formation of cytoplasm or to its redistribution within the cell. Cytoplasm moves toward a point of injury within a cell (see Aist 1976), and in neighbouring cells it moves toward an injured or necrotic cell (Tangl 1884, Ritter 1911) within minutes or hours. By the time two days have elapsed, however, there has been plenty of time for production of new cytoplasm, as shown by the sheer volume of cytoplasm in many cells (figs. 18, 19, 20, 46).

The attraction of cytoplasm toward a wound was first thought to be based on simple chemistry, such as change in pH, until Ritter (1911) showed that similar responses elicited in the absence of wounds by application of the suspected chemicals, were far too slow to share a

common mechanism. Similar tests making use of modern knowledge of plant hormones might cast more light on the nature of the attracting stimulus. More recent experiments show that wounding is followed by a rapid depolarization of cell membranes that is propagated through the plant within seconds or minutes (Van Sambeek and Pickard 1976), then by a slow hyperpolarization of the membranes of the few cells nearest the wound over several hours (Koopowitz et al. 1975), but no study has yet related these electrical phenomena to cytoplasmic movement. Such rapid changes could not be expected to set up the gradient required to inform each cell of the direction in which the wound lies.

Two other aspects of the distribution of the accumulation of cytoplasm need mentioning. First, the vascular parenchyma generally is richer in cytoplasm than is the cortex or the pith. Second, there appears to be more accumulation of cytoplasm in the scions and cuttings than in the stocks and stumps.

The concentration of cytoplasm in the vascular parenchyma is probably due to the proximity of the nutrient- and hormone-rich vascular conduits, which is also likely the cause for the large number of divisions and large amount of cell expansion in this tissue, discussed below. By day 6 enough of the active compounds have leaked out to the cortex for it to produce more cytoplasm. The reason for the earlier peak of cytoplasmic content in the covered wounds is not clear.

The explanation of the second aspect may be the following. The poor development of the cytoplasm in the isolated internode grafts may be due partly to the presence of wounds at the ends of the internode. Since the cytoplasmic accumulation, even where best developed, appears only in the few cell layers nearest the wound, comprising, at most, less

than a millimeter, and since the wounds at the ends of the internode are about 10 mm away from the graft, it is assumed that any effect of the remote wounds must be small. Thus the isolated internode tissue appears to be inherently incapable of producing a great deal of cytoplasm, an influx of nutrients from other parts of the plant being necessary. As in most other grafting systems (see Stoddard and McCully 1980 for a discussion of this point), there are more nutrients available from the greater amount of photosynthetic tissue in the scions, and thus in the cuttings, than in the stocks and stumps.

If it is possible for isolated internode grafts to heal with little development of new cytoplasm, then what is the role of greater development? Perhaps it is a case of "more" being better than "some". The early rush of cytoplasm toward a wound is associated with the deposition of wall material as wound plugs (Tanl 1884, Nims et al. 1967) generally containing callose (Currier 1957) which in a few hours is obscured by other wall components (Aist 1976), which explains why aniline blue positivity is seen only infrequently in the Coleus stems (figs. 4, 5).

Since the original accumulation of cytoplasm is related to deposition of wall material, and disperses when that deposition is complete (Nims et al. 1967, Aist 1976), it seems reasonable to assume that the continued abundance of cytoplasm near the wound is associated with the continued production of wall material. Two patterns of expansion of the wall follow the peak of cytoplasmic content: in some wounds, it is all over the growing cell, lifting the overlying dead cell layers (figs. 53, 68); in the other grafts and wounds the cell ends grow invasively (e.g., figs. 23, 24, 38).

A further possible role for the cytoplasm is the provision of proteins for the recognition of a suitable graft partner. This suggestion requires that recognition be based upon contact between cells, as could be provided by plasmodesmata, or upon the release of specific proteins into the walls.

The present work casts no light on the question of bridging of the graft by plasmodesmata. Pit fields were found but infrequently between cells within a graft partner, and not at all at any site of contact between partners. It seems unlikely that there should be no symplastic connection across a graft, for the following reasons. In successful orchid grafts, the scion remains alive without vascular connection to the stock, i.e., with only parenchymatous connections (Muzik 1958). Similarly, in graft-induced periclinal chimaeras, "connecting threads" have long been observed between cells of the partners (e.g., Hume 1913), implying the presence of pit fields. Burgess (1972) reports half-plasmodesmata in electron micrographs of apparently normal pit fields in the walls between the chimaera partners of Cytisus adami (epidermis of C. purpurea around Laburnum vulgare). He shows one convincing electron micrograph (his fig. 10) wherein continuity "appears to consist of half-plasmodesmata joined by an indirect bridge between their ends" (Burgess 1972, p. 454). It remains to be shown at what stage in graft development plasmodesmata form, largely because no system has yet been available in which the stock-scion interface could be identified reliably. Perhaps electron microscopy of parts of grafts of isolated internodes, where that interface is frequently conspicuous, can resolve this problem.

1d. Other similarities. -- Both grafts and wounds show some develop-

ment of dense plugs and foam in the xylem, slime in sieve tubes, and thin refractile layers.

The location and appearance of the slime in the sieve tubes make it probable that it is P-protein, which is well known for sealing severed sieve tubes (Capny 1973, Esau 1977).

The dense plugs, which appear most often in sections or cuttings, are reminiscent of the true "wound gum" which blocks cut xylem in many wound situations (e.g. Swarbrick 1926, Moore 1978a). The term wound gum was first applied to this type of material, which is itself variable, and was later erroneously applied to the necrotic material formed on cut surfaces from killed cells (Mendel 1936). Necrotic material is a complex of dead walls and contents, while wound gum has distinct optical and histochemical properties (Swarbrick 1926, 1927, Moore 1978a). The dense plugs clearly bear little histochemical similarity to wall material, and as shown by the autofluorescence and toluidine blue staining, have a large content of phenolic substances, which may interfere with other histochemical reactions, such as causing acid fuchsin positivity regardless of the presence or absence of protein.

The xylem foam, while sharing a general positivity for phenolic reactions with the dense plugs, is quite different from them and from classical wound gum. The toluidine blue reactions of the two types of xylem inclusion differ greatly, as do their physical appearances, and their intensity of acidophilia. The continuity and similarity of the foam and thin refractile layer suggest that they are one and the same material, the nature of which is not altogether clear. Positivity to acid fuchsin would suggest a high protein content, except that the mild acidophilia of the lignin in xylem wall thickenings casts doubt on

the specificity of the reaction. The lack of reactivity to Schiff reagent alone (without preceding oxidation of the specimen) implies that the refractile material has a low content of aldehyde groups in contrast to the lignin of the xylem, so it is not correct to speak of the layer as being lignified. The properties of refractility and birefringence indicate a fairly well-ordered structure. Thin refractile layers are not mentioned in other work on wounding or grafting, so they may be unique to the present graft system. On the other hand, no work of the present resolution has been seen on any wound or graft in a member of the Labiatae. Certainly the phenolic reaction of the refractile material might have contributed to the opinion that the necrotic material, on which it sits, was lignified (see references in Bloch 1937).

The refractile material is probably unstable in the presence of air, since it thins and disappears from the uncovered cuttings and stumps by day 6, while it remains on the control grafts through day 10. Its general absence from isolated internode grafts suggests its synthesis either along an extended length of stem, or in organs other than the internode itself, or from materials imported from those organs, while its absence from the covered cuttings and stumps may simply indicate adhesion to the parafilm which was removed in preparing the tissue. Its location in the xylem, in particular the way the material often coats the wall thickenings (fig. 10), implicate the adjacent cells in its synthesis. Ordinary upward flow in the xylem could suffice to bring the material to the cut surfaces of stumps and stocks, whence it could be transferred to scions, it being absent from cuttings (tables 2 to 5). The importance of the material to the plant is not clear, unless it is as a sealant against microbial invasion, for which the

phenolic content might help.

2. Later differences. - The differences in developmental anatomy between grafts on the one hand, and wounds on the other, are best demonstrated in the patterns of cell division and proliferation.

2a. Cell divisions and callus formation. - There are three primary planes of cell division: transverse to the axis of the plant (hereafter, "transverse") or parallel to it ("longitudinal" or "axial"), or parallel to the cut surface, or to the living surface in those cases in which its location has changed due to death of some outlying cells ("parallel") (e.g., figs. 49, 68). Divisions perpendicular to the living surface are very rare.

As in most graft situations (Wright 1893, Mendel 1936, Juliano 1941, Copes 1969, Stoddard and McCully 1979), early divisions close to the wound are predominantly parallel to the living surface, with rather fewer in the other planes. Farther back from the wound, planes of division are more random. Between days 6 and 10, planes of division in the grafts become more random, forming a callus, while in the wounds divisions become even more predominantly parallel to the living surface, developing a wound periderm. Thus a visible difference in primary plane of cell division develops between grafts with living partners on the one hand, and wounds, without living partners, on the other. The pith parenchyma in many grafts, however, shows a periderm-like proliferation (see figs. in Stoddard and McCully 1980). Hence a graft partner provides more than the simple protection from the environment substituted by the parafilm covering.

Most cell divisions are found in the vascular parenchyma, possibly for the same reasons discussed for cytoplasmic concentration. The cam-

bium, along with the epidermis and, if present, endodermis, rarely contributes significantly to the callus of grafts, even in woody stems, the bulk coming from the vascular parenchyma, some from cortex and pith (Vöchtling 1892, Wright 1893, Kabus 1912, Sass 1932, Sharples and Gunnery 1933, Crafts 1934, Mendel 1936, Juliano 1941, Buck 1954a, 1954b, Warren Wilson and Warren Wilson 1963, Copes 1969, Thair and Steeves 1976, Stoddard and McCully 1979). In the isolated internodes there were very few divisions outside the vascular parenchyma, which again is probably related to the lack of influx of nutrients or hormones, although the basal ends are capable of forming extensive wound periderms (fig. 63).

Because isolated internode grafts never reach 100 % cohesion (50 % in Stoddard and McCully 1980, 40 % in the present study), some of the younger grafts examined might never have cohered and thus might have contributed information not entirely consistent with eventual cohesion. The general uniformity of the grafts at any one age suggests, however, that the differences leading to cohesion may be quantitative rather than qualitative. For example, there were rather more divisions in the isolated internode grafts which cohered at any given age, than in those that did not cohere. Nevertheless, as before (Stoddard and McCully 1980) cohesion often takes place in areas of the pith where there are very few if any divisions.

2b. Cellular hypertrophy. - Grafts of isolated internodes are distinguished by the presence of large hypertrophied cells which have but infrequently divided (figs. 26, 27, 37 to 39). Such protuberances have been reported for wounded leaves maintained in damp atmospheres (LaRue 1937, Rosenstock and Sondermann 1974), and for some grafts of monocotyledonous plants (Krenke 1933, Muzik and LaRue 1952) unless prepared by

breaking an active intercalary meristem (LaRue 1944, Muzik and LaRue 1952).

Some expansion (in addition to tylosis formation, which is important in all cases for sealing severed xylem vessels) is found at some stage in all graft and wound types, but generally with some cell division. In the early stages of grafting and wound healing, this expansion tends to be at the ends of the cells facing the gap, producing large bulbous protuberances. In later stages, however, much expansion in wounds is behind the cut surface, bodily lifting the outlying cells (figs. 53, 68), while in grafts expansion continues to be beyond the cut surface. A similar differentiation between subsurface divisions and expansion on the one hand, and surface expansion with infrequent division on the other, is seen in Sedum leaves (Rosenstock and Sondermann 1974). Periderm formation was enhanced by low humidity, in older leaves, and by the presence of dead cell material, while papilla growth was promoted by the opposite conditions (Rosenstock and Sondermann 1974). The authors interpret their results on the basis of an osmotic gradient, any contributor to water loss enhancing periderm formation at the expense of papilla growth. Such an explanation might apply in the early stages of graft formation and wound healing in Coleus stems, where the uncovered wound produces very few papillae and first starts periderm formation. Later stages, however, where wounds are covered and yet produce periderm, defy this explanation.

Localization of hypertrophy follows the same sequence as amplification of cytoplasm: scions more than stocks, vascular parenchyma more than cortex or pith, and largely at the wound-facing (i.e., free) ends of cells, and again one may recall the explanation proposed on the basis of proximity to the vascular conduits. Two questions are then raised: first, what makes the lower end of a cell more likely to expand than the

upper, as implied by the greater number of cells expanding from scions than from stocks? It is not a case of capability to expand, since the amount swollen by any cell is independent of whether it is in stock or scion. Second, what causes the great amplification of cytoplasm at the swelling ends of cells in some situations, such as control grafts, while other cells seem capable of great expansion without much increase in cytoplasmic content, as in the isolated internodes? Is the amplification of cytoplasm a secondary effect, a way of making use of the available nutrients when present, or is the wall made in its absence somehow inferior? Such inferiority could be used to explain the decreased rate of cohesion of isolated internode grafts described previously (Stoddard and McCully 1980), as some individual grafts would be so weakened as not to cohere.

Cell expansion, with or without concomitant cell division, is necessary if a new cell surface is to be exposed, as has classically been assumed to be prerequisite for cohesion of grafts (see Discussion, Stoddard and McCully 1980). The validity of this assumption is again called into question by the presence of places at graft lines where a new surface is not exposed, yet certainly no graft gap remains (figs. 56, 58, 59). There is as yet no way to determine the relative contributions of different contact zones, with intervening necrotic material or between new surfaces, to cohesion. Some technique is necessary to gently relax the tissue so areas in contact, but not cohering, can separate without disrupting genuine zones of cohesion, and the tissue can then be examined microscopically.

Either type of cohesion, however, depends on the propagation of some adhesive material which must penetrate at least the primary cell

wall, if not an additional overburden of necrotic materials. This sup-  
 position is based on the evidence that the middle lamella, which appears  
 to be the cement between cells in ordinary circumstances, is the first  
 cell wall layer to be produced after division (Esau 1977). There is evi-  
 dence, based upon autoradiography and reasonable conjecture, that the  
 pectic substances and hemicelluloses (an old term with unfortunate con-  
 notations of uniformity, but still applied to the range of mixed poly-  
 saccharides including galactomannans, glucuronoxylans, etc. [Preston  
 1979]) enter the wall by intussusception rather than by layering as for  
 cellulose (Preston 1974). There is further evidence that auxin, which  
 is apparently in abundance at the bases of cuttings and scions (see  
 Discussion, Stoddard and McCully 1980), enhances the production of the  
 monosaccharide components of hemicelluloses and pectins much more than  
 that of glucose, in coleoptiles of Hordeum (Sakurai and Masuda 1978).  
 The question is thus posed, if these two groups of polysaccharides can  
 be forced part way into a wall, could they not be forced right through  
 it, making the outer surface sticky to a greater or lesser degree?

There is possible evidence for such an extrusion in the material  
 appearing between some graft partners (figs. 42, 56) and also in sections  
 of some scions and covered wounds. This substance is immediately dis-  
 tinguishable from somewhat similar material associated with bacteria by  
 the latter's stronger PAS positivity, and by the distinct absence of  
 anything resembling the dark-staining rod-shaped bacteria found in the  
 latter. The clear pink to purplish-pink colour of this substance when  
 stained with toluidine blue, and its weak alcian blue positivity, sug-  
 gest weakly acid polysaccharides (O'Brien et al. 1964, Pearse 1968).  
 Calcofluor negativity implies an absence of pectins, callose or cellu-

lose (Hughes and McCully 1975), while weak PAS positivity indicates low content of vicinal glycol groups as found in pectins, starch and cellulose (Pearse 1968), and absence of birefringence, a lack of alignment of microfibrils. Finally, the presence of small holes in the otherwise amorphous material and its apparent stretching between points of attachment indicate a fairly cohesive material, in which any fibres present form an interlocking network. On these bases, it is suggested that the substance is a wall component, possibly well-branched hemicelluloses.

There is no evidence to suggest or to deny that this material has a role in cohesion. These results should be compared with those of Lindsay (1972), who used a variety of treatments in an effort to separate day-old tomato grafts which had already cohered. Some of the treatments suggested a small role for pectins in cohesion. A pectinase solution caused separation of some 20 % of his grafts, and only hypotonic buffer solutions sufficed to separate his grafts consistently. He collected material staining by the hydroxylamine- $\text{FeCl}_3$  method for pectins by inserting cellophane between the graft partners, but this result is questionable because separation causes changes in the grafting process in Coleus (figs. 75, 76). His results are generally difficult to interpret since his techniques had such low rates of success, and differed so widely from the standard methods of wall fractionation (Jensen 1962, Siegel 1962, Preston 1974) which have yet to be used to investigate the cohesive substance.

2c. Callus cell differentiation. - Two types of differentiation of the callus cells were seen. The first, in context not unremarkable, is the differentiation of xylem elements (apparently without phloem) in the callus of parafilm covered cuttings, which could be thought to indicate

that the covered cutting was acting as if it had a graft partner. This idea is best countered by the difference in appearance of callus of control grafts, where divisions are effectively random below the vascular parenchyma, from the incipient wound periderms of the covered wounds with their highly ordered parallel divisions. It is more likely that this formation of xylem is disordered, as seen in failed pea root grafts where much vascular tissue is produced in the absence of cohesion (Stoddard and McCully 1979) and as associated with the accumulation of stimuli inducing differentiation of vascular tissue at the bases of scions (Stoddard and McCully 1980) and therefore presumably of cuttings.

The second type of differentiation, which is entirely restricted to wounds, both covered and uncovered, is suberization. Cells which were presumably suberizing (fig. 28) showed other related histochemical reactions, i.e., positivity to the phenolic component of the suberized lamella (Kolattukudy et al. 1975) with toluidine blue and acid fuchsin, and autofluorescence, before Sudan black positivity to the lipid components (Pearse 1968) developed. These reactions, which are not associated with any apparent cytoplasmic necrosis, may result from release of phenolic precursors of suberin (Martin and Juniper 1970, Kolattukudy et al. 1975) into the cytoplasm during fixation.

In covered stumps, the one to three intact layers closest to the wound and overlain by many dead cell layers suberize. In the cuttings, suberization is usually confined to the cortical cells, again the live cell layers nearest the wound being involved. Even the covered cuttings which had xylem differentiating in the callus had some suberization taking place in the proximal live cell layer. This reduction in suberization may result from the continuous enclosure of the cuttings, whereas

the stumps and accompanying grafts were gradually exposed to atmospheric humidity. In no graft, however, was suberization of any layer of intact cells seen within the epidermis, aside from a few collenchyma cells where the epidermis and outer collenchyma had been crushed in preparing the graft.

Thus there seems to be a clear difference between patterns of cell division and differentiation in the vascular and cortical parenchyma of grafts and wounds, although the callus of wounds and that of grafts are indistinguishable at their early stages (see also Bradford and Sitton 1929), and the callus in the pith of many Coleus grafts is reminiscent of wound periderm in its array (fig. 13 in Stoddard and McCully 1980). Where a partner is present, the gap closes by somewhat random callusing with extensive hypertrophy of cells and without suberization of intervening layers, though it may be presumed that the new epidermal layer around the graft gap eventually forms a suitable protective barrier. The plant part must have recognized absence of a partner before day 4, when suberization is already well initiated, and when the division plane is commonly parallel to the living surface. At an early stage, thus, the plant must be able to discriminate between a wound closed by living plant tissue and one not so closed, which has obvious adaptive significance for the plant. It would be interesting to know just what is the stimulus informing a wounded plant that a partner is available.

There are methods other than provision of a graft partner to repress wound periderm formation. In potato tuber tissue, for example, the periderm may be replaced by a proliferation in all superficial cell layers similar to a graft callus, after careful separation of tissue with disruption of the minimum possible number of cells, followed by rinsing

away the débris, then keeping the tuber pieces in an atmosphere resembling the intercellular environment, with 100 % relative humidity and 10 % CO<sub>2</sub> content (Lange et al. 1970). Necrotic material must be present for at least four days from wounding to allow suberization of potatoes (Soliday et al. 1978). In contrast, abscisic acid alone suffices to initiate suberization in cultured potato tuber tissue (Soliday et al. 1978).

Grafting experiments show that wound periderm formation is repressible in the presence of large amounts of cell débris if a graft partner is present. A parafilm covering, and enclosure in a container, clearly provided 100 % relative humidity for the wounded Coleus stems. Amounts of CO<sub>2</sub> were presumably similar in control grafts and grafts with parafilm between the partners, which nevertheless suberize (fig. 75), and in incompatible grafts which often heal like wounds with a periderm (Proebsting 1926, 1928, Bradford and Sitton 1929, Argles 1937, Mosse 1962, Lachaud 1975). Furthermore, an apparently normal, suberized periderm completely encloses the cavity in potatoes afflicted with the physiological disorder, "hollow heart" (Dean et al. 1977). Thus simple absence of an adjacent living graft partner, all other factors being equal, is sufficient to allow wound periderm formation and suberization in Coleus. It may be that this graft partner provides a sink for abscisic acid, preventing it from reaching suberization-inducing levels.

It may be worthwhile to spend a few sentences here speculating on why plants graft. There is in nature, after all, no case wherein a plant could conceivably become completely severed and regrown together. Various steps in the grafting process are mirrored in wound healing, others in natural approach grafts. Formation of necrotic layer and callus are aspects of healing of any wound, and bridging by vascular

tissue is found in healing of deep narrow wounds (Jacobs 1952, 1954, LaMotte and Jacobs 1963, Thompson 1966). Pollination incompatibility shows a strong but not perfect correlation with grafting incompatibility (Addison and Tavares 1952, Evans and Denward 1955), and in natural subterranean root grafts connections are virtually always among members of a single species (LaRue 1934, Bormann and Graham 1959, Keane and May 1963, Bormann 1966, Graham and Bormann 1966, Wood and Bachelard 1970, Eis 1972). Cohesion is also seen in the developmental fusion between flower parts (Esau 1977). Thus the grafting process may be considered as a fortuitous combination of a number of processes which can happen naturally in various parts of a plant.

### 3. Graft hybridization, incompatibility and disease transmission.

The hope has long been held that by grafting two plants together one could somehow get a hybrid. In higher plants one may assume that ordinary uninucleate cells are killed when they are cut. If death is not instantaneous, it should surely take place in the several minutes required to get stock and scion together. Furthermore, in most grafts examined (e.g., Crafts 1934, Mendel 1936, Buck 1954a, 1954b, Fletcher 1964, Stoddard and McCully 1979) it is several days before there is cellular contact between graft partners, although Lindsay (1972) found some cohesion at day one. Even if two cells did fuse, one is left with the problem of getting a plant from the product. Certainly the tissues near the union could be cultured, but there would be little guarantee that hybrids grown from the culture were formed in the graft.

There are few exceptions to the rule of death following cutting of cells. Living syncytia, for example, must be able to repair themselves, otherwise small injuries would lead to massive losses of plant tissue.

Thus, in the siphonaceous algae, cut coenocytes may form wound plugs of highly variable composition (Bryopsis, Burr and West 1971; Caulerpa, Dawes and Goddard 1978). Wounded phloem of higher plants plugs itself with P-protein (Canny 1973, Esau 1977) but the sieve tubes are enucleate and thus could not be expected to contribute to graft hybridization.

The case for laticifers is less clear. Chemical and microscopical analyses of latices of graft partners indicate that they do not mix in the plant whether the laticifers are articulated (Hevea, Hoop and Ostendorf 1932, Taylor et al. 1951; Taraxacum, Prokofiey 1944) or non-articulated (Parthenium, Taylor et al. 1951), which would seem to indicate lack of fusion of the laticifers. Artschwager's description of the developmental anatomy of the graft unions in several Compositae (in Taylor et al. 1951) appears to neglect the problem of the non-articulated laticifers bridging the graft. Nevertheless, one is still left with the question of whether joining of laticifers across a graft line, even if it does occur, would constitute a graft hybrid simply because of the presence of two types of nuclei in the cell, which could not be expected to produce a plant in ordinary circumstances.

If cut cells are unlikely to unite and form a dikaryon, then, are there other ways for transfer of genetic information across a graft union? Michurin developed a "Mentor" hypothesis wherein he required that the scion be genetically labile when inserted into the stock, and then paradoxically become pronouncedly stable (Brabec 1965). In general, any explanation of graft results on this basis can equally well be explained, for example, by nutritional or osmotic changes brought about by different requirements for nutrients by stock and scion (Brabec 1965, Lipetz 1970). Furthermore, the changes are usually not heritable. In

the few cases where heritability has been shown, it has usually been a matter of young seedlings from self-fertilized scions having an alkaloid content indicative of a stock-scion hybrid. In a short time, however, the seedling establishes its own alkaloid production system and the foreign alkaloids laid down in seed synthesis are either destroyed or diluted into insignificance, and do not appear in later generations (Brabec 1965). If this proviso does not hold, examination of ungrafted plants shows that inherent genetic variability explains any change supposedly wrought by a mentor (Brabec 1965).

A fairly recent paper on heritable changes in Capsicum peppers purports to show transmission of fruiting characteristics from stock to scion (Ohta and Chuong 1975). Controls, however, are inadequate, as there is no attempt to examine the rate of change of fruiting characteristics in autografts or in ungrafted plants.

Grafting is frequently put to use for the detection and indexing of diseases, usually of virus origin. A leaf or some small part of the plant to be tested for disease carriage is grafted onto a susceptible indexing plant, and any development of symptoms noted, usually after a couple weeks (e.g. Schneider 1971, Frazier 1974) by which time the connections between graft partners are presumably complete. While diseases may be graft-transmissible, there is absolutely no evidence for transmissibility of disease resistance (Arroyo and Selman 1977), which would be a form of transfer of genetic information across a graft. When a stock, for example, is used to impart disease resistance to the plant, as in Phylloxera attack on Vitis vinifera, it is simply a matter of blocking entry of the parasite.

This transmissibility of viruses through grafts is responsible for

one type of graft incompatibility, which is not eliminated by the use of a length of a mutually compatible variety called an interstock. Evidently, viruses from a tolerant carrier graft partner are transmitted to a susceptible partner (Mosse 1962).

Incompatibility can be caused by a number of other factors. A particularly well studied example is the combination of pear on quince, the compatibility of which depends on the cultivars of the partners. Basing his argument partly on some very bad electron and light micrographs, Buchloh (1960, 1962) developed a model involving hydrolysis of the glycoside of pears, arbutin, to a hydroquinone which oxidizes to an inhibitor of lignification, compatibility being allowed by rapid reduction and sequestering of the oxidation product. A model with considerably more supporting evidence has since been developed, based on hydrolysis of prunasin, a graft-transmissible glycoside of quinces, by the beta-glycosidases in pear that ordinarily act on lignin precursors. The hydrolysis product of the prunasin decomposes, releasing cyanide, unless the compatible partners are able to detoxify it (Gur et al. 1968). The cyanide injures the incompatible scion's base, reducing translocation of sugars to the stock, so the cells of the stock are starved and start using the sugar of the prunasin, allowing further release of cyanide and poisoning the stock. Grafts may live for years and then break suddenly, survival time apparently depending on a wide variety of factors; for example, production of prunasin is higher in spring and fall, lower in summer and winter (Gur et al. 1968, Brian and Duron 1971).

Incompatibility in grafts may also be evidenced by a complete absence of cohesion (e.g., tomato on Pelargonium, Wright 1893), failure to establish cambial continuity following callus fusion (several com-

binations of fruit trees, Argles 1937, Brian and Duron 1971), or breakage of the cambium followed by parenchymatous proliferation, the cambia of stock and scion then running parallel to the graft and producing bark parallel to the graft line which then separates (Proebsting 1926, 1928, Bradford and Sitton 1929, Argles 1937, Mosse 1962, Lachaud 1975). This last case is an interesting example of a graft reaction converting to a wound reaction. Frequently the xylem union is evidently perfect while the phloem degenerates (Duffield and Wheat 1964, Silen and Copes 1972).

On the basis of production of "precipitins" by graft partners, Kostoff (1928) proposed an antigen-antibody mechanism for graft incompatibility, which has been rejected repeatedly since (Argles 1937, Herrero 1951, Mosse 1962, Lachaud 1975) because of absence of evidence. Recently, Yeoman et al. (1978) have speculated on a role for lectins in mediating the compatibility reaction, while admitting that there is no direct evidence supporting this idea.

Because of the complex nature of the mechanism, many communications on graft incompatibility are necessarily restricted to a detailed description of an individual incompatible combination (e.g., Amos et al. 1936). The study of incompatibility has contributed but little to the knowledge of the grafting mechanism.

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abstract no. 18582)

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## CONCLUSION

The principal conclusion of this work is that the grafting process is separable into a number of components, each under apparently independent control. These processes are cohesion; callus formation, composed of the independent sub-processes of expansion and division of cells; formation and subsequent removal of the necrotic layer; bridging of the graft by xylem, by phloem and by a cambium; and either a repression or an absence of stimulation of wound periderm formation and suberization. It is further shown that wounds and grafts, following some initial similarities, heal in different ways, with different patterns of cell division as well as differentiation established as soon as four days after wounding.