DEVELOPMENTAL BIOLOGY OF INTERCLONAL *JUNIPERUS* L. GRAFTS

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THESIS

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ABSTRACT

DEVELOPMENTAL BIOLOGY OF INTERCLONAL
JUNIPERUS L. GRAFTS

By

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Using several clones of *Juniperus* L., a series of experiments were conducted to study various aspects of self and interclonal grafts during their development.

Cytological studies produced new chromosome counts for three previously uncounted juniper clones.

An anatomical study revealed an increase in medullary ray cell size with increase in ploidy level.

In developing grafts, no basic difference was noted between clones used, only differences in the time each stage was reached. The healing sequence, beginning at 20 days, was as follows: filling of voids with callus originating outside the xylem; overwalling of injured xylem surfaces with isodiametric cells originating near the cambium; division and differentiation of overwalling cells into typical vascular and cambial cells; and cell production from the new cambial initials. Abnormally large numbers of pits and occasional "blind pits" were observed in mixed graft tissue.

Mineral distribution studies of entire graft components using ashing and photometry techniques revealed that apparent rate of translocation at the graft union was in decreasing order: potassium, calcium, and magnesium. Greatest accumulation of potassium occurred in *Juniperus chinensis* 'Hetzii' scions and lowest in scions of *Juniperus horizontalis* 'Plumosa Compacta' self grafts.

Mineral distribution, using the electron microprobe X-ray analyzer, showed translocation rates to be in decreasing order: magnesium, potassium, and calcium. Greatest blockage of calcium appeared to be at the xylem union, while potassium and magnesium moved relatively unimpeded. In phloem and cortical tissue, calcium accumulated in the scion while other elements were equally distributed between stock and scion.

Serological techniques, using fluorescein labelled antibody obtained by immunizing inbred mice with juniper leaf and stem extract, made possible the identification, in mixed graft tissue, of cells belonging to each component of interclonal grafts. Although antibody specificity was greatest in parenchymatous tissue, the greatest fluorescence occurred in the xylem of Juniperus horizontalis 'Fountain'.

Cultural studies of self and interclonal grafts revealed greatest success in 'Fountain' self grafts and those involving this clone as a component, and lowest in self grafts and those involving 'Pfitzeriana Kallay'.

Experiments in which the leaf-stem extract of one juniper was applied to cut surfaces of self grafts, at grafting, showed that graft success of 'Pfitzeriana Kallay' could be increased by application of 'Fountain' extract. When 'Pfitzeriana Kallay' extract was applied to 'Fountain' or Juniperus chinensis 'Hetzii' self grafts, success and vigor was reduced.

Cell measurements revealed distinct differences in ray cell dimensions and tracheid length among the clones. Tracheids formed subsequent to graft wounding were larger than those in uninjured tissue.

Cell counts indicated that generally greater numbers of tracheids arose from understocks in typical grafts than from scions. In self grafts greatest cell contributions occurred in graft components of 'Hetzii'. New tracheid production was delayed in 'Pfitzeriana Kallay' self grafts until after 30 days, while a shorter delay occurred in 'Fountain' self grafts. Although occurring at different periods in graft development, plateaus in new cell production were noted in 'Hetzii' self grafts.

Scion cell production increased significantly with increasing ploidy level of the understock except where 'Pftizeriana Kallay' was the scion. 'Hetzii' scions and 'Pfitzeriana Kallay' understocks produced greatest amounts of new cells in uninjured wood, while in wound tissue more cells arose from 'Pfitzeriana Kallay' scions.

Histochemical studies using Ruthenium Red staining showed pectic substances to be concentrated in the middle lamella of mature tracheids but very diffuse in undifferentiated tissue.

In addition to increasing our basic knowledge of the graft healing sequence of certain Juniperus L. clones, the present study brought to light new avenues of approach to graft union study.

DEVELOPMENTAL BIOLOGY OF INTERCLONAL *JUNIPERUS* L. GRAFTS

By

George Edward Evans

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Guidance Committee:

Sections I, II, III, and IV are segments of related thesis research information condensed into formats suited, and intended for publication in The Botanical Gazette, The American Journal of Botany, Journal of the American Society for Horticultural Science, and The Botanical Gazette, respectively.

SECTION I

Chromosome Counts In Three Cultivars of Juniperus L.

Introduction

The first cytological studies within Juniperus L. were conducted less than four decades ago (12). Considering the size of this genus, it is surprising that documented chromosome counts exist for only 11 different species.

Early studies indicated Juniperus L. to have a base chromosome number of n=11; one found to be consistant among other genera of the Cupressaceae, but differing by one chromosome from what is believed to be the original base number of n=12 for gymnosperms (12).

A compilation of documented chromosome numbers for Juniperus L. is given in Table 1. This includes the counts reported by Darlington and Wylie (2) as well as the Index to Plant Chromosome Numbers (1). All counts listed as haploid resulted from gametophyte counts; the remainder being from mitotic counts of root tips or seed endosperm.

Polyploidy is considered rare in Juniperus L., the only documented counts being those reported for Juniperus chinensis and its cultivar 'Pfitzeriana' which were considered to be autotetraploids with 2n=44 (12). Incidental to a study of tetraploidy in Sequoiadendrom giganteum, Jensen and Levan (4) reported a chromosome number (unpublished) of 2n=44 for Juniperus squamata 'Meyeri'. A single case of triploidy in juniper has been reported by Stiff in 1951 (13). This juniper was discovered among open pollinated seedlings of Juniperus virginiana and had a chromosome count of 3n=33. No postulate was advanced by the author concerning the possible pollen parent of this triploid.

Table 1. A compilation of documented chromosome counts for Juniperus L.

Species	Chromosome Count	Contributor
Juniperus bermudiana	n=11	Mehra and Khoshoo (9)
Juniperus chinensis	2n=44	Sax and Sax (12)
Juniperus communis	2n=22	Sax and Sax (12)
Juniperus communis	2n=22	Löve and Löve (8)
Juniperus communis v. montana	2n=22	J♦rgensen and S♦rensen (6)
Juniperus horizontalis	2n=22	Ross and Duncan (11)
Juniperus phoenicea	n=11	Mehra and Khoshoo (9)
Juniperus procera	2n=22	Mehra and Khoshoo (9)
Juniperus rigida	2n=22	Sax and Sax (12)
Juniperus sabina	2n=22-24	Reese (10)
Juniperus virginiana	2n=22,33	Stiff (13)
Juniperus virginiana v. scopulorum	n=11	Mehra and Khoshoo (9)

Materials and Methods

The plant material employed in this study was obtained as clonally propagated stock from a commercial nursery[1]. The cultivars chosen for study were Juniperus chinensis 'Hetzii', Juniperus horizontalis 'Plumosa Compacta', and Juniperus sabina 'Von Ehron'.

Root tips 2-3 mm. in length were collected and placed in several pretreatment solutions to determine a suitable one for arresting chromosomes at metaphase. Test solutions included 0.002 M 8-hydroxyquinoline (14) or 0.2% aqueous colchicine for 4 or 6 hours. After pretreatment the root tips were killed and fixed in modified Carnoy's (5) solution (absolute ethanol, chloroform, glacial acetic acid, 6:3:1 v/v) prior to cytological examination.

Fixed root tips were treated with 50% HCl for 10 minutes to facilitate tissue maceration. The tissue was smeared on a microslide and stained with 5% aceto-carmine and the coverslip temporarily sealed with petroleum jelly. Sufficient numbers of preparations were examined to obtain accurate chromosome counts. Photomicrographs were taken for a permanent record of the observations.

Results and Discussion

Eight-hydroxyquinoline appeared to provide the best chromosome preparations of the two pretreatments used. A 4 hour pretreatment was sufficient for Juniperus chinensis 'Hetzii' while 6 hour

[1] Monrovia Nurseries, Azusa, California

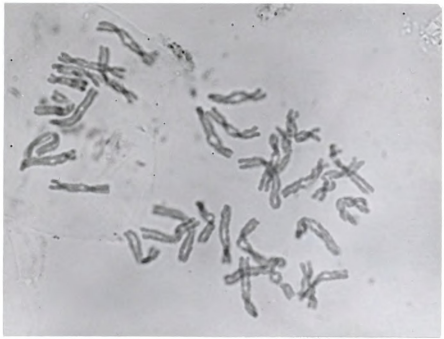

Figure 1 - Photomicrograph of the triploid (3n=33) chromosome complement of Juniperus chinensis 'Hetzii'. Root tips pretreated 4 hours with colchicine. X 1000.

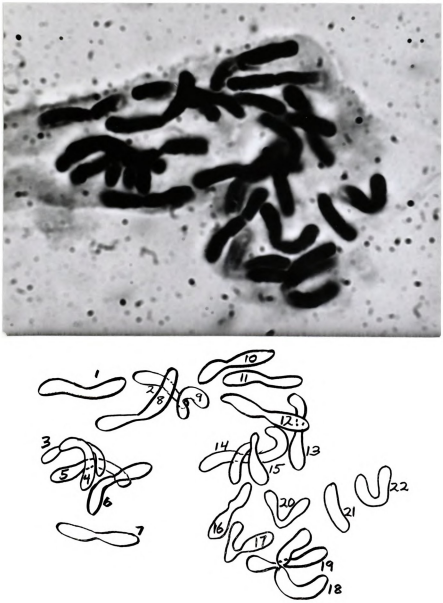

Figure 2 - Photomicrograph and interpretive drawing of the diploid (2n=22) complement of Juniperus horizontalis 'Plumosa Compacta'. Root tips pretreated 4 hours with 8-hydroxyquinoline. X 1900.

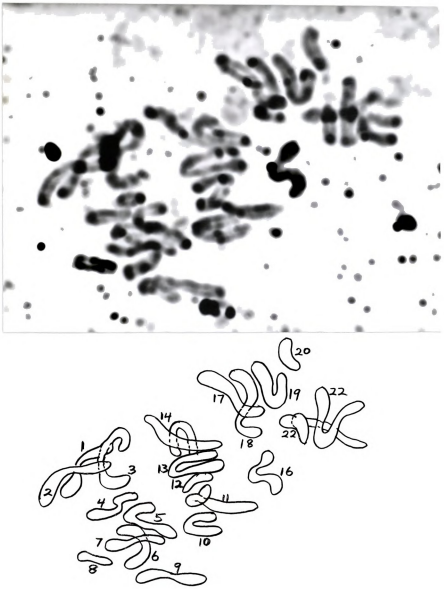

Figure 3 - Photomicrograph and interpretive drawing of the diploid (2n=22) complement of Juniperus sabina 'Von Ehron'. Root tips pretreated for 6 hours with 8-hydroxyquinoline. X 2100.

pretreatment resulted in better preparations for Juniperus horizontalis 'Plumosa Compacta' and Juniperus sabina 'Von Ehron'.

Of greatest interest was the observation of a triploid chromosome count for Juniperus chinensis 'Hetzii'. On the basis of the known tetraploid count for Juniperus chinensis (12), one would also expect the cultivar 'Hetzii' to be 2n=44. One of the 10 smears clearly showing the 3n=33 chromosome complement is presented in Figure 1.

Since this cultivar has been solely propagated by vegetative means since its discovery, the unbalanced chromosome complement has been maintained. The parentage of Juniperus chinensis 'Hetzii' is, at the present, a matter of conjecture. According to Den Ouden (3), it was obtained by Fairview Evergreen Nurseries, Fairview, Pa. in 1920. However, it was suggested by Leiss (7) that this variant seedling was obtained from the West coast, from a cross between Juniperus virginiana glauca as seed parent, and Juniperus chinensis 'Pfitzeriana' as pollen parent.

During the course of this study, a diploid count of 2n=22 was obtained for Juniperus horizontalis 'Plumosa Compacta' as shown in the photomicrograph of Figure 2. It was possible to clearly obtain the diploid count in the 15 individual cell smears found sufficiently well prepared for accurate counting. This count was in agreement with the diploid 2n=22 count reported for the species by Ross and Duncan (11).

Cytological studies of Juniperus sabina 'Von Ehron', consistantly yielded a chromosome count of 2n=22 in 12 individual cell smears found suitable for counting. The count is illustrated by the photomicrograph of Figure 3, and supports the 2n=22-24 count reported for Juniperus

sabina by Reese (10).

Summary

Somatic chromosome counts, by examination of root tip cells were established for three cultivars of the genus Juniperus L.. A triploid mitotic count of 3n=33 was obtained for Juniperus chinensis 'Hetzii'. This was unexpected since this species has a previous documented count of 2n=44 (12).

The 2n=22 obtained for Juniperus sabina 'Von Ehron' is in accord with an earlier report of 2n=22-24 for the species (10). It is quite possible that unbalanced genomes for junipers could survive since they are propagated exclusively by vegetative means.

A somatic chromosome count of 2n=22 was found for Juniperus horizontalis 'Plumosa Compacta'.

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SECTION II

Anatomical Changes in Developing Graft Unions of Juniperus L.

Until the advent of the electron microscope and radioactive tracers, most graft studies were made at the morphological or gross anatomical levels. Histological studies, however, date back to Waugh's (1904) investigation of graft union callus tissue.

Although graft callus tissue has received much study, questions concerning the relative proportions of callus produced by the scion, the point of origin of callus tissue cells and its ultimate function remain the object of controversy.

Aside from the function of wound protection, it has been suggested (Sharples and Gunnery, 1933) that callus may serve (1) as temporary nutrient transport from stock to scion, and (2) as a storage and nutrient supply for regenerating vascular tissue. The reality of the first listed function is demonstrated by Muzik's (1958) studies which showed that vanilla grafts could survive and grow for at least 2 years on parenchyma cell unions. This suggests the capability for both acropetal and basipetal transport through these cells. Braun (1961) credited callus tissue with the function of water supply to the scion until a permanent vascular bridge was formed.

Kac (1931) believed that scions produced the greater amount of callus tissue, while other workers (Kostoff, 1928; Mergen, 1955) concluded that it was of stock origin. Sharples and Gunnery (1933), on the other hand, claimed equal contributions from stock and scion. Because of its more intact nature and greater food reserves, the stock would logically be in better position to contribute to callus formation.

This would especially be true for conifers grafted with techniques in which the entire stock is left intact until healing is complete.

Although callus would be expected to develop from any parenchymatous cell near the graft union, it is generally agreed that it arises from tissue outside the cambial ring in grafts of woody perennial plants.

Apple graft callus has been described by Sass (1932) as arising from tissue external to the xylem cylinder, exclusive of the periderm. Other investigators (Sharples and Gunnery, 1933; Juliano, 1941) felt that tissue within the xylem cylinder also contributed to callus production. In cleft grafts of Nothopanax sp., Juliano (1941) found callus to be first produced in the cortex, pith and in ray cells. Medullary rays of the xylem were found to produce at least a portion of the callus pad formed on xylem surfaces exposed by stripping the bark of Hibiscus rosa-sinensis L. (Sharples and Gunnery, 1933). This suggests that callus may arise from recently produced secondary xylem and secondary phloem in the immediate vicinity of the union.

Within recent years, techniques have been perfected by which callus tissues may be dissected from various plant parts and cultured in vitro. Studies by Barker (1953), using tissue cultured from Tilia americana, showed that medullary ray cells had the capacity to form callus. This tends to support the hypothesis that the xylem may contribute callus in woody plant grafts.

White's (1964, 1967) studies with Picea glauca explants, supported the theory that callus developed from the cambium, as well as the new phloem. In old cultures, callus occasionally arose from xylem resin

duct lining, but not from medullary ray tissue.

Although some doubt exists about the origin of cambial bridges between stock and scion, certain authors (Sass, 1932; Mosse, 1962) consider new cambial cells to arise through differentiation of callus; a phenomenon also observed by Mosse and Labern (1960) in apple bud unions and by Mathes (1967) in cultured explants of maple.

A chicory scion grafted into a root piece with mismatched cambiums induced the formation, by dedifferentiation, and redifferentiation, of a cribro-vascular system which connected existing vascular systems of stock and scion (Camas, 1949). This unexplained phenomenon was also illustrated by Kuroda (1960a and 1960b) utilizing tissue culture grafts of carrot. In this case, a scion containing xylem and phloem tissue was grafted to stock tissue containing only phloem; the scion subsequently inducing formation of xylem elements in the stock. A review by Gautheret (1966) suggested that by using tissue culture procedures and adequate growth promoting substances, it should be possible for virtually any cell type, including collenchyma, schlerenchyma, and degenerative cells, to dedifferentiate.

Kostoff (1928), in studying callus, pointed to the possibility that newly formed cambial initials could arise through radial divisions of the cambial initials at the original point of incision. Kac (1931) also suggested that new cambial derivatives pushed their way through the wound callus.

To date, much has been learned about the general histology of the graft union, but many questions are unresolved as to the nature of common walls of mixed stock and scion cells. In intact homogeneous

cells, pit fields and subsequently formed pits appear to function as sites of transport between cells. In a graft union, where each cell is under different genetic control, these structures may pair at random. If so, this may explain reduced water and mineral element transport across less compatible grafts.

The pit field distribution in parenchyma cells of Avena coleoptiles, were found to remain constant in number and distribution during cell extension (Wardrop, 1955). Wilson (1957) found that in mature Elodea canadensis internodes longer than 0.5 mm. pit field behavior in the primary cell walls, was similar to that in Avena coleoptiles described by Wardrop. Applying these findings to graft union formation may be difficult since each of the heterogeneous adjoining cells would have to redistribute and pair pit fields following their formation during cell division. Unless chance matching of pit fields had occurred, translocation between cells would be greatly reduced, or limited to diffusion through membranes.

Material and Methods

The genetically stable clonal forms Juniperus horizontalis 'Fountain', Juniperus chinensis 'Hetzii', and Juniperus chinensis 'Pfitzeriana Kallay' were used in this study and are referred to hereafter by their cultivar names. Ploidy levels of these junipers are 2n, 3n, and 4n respectively (Evans, 1969).

On October 24, 1966, the above listed junipers were obtained from a commercial nursery[1/], and after suitable cold treatment, potted in

[1/] D. Hill Nursery Co., Dundee, Illinois.

4 inch clay pots and benched in a 68-70° F. night temperature greenhouse. Scion wood, from the same nursery source, was stored at 35° F. to break its rest period and assure continued dormancy. Stock and scion wood were approximately one year old from cuttings; stem diameters being 1/8 to 3/16 inches.

Between January 15 and January 17, 1967, thirty each of the following listed graft combinations were prepared using a modified side graft:

	Stock	Scion
1.	J. horizontalis 'Fountain'	J. horizontalis 'Fountain'
2.	J. chinensis 'Hetzii'	J. chinensis 'Hetzii'
3.	J. chinensis 'Pfitzeriana Kallay'	J. chinensis 'Pfitzeriana Kallay'
4.	J. chinensis 'Pfitzeriana Kallay'	J. chinensis 'Hetzii'
5.	J. chinensis 'Hetzii'	J. chinensis 'Pfitzeriana Kallay'
6.	J. horizontalis 'Fountain'	J. chinensis 'Hetzii'
7.	J. chinensis 'Hetzii'	J. horizontalis 'Fountain'
8.	J. horizontalis 'Fountain'	J. chinensis 'Pfitzeriana Kallay'
9.	J. chinensis 'Pfitzeriana Kallay'	J. horizontalis 'Fountain'

The grafted plants were arranged in a randomized complete block design incorporating 3 complete blocks; each treatment within the block being replicated 10 times. All graft unions were covered with damp sphagnum moss, and the entire bench covered with 4 mil clear polyethylene supported on a wooden frame 24 inches above the plants.

The understock plants were cut back to one-half their mass 40 days after grafting; the remainder being removed at the union 60 days

after grafting.

Progressive anatomical changes during healing were studied in stem segments containing the graft union and collected at random from the original grafted plants. Three samples of each combination were collected at 10 day intervals throughout the experiment. Upon harvest the samples were immediately frozen in polyethylene bags to await sectioning.

Graft samples were evacuated and infiltrated for one hour in a 1.5% solution of Reten[2/] using a venturi type vaccuum aspirator. Sections were cut at 10 microns on a Model CTD refrigerated microtome (cryostat)[3/] at -15°C.. Sections were picked up directly from the microtome blade using microslides which had been previously coated with ARG adhesive (Jensen, 1962) and a thin film of 0.75% Reten.

Sections were stained with safranin (15 minutes) and counter-stained with aniline blue (1 minute): a stain combination considered to be ideal for gymnosperms (Jensen, 1962).

The staining procedure was as described by Jensen (1962) but included a stepped alcohol dehydration series between safranin and aniline blue. Otherwise, poor preparations resulted when the sections were placed in clove oil-absolute alcohol-xylene solution. The cover slips were mounted with Permount[4/].

2/ A cationic, water soluble polymer from Hercules Powder Co.

3/ International Equipment Company

4/ Fisher Scientific Company

Results and Discussion

General Observations

The ray cell size in each juniper clone studied was markedly different. The tangential ray cell diameter differed considerably from clone to clone, and was positively correlated with increasing ploidy level: 'Fountain', 'Hetzii' and 'Pfitzeriana Kallay' (Evans, 1969). Differences in ray cell size in longitudinal section of a 40 day old graft of 'Fountain' (2n=22) on 'Pfitzeriana Kallay' (4n=44), arrows indicating ray cells on either side of mixed graft union tissue, are visible in Figure 1A.

Developmental Graft Histology

Typical progressive histological changes which occur at the graft union during the healing period are presented in Figure 1. Photomicrographs were prepared which illustrate how the typical graft union appeared at each of five dates following grafting. Figure 1 (A through H) demonstrates the events noted during the period of zero to sixty days after grafting. Observations of tissue production for all combinations and dates are given in Table 1, and serve to compare the interclonal grafts with respect to graft development.

Typical 10 day old grafts were characterized by a lack of activity in most combinations; the most notable exception being the well formed callus in 'Fountain' on 'Hetzii' combinations. The reciprocal of this combination and 'Hetzii' on 'Pfitzeriana Kallay' produced moderate amounts of callus tissue during the first 10 days.

Callus tissue was noted to arise rapidly from phloem and cortical parenchyma during the initial stages of healing (Figure 1B and C) , an

observation documented by other workers (Sass, 1932; Juliano, 1941; Buck, 1954).

Callus tissue in 20 day old grafts had partially filled the void between cut surfaces (at arrows, Figure 1B) with contributions coming from both flanks of the graft incision. A limited amount of new tissue had begun to form (Figure 1C, lower right) among the callus cells. Mature tissue appears to be vertically bisecting this longitudinal section. Such isolated tissue may have become separated from one of the graft partners during grafting. The greatest contribution to new xylem came from the understocks of 'Fountain' and 'Hetzii' self grafts, whereas the interclonal grafts produced only a limited number of new tracheids. Grafts involving 'Pfitzeriana Kallay' as a scion had not produced new xylem by 20 days, and only small contributions of callus from the scion had occurred at this date.

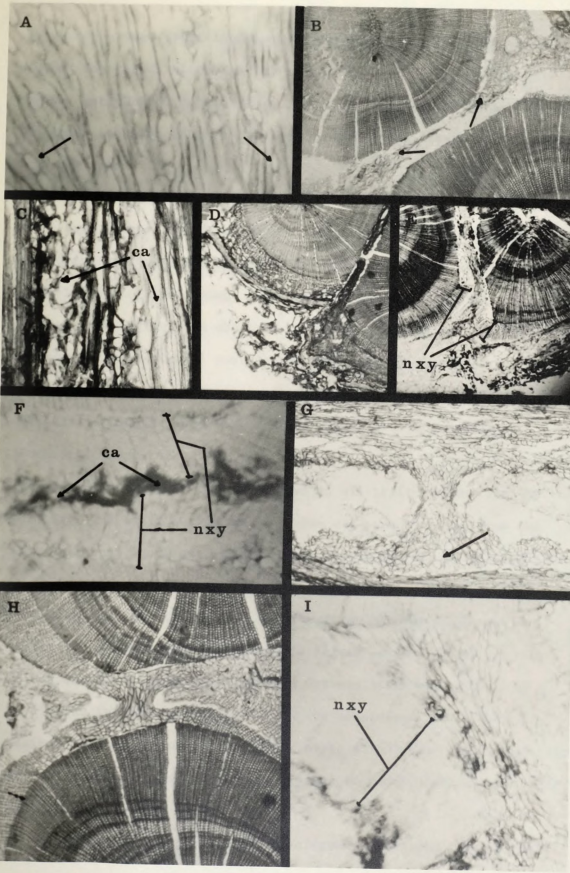
Immediately following or coincident with development of callus tissue, certain cells, hereafter referred to as new xylem, were observed to arise directly from the uninjured cambial surface. This substantiates the observations of Sharples and Gunnery (1933) on wound studies of Hibiscus rosa-sinensis, but is not in agreement with the views of other workers (Sass, 1932; Mosse, 1962). A portion of this tissue may arise directly from medullary rays as illustrated at the arrows in Figure 2A. Such an occurrence has been reported by Barker (1953) in tissue cultures of Tilia americana, and by White (1964, 1967) in explants of Picea glauca. These cells were isodiametric (Figure 1G & 2A).

By an alternating series of radial and tangential cell divisions,

Table I. Histological observations on Juniperus grafts at six dates following grafting

Scion Stock	'Fountain' 'Fountain'	'Hetzii' 'Hetzii'	'Pfitzer' 'Pfitzer'	'Fountain' 'Hetzii'	'Hetzii' 'Fountain'	'Hetzii' 'Pfitzer'	'Pfitzer' 'Hetzii'	'Fountain' 'Pfitzer'	'Pfitzer' 'Fountain'
10 Days Callus	None	None	Slight	Well Formed	Moderate	Moderate	Slight	None	None
New xylem	None	None	None	None	None	None	None	None	None
20 Days Callus	Moderate	Moderate	Slight	Moderate Well Form	Slight	Moderate	Moderate	Slight	Moderate
New xylem	Scion=+ Stock=++	Stock++	None	Stock=+ Scion=+	Stock=+	Stock=+	None	Stock=+	None
30 Days Callus	Moderate	Moderate Well Dev	None	Moderate Well Dev	Well Developed	Well Developed	Moderate	Moderate	Moderate
New xylem	Scion=+	Stock=++	None None	Stock=++	Stock=++ Scion=+	Scion=+	Stock=++ Scion=+	Stock=++	None
40 Days Callus	Well Dev.	Well Dev.	Mod. To Well Dev	Well Dev.	Well Dev.	Very Well Dev.	Well Dev.	Very Well Dev.	Well Dev.
New xylem	Stock=++	Stock ++	Stock=+ Scion=+	Stock=++ Scion=+	Stock=+ Scion=++	Stock=++ Scion=+	Stock+++ Scion+++	Stock ++ Scion +	Stock++to +++ Scion +
50 Days Callus	Very Well Dev	Very Well Dev	Well Dev.	Very Well Dev	Well Dev.	Very Well Dev	Well Dev.	Very Well Dev	Moderately Well Dev
New xylem	Stock+++ Scion++	Stock++ Scion++	Low vas- cular Act	Stock++ Scion+	Stock++ Scion+++	Scion+++ Stock++	Stock+++ Scion+	Scion+ Stock++++	Stock+ Scion++
60 Days Callus	Very Well Dev	Very Well Dev	Well Dev.	Very Well Dev	Very Well Dev	Very Well Dev	Moderate Well Dev	Very Well Dev	Very Well Dev
New xylem	Scion++ Stock+++	Scion+++ Stock ++	Stock++ Scion+++	Stock+++ Scion++	Scion+++ Stock++	Stock+++ Scion ++	Scion+++ Stock++	Stock+++ Scion++	Stock+++ Scion++

Figure 1 - Progressive histological change of juniper grafts during the period zero to 60 days after grafting. (A) 'Fountain'/'Pfitzeriana Kallay', 40 days old, longitudinal section (X 413). Mixed new vascular elements illustrating distinguishable difference in ray cell size between clones. (B) 'Fountain'/'Fountain', cross-section, 20 days old (X 41). Beginning callus formation (at arrows) from phloem and cortex. (C) 'Fountain'/'Fountain', longitudinal section, 20 days old (X 100). New vascular elements forming at mature xylem-callus junction (lower right). (D) 'Pfitzeriana Kallay'/'Fountain', cross-section, 30 days old (X 30). Callus tissue continuing to fill voids. (E) 'Fountain'/'Pfitzeriana Kallay' 40 days old, cross-section (X 25). New vascular tissue (nxy) forming from stock and scion. Largest increment from stock (right). (F) 'Fountain'/'Hetzii', 50 days old, cross-section (X 165). Contribution to new xylem (nxy) from stock and scion. Callus (ca) crushed by advancing vascular elements. (G) 'Fountain'/'Fountain', 40 days old, longitudinal section (X 83). Typical view of vascular bridge with undifferentiated isodiametric cells (arrow). (H) 'Fountain'/'Fountain',60 days old, cross-section (X 35). Typical view of vascular bridge at later stage of development. (I) 'Hetzii'/'Pfitzeriana Kallay', 60 days old, cross-section (X 165). Cambial bridging of union and subsequent formation of normally oriented new xylem (nxy).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the wound between graft incisions became filled by new xylem which crushed the callus tissue in its path (Figure 2B). Production of such cambial derivitives continued by tangential division from each end of the graft incision (Figure 2C), until all injured tissue was overlain with new xylem.

Callus tissue had nearly filled all voids between graft partners at 30 days except in 'Pfitzeriana Kallay' self grafts (Table 1). Thirty day grafts were more sound and maintained their structural integrity during sectioning. Graft incisions were, however, still well defined, not having been obscured by newly formed xylem. At this date most new xylem was still contributed primarily by the stock but the scions showed increased cell production. No xylem production was noted for 'Pfitzeriana Kallay' self grafts or grafts of this clone on 'Fountain' understocks.

Callus tissue was well developed in all graft combinations by the 40th day, and contribution to new xylem was predominately from the stock (greater increment, Figure 1E). Callus and new xylem production in 'Pfitzeriana Kallay' self grafts and in 'Pfitzeriana Kallay' on 'Fountain' grafts was observed for the first time at 40 days. An example of such tissue can be seen in Figure 1E extending from the flanks of the incision into the void between stock and scion. As a result the intervening callus tissue was compressed into a darkly stained mass of tissue (Figure 1F). Mosse (1962) also reported darkly stained crushed cells between the tissues of stock and scion of fruit bud unions.

At this point juniper graft development resembled closely that

found in wound healing of woody plants, especially in poorly matched grafts. Bradford and Sitton (1929) drew a parallel between graft healing and wound healing in such mismatched grafts. In closely fitted grafts, production of new xylem between graft partners was considerably reduced.

At 50 days the contribution to new xylem tissue was balanced between stock and scion (Table 1). The tendency for 'Pftizeriana Kallay' self grafts to produce limited xylem, was still evident. Concomitantly grafts involving this clone as a scion, heretofore lagging in xylem production, produced moderate amounts.

At points along the graft incision where the stock and scion cambia were close newly formed tissue became mixed to form "bridges" (Figure 1G and 1H). Cambial initials within vascular bridges began by tangential cell division to form normally oriented tracheids. The mixed tissue lost the isodiametric shape of earlier formed cells and assumed the typical linear form of tracheids (Figure 1A). The appearance of the new xylem (nxy) is shown in Figure 1I.

Graft Union Tissue Structure

Concurrent with developmental sequence studies, were detailed observations of mixed stock and scion tissue in the graft union. Although this study neared the limits of resolution for the light microscope, some revealing observations were made.

The common walls of mixed tissue tended to remain un-modified. Walls of these heterogeneous cells appeared to have normal secondary wall thickenings and middle lamellae, resulting in a four layered structure (arrows, Figure 2E and F).

Heavy pitting occurred in mixed stock and scion cells at the point of union; a characteristic noted in both self and interclonal grafts (Figure 3A). At higher magnifications, heavy pitting was observed in the early formed cambial derivitives (Figure 3B) as well as in later formed graft union tissue (Figure 3C). These observations were contradictory to those of Preston (1955) who reported pits lacking or difficult to observe. Such a lack of pitting might only be expected in parenchymatous cells containing only pit fields; structures less easily observed.

The significance of heavy pitting at the graft union is a matter of conjecture, but it seems plausible that it may be an adaptive mechanism by which the grafted plant may overcome the reported problem of restricted flow through the graft union (Roach, 1931; Colwell, 1942; Gur & Samish, 1965). If pits remain constant in number and distribution during cell extension (Wardrop, 1955), those produced independently by stock and scion but in association with one another would be expected to align their pit pairs only at random. Such random alignment would reduce efficiency of translocation. Therefore, an increase in pit numbers would increase the probability of pit pair alignment.

Less noticeable were the presence of what appeared to be blind pits which were believed associated with common walls of stock and scion (Figure 3D). By reason of random pit alignment, the occurrence of pits permeating the wall of one graft partner and terminating against a non-pitted site on the wall of the adjoining cell would be expected.

Figure 2 - Fine structure and origin of graft union tissues. (A) 'Fountain'/'Hetzii', 20 days old, cross-section (X 400). Proliferation of medullary ray cells of cambium and at the graft incision (gi) Cells arising from medullary rays shown at arrows. (B) 'Fountain'/'Pfitzeriana Kallay', 40 days old, cross-section (X 413). New vascular derivatives coursing over injured xylem surface at graft incision (gi). (C) 'Fountain'/'Fountain', 40 days old, cross-section (X 100). New vascular tissue over-walling graft incision. Advanced stage of illustration B. (D) 'Fountain'/'Pfitzeriana Kallay', 50 days old, longitudinal section (X 1000). Detail of common cell walls of stock and scion. Arrows showing paired, double secondary wall structures of mixed graft tissue. (E) 'Fountain'/'Hetzii', 60 days old, longitudinal section (X 1000). Paired wall structure of adjoining stock and scion ray and tracheid cells. Void between paired wall structure indicated by arrows. (F) 'Fountain'/'Pfitzeriana Kallay', 60 days old, cross-section (X 1000). Common walls of mixed new tracheids. Point of cell contact at arrows.

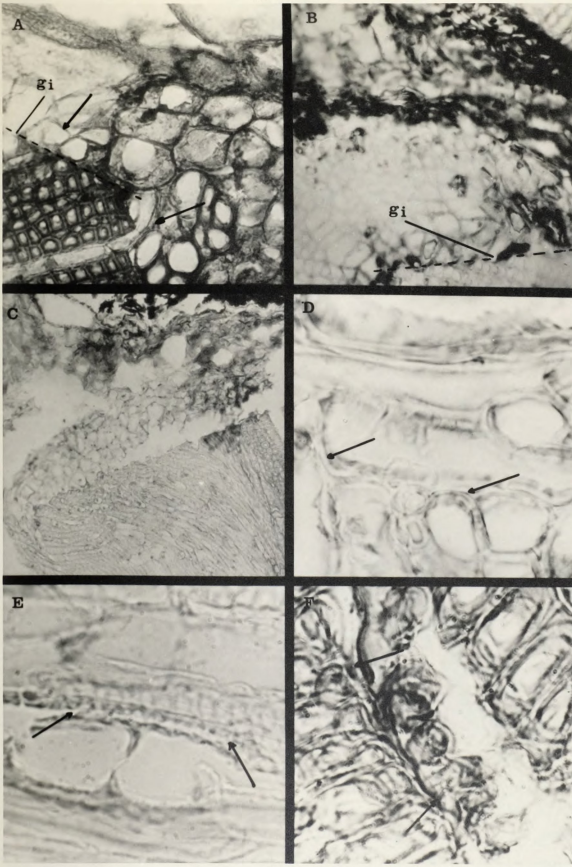
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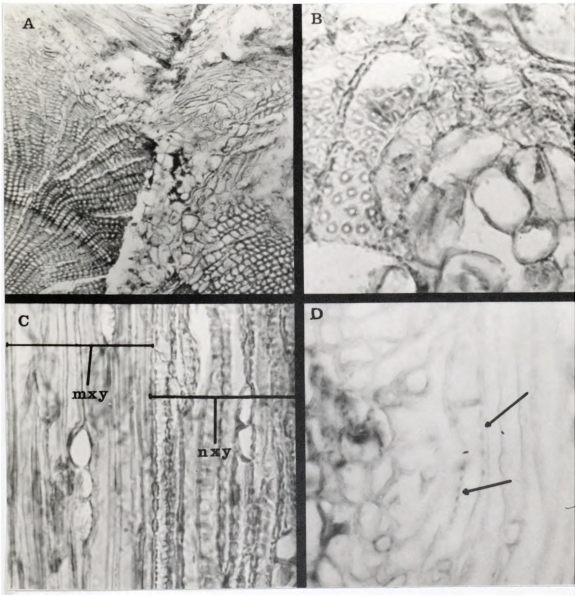
Figure 3 - Pitting behavior among mixed vascular cells of stock and scion. (A) 'Fountain'/'Hetzii', 40 days old, longitudinal section (X 100). (B) 'Fountain'/'Fountain', 50 days old, longitudinal section (X 400). Pitting in partially differentiated, early formed cells at the graft union. (C) 'Fountain'/'Fountain', 50 days old, longitudinal section (X 400). Comparison of pitting in mature xylem (mxy) and new, recently differentiated xylem (nxy) at the graft union. (D) 'Fountain'/'Pfitzeriana Kallay', 60 days old, longitudinal section (X 825). Occurrence of "blind pits" in common walls of new stock and scion tracheids.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Summary

Anatomical and graft healing sequence studies of three clonal junipers differing in chromosome number (Juniperus horizontalis 'Fountain', 2n=22; Juniperus chinensis 'Hetzii', 3n=33; and Juniperus chinensis 'Pfitzeriana Kallay', 4n=44) revealed differing basic anatomy and established the typical structural changes occurring during initial stages of graft union development. Also of interest were observations of pitting behavior at the graft union.

Anatomical study of the three junipers revealed visible differences in tangential medullary ray cell diameter; this dimension increasing with rising ploidy level.

Interclonal grafts were studied histologically at 10 day intervals for 60 days from time of grafting to determine possible differences in their behavior during healing. Although there were no basic differences in the developmental sequence during this period, the rates at which these stages appeared was variable among graft combinations.

Between the 10th and 20th day following grafting, voids between stock and scion began to fill with callus. This was first observed in grafts involving 'Fountain' and last in those containing 'Pfitzeriana Kallay' as a component.

Isodiametric cells from uninjured cambia appeared by the 20th day except in grafts with 'Pfitzeriana Kallay' as the scion. These cells by a series of tangential, radial, then tangential divisions over-walled the injured surfaces. Interclonal grafts with 'Pfitzeriana'

Kallay' as a scion did not produce this tissue until 30 days.

Overwalling of the graft incision was most pronounced in unions not in close contact; the process resembled surface wound healing. Overwalling did not occur in closely matched grafts.

Between 40 and 50 days, the graft union, by radial divisions of the overwalling cells, began filling the area between graft partners with well organized tissue while crushing existing callus. In contrast to previously formed tissue, these cells were produced radially with respect to the graft component from which it arose.

During the 50 to 60 day period a mixing of tissue occurred at points opposite the cambial rings of stock and scion. These cells lost their isodiametric shape and assumed the elongated form of tracheids. Subsequent to this "cambial bridging", new xylem formed in normal centrifugal direction with relation to the total graft union.

Newly formed tissue in a typical graft arose principally from the stock prior to the 50th day following grafting. After 60 days, contribution of new tissue was nearly equal from stock and scion.

Within mixed graft tissue the walls of adjoining stock and scion cells were largely un-modified. Typical cell pairs appeared as paired double wall structures, each with middle lamellas and secondary wall thickenings. Abnormally large numbers of pits were observed in graft union tissues; a condition suggested to be an adaptive mechanism by which reduced translocation could be corrected through increased probability of random pit pairing. "Blind pits" were occasionally observed and appeared to be the result of un-aligned pit pairs of adjacent stock and scion cells.

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SECTION III

Distribution of Calcium, Potassium, and Magnesium in Interclonal Grafts of Juniperus L.

Since before the turn of the century investigations have been aimed at the mode of mineral translocation in intact plant tissues (Clements and Engard, 1938; Phillis and Mason, 1940; Stout and Hoagland, 1939). In grafted tissue, however, this is primarily a matter of speculation.

First reference made to the relationship of grafting to mineral transport came when plant physiologists were proposing that water and minerals moved freely through the plant in a semi-closed system. Hales (1769) argued that, "The instance of the ilex upon the English oak, seems to afford a very considerable argument against circulation; for, if there were a free uniform circulation of the sap through the oak and ilex, why should the leaves of the oak fall in winter, and not those of the ilex".

Near the turn of the century, it had been generally accepted that circulation in plant tissues occurred through two distinctly different tissue systems, the xylem and phloem; the former being primarily responsible for water and mineral transport in at least an acropetal direction and the latter for basipetal transport of photosynthates and minerals. Early evidence for water and mineral translocation through the xylem resulted from ringing experiments with woody perennial plants (Malpighi, 1675 and 1679; Clements and Engard, 1938; Phillis and Mason, 1940). In these studies, all tissue exterior to the xylem was removed and the subsequent response observed. No water stress was observable in plants thus treated until the xylem was also severed.

Radioactive isotope work by Stout and Hoagland (1939) illustrated that element movement occurred primarily in the xylem but that comparatively free lateral movement between xylem and phloem also occurred.

Harvey (1931) demonstrated that not only did water move in the xylem, but that it was limited to the newly differentiated vessel elements. It was, therefore, concluded that scions could not directly utilize old xylem elements.

The relationship of water movement through graft unions and ultimate compatibility have, for years, been the object of study. Some investigators considered restricted water movement across the graft union to be one of a limited number of prime symptoms of incompatibility (Chang, 1937; Randahawa & Upshall, 1949; Evans & Hilton, 1955; Evans & Hilton, 1957; Braun, 1961). Brown (1961) described the scion as experiencing two periods in its water regime; 1) utilization of water present in the scion and 2) utilization of water traversing the union from the stock. Evans and Hilton (1957) found significant differences in rate of water flow to scions grafted on different apple varieties.

Most translocation studies have been done with radioactive phosphorus. In studies using ringed squash plants, Colwell (1942) demonstrated that basipetally translocated phosphorus accumulated above the deleted bark ring, in both phloem and xylem, and thereby concluded that lateral translocation of phosphorus occurred. Dickenson and Samuels (1956) also demonstrated downward blockage of phosphorus in apple graft phloem.

Daniel and Thomas (1902) demonstrated that the rootstocks of intervarietal bean grafts absorbed less than intact plants of either

variety. Roach (1931), using Lanes' Prince Albert apple budded on dwarfing (EM IX) versus non-dwarfing (EM XII) understocks, found that only molybdenum and lead of the twenty-one elements analyzed were not transported through the union. Bukovac, Wittwer, and Tukey (1958) found ^{45}Ca and ^{32}P to be translocated through apple grafts, with no accumulation at the union. Similarly they found no blockage to occur in well made tomato self-grafts (1957). These data indicate that most elements are potentially translocatable through graft unions.

Zinc was found by Gur and Samish (1965) to accumulate at the union of 10 year old pear on quince rootstocks. This resulted from the girdling effect of uncongenial unions.

Present evidence suggests that uptake and translocation of minerals may be controlled to some extent by the understock used (Warne and Wallace, 1935). The calcium content in leaves of apple cultivars grafted on Hibernal was higher than when grafted on French crab rootstock (Sistrunk & Campbell, 1966). Berry (1939) suggested that a close correlation may exist between understock vigor and mineral uptake.

Mason and Maskell (1931) found calcium and phosphorus to accumulate in significant amounts above the bark ring in cotton plants, whereas potassium was not found to accumulate.

The objective of the present study was to:

a. Study calcium, potassium and magnesium distribution at, and in both components of self grafted plants.
b. Study distribution of these minerals in graft components of interclonal grafts.
c. Study changes in balance of these elements in self and interclonal grafts as affected by time after grafting.

Materials and Methods

Experiment I:

Vegetatively propagated clones[1/] of Juniperus horizontalis 'Plumosa Compacta', Juniperus chinensis 'Hetzii', and Juniperus sabina 'Von Ehron', were chosen as biological material for this study. The plants were approximately one year from cuttings, and 1/8 to 1/4 inch in stem diameter. Scion wood of equivalent age and from the same plants as the understocks, was held at 35 C until time of grafting.

Between May 24 and May 28, 1966, the junipers were grafted, using a modified side-veneer graft (Hartmann and Kester, 1968) in nine combinations:

	Scion	Stock
1.	J. chinensis 'Hetzii'	J. chinensis 'Hetzii'
2.	J. chinensis 'Hetzii'	J. sabina 'Von Ehron'
3.	J. chinensis 'Hetzii'	J. horizontalis 'Plumosa Compacta'
4.	J. sabina 'Von Ehron'	J. chinensis 'Hetzii'
5.	J. horizontalis 'Plumosa Compacta'	J. chinensis 'Hetzii'
6.	J. sabina 'Von Ehron'	J. sabina 'Von Ehron'
7.	J. horizontalis 'Plumosa Compacta'	J. sabina 'Von Ehron'
8.	J. horizontalis 'Plumosa Compacta'	J. horizontalis 'Plumosa Compacta'
9.	J. sabina 'Von Ehron'	J. horizontalis 'Plumosa Compacta'

All graft unions were tied with 4 inch rubber grafting strips, packed with moist sphagnum moss, and cultured under a 4 mil polyethylene canopy 24 inches above the plants during the period of investigation.

[1/] Monrovia Nursery Co., Azuza, California

One-half of the understock plant was removed 6 weeks following grafting and the remainder removed 3 weeks later.

Ten collections of 3 samples of each combination were made at 5 day intervals starting June 2, 1966. Stocks and scions were harvested separately, oven dried, and analyzed for calcium, potassium, and magnesium using standard dry ashing and photometry tests (Greweling, 1960). Results are expressed as stock-scion ratios based on each elements' percentage by weight of sample tested.

Experiment II:

Plant materials for this phase of the investigation included Juniperus horizontalis 'Fountain', Juniperus chinensis 'Hetzii', and Juniperus chinensis 'Pfitzeriana Kallay'. The plants were one year old, clonally propagated[2/], had stem diameters of 3/32 inch to 1/8 inch. Scion wood in clonal form and the same age as the understocks upon arrival were held at 35 C until grafting. Using grafting and culture techniques like those of Experiment I, the following graft combinations were made between January 14 and January 16, 1967:

	Scion	Stock
1.	J. chinensis 'Hetzii'	J. chinensis 'Hetzii'
2.	J. horizontalis 'Fountain'	J. chinensis 'Hetzii'
3.	J. chinensis 'Pfitzeriana Kallay'	J. chinensis 'Hetzii'
4.	J. chinensis 'Pfitzeriana Kallay'	J. chinensis 'Pfitzeriana Kallay'
5.	J. chinensis 'Hetzii'	J. chinensis 'Pfitzeriana Kallay'
6.	J. horizontalis 'Fountain'	J. chinensis 'Pfitzeriana Kallay'

[2/] Provided by D. Hill Nursery Co., Dundee, Illinois

7. J. horizontalis 'Fountain' J. horizontalis 'Fountain'

8. J. chinensis 'Hetzii' J. horizontalis 'Fountain'

9. J. chinensis 'Pfitzeriana Kallay' J. horizontalis 'Fountain'

At six 10 day intervals, following grafting, three 3/4 inch stem segments containing the graft union were collected for each combination, and immediately frozen in polyethylene bags.

Prior to histochemical study, the tissue was thawed and infiltrated with Tissue-Tec[3] embedding medium under vacuum in a venturi type aspirator. The infiltrated material was mounted and frozen in the same media, sectioned longitudinally and transversely at 18 microns using a refrigerated microtome (cryostat). Serial sections were affixed to highly polished pure aluminum discs (1/8 inch thick by 1 1/4 inch diameter) for subsequent analysis. The mounting medium served as an adhesive.

The samples were analyzed for calcium, potassium and magnesium content using an ARL[4] electron microprobe X-ray analyzer, Model EMX-SM. This instrument combines the advantages of a scanning electron microscope with X-ray spectrographic analysis. Instrument conditions were 14.5 KV accelerating voltage and 0.026 microamperes sample current resulting in an electron beam diameter of approximately 0.5 microns. Approximate penetration of the electron beam into the material was 15 microns.

Microprobe analyses of the elements in question were made using line scans across the sample. The X-rays (K^{α} radiations) with characteristic wave lengths for each element (Calcium, 3.359 Å; potassium,

[3] Ames Company, Elkhart, Indiana
[4] Applied Research Laboratories

3.742 Å; magnesium, 9.889 Å) were displayed and photographed from a cathode ray tube (CRT) or recorded on an X,Y recorder.

Oscillograms (photographs) were prepared from images produced on the CRT screen by secondary electron detection. The oscillograms, recorded on Polaroid film, delineated the exact area being examined as well as the position of the line scan on the sample. In all cases, magnification was 250 diameters (40 microns per centimeter). Direct comparisons of the ocsillograms of the tissue with the curves from the X,Y recorder resulted in precise element localization.

Semi-quantitative information from the line scan data was obtained by determining the area beneath the curves with the aid of a planimeter. Comparison of the appropriate oscillogram and its corresponding sample allowed categorization of each curve with respect to the tissue through which the analysis was made, thus making it possible to semi-quantitate the mineral level for each tissue, for each date, and for each graft combination. The quantity of each tissue was not constant from sample to sample; therefore, all planimeter values were converted to counts per centimeter of sample traversed by the line scan.

Results and Discussion

Experiment 1:

Results of plant ashing studies, expressed as stock-scion ratios, are presented in Tables 1, 2 and 3. Statistical analysis of the data in Table 1 (Appendix Table B1) for calcium indicated no significant differences among dates or interactions between dates and graft

combinations. Combinations were, however, significant at the 1 percent level.

The mean stock-scion ratio for calcium (Table 1) fell between those for the other two elements. Although Bukovac et al. (1958) did not observe blockage of Ca^{45} in apple grafts, differences in methods of analysis and plant taxa may explain lack of correlation of their results with those obtained in this study.

Examination of the means for graft combinations (Table 1) revealed that calcium accumulated primarily in understocks except when Juniperus chinensis 'Hetzii' was used as a scion. Self grafts of this clone accumulated significantly greater amounts than the other self grafts which contained statistically equivalent amounts. The observed consistent calcium concentration in the graft partner containing this clone lead to the assumption that either 1) its calcium requirements exceed that of the other clones or 2) it tends toward "luxury consumption".

Lack of significant differences between values for each clone self grafted and grafted on other clones suggested that calcium translocation was not influenced by the stock used.

Statistical analysis of the potassium data of Table 2 (Appendix Table B1) indicated greater stock accumulation than was observed for magnesium and calcium. Similar to calcium, the ratios were not significantly different for dates, or dates times combination interaction, whereas, graft combinations were significantly different at the 1 percent level.

Contrary to the calcium data 'Plumosa Compacta' self grafts accumulated significantly less potassium in the understocks than did

Table 1. Stock-scion ratios for calcium content of juniper graft combinations sampled at regular intervals after grafting.*

COMBINATION	DATES 6/3	6/7	6/12	6/17	6/22	6/27	7/2	7/7	7/12	7/17	MEANS	DMR(5%)
'Hetzii'/ 'Von Ehron	1.51	.96	.97	.96	.83	1.03	1.25	.63	.77	.91	.98	c d
'Von Ehron'/ 'Hetzii'	1.46	1.43	.82	.92	1.40	1.15	1.14	1.43	1.12	.93	1.18	b c
'Plumosa'/ 'Von Ehron'	.93	1.61	1.09	1.46	1.26	1.46	1.39	1.37	.90	3.41	1.49	a
'Von Ehron'/ 'Plumosa'	1.04	1.37	1.33	.90	1.15	1.26	1.31	1.70	1.64	1.17	1.29	b
'Hetzii'/ 'Hetzii'	.69	.76	.84	.72	.86	.86	.88	.50	.90	.92	.79	d
'Plumosa'/ 'Hetzii'	1.28	1.57	1.28	1.30	1.13	1.79	1.16	.91	1.66	1.05	1.31	a b
'Hetzii'/ 'Plumosa'	.94	1.00	.90	1.12	.83	.75	.97	1.01	.54	.99	.91	d
'Plumosa'/ 'Plumosa'	1.15	1.36	1.76	1.10	1.43	1.49	.97	1.98	.85	1.50	1.36	a b
'Von Ehron'/ 'Von Ehron'	1.56	1.45	.62	1.27	1.25	1.11	1.43	1.31	1.42	.92	1.23	b c
MEANS	1.17	1.28	1.07	1.08	1.13	1.21	1.17	1.20	1.09	1.31	1.17	

*Note: Analysis of variance based upon arcsin transformed data; not table values. Means followed by identical letters not significantly different at the 1% level.

Table 2. Stock-scion ratios for potassium content of juniper graft combinations sampled at regular intervals after grafting.*

COMBINATION	DATES 6/3	6/7	6/12	6/17	6/22	6/27	7/2	7/7	7/12	7/17	MEANS	DMR(5%)
'Hetzii'/ 'Von Ehron'	1.10	.93	1.14	1.06	1.11	1.48	1.15	1.16	1.01	1.39	1.15	b c
'Von Ehron'/ 'Hetzii'	1.02	1.40	1.33	1.45	1.27	1.40	1.80	1.30	2.35	1.25	1.46	a b
'Plumosa'/ 'Von Ehron'	1.02	1.24	1.07	1.25	1.55	1.31	1.07	.98	1.17	.81	1.15	b c
'Von Ehron'/ 'Plumosa'	1.45	1.17	1.30	1.07	1.40	1.02	.75	1.77	1.45	1.83	1.32	b c
'Hetzii'/ 'Hetzii'	1.40	.98	1.12	1.59	1.15	1.72	1.68	1.02	1.56	1.78	1.40	a b
'Plumosa'/ 'Hetzii'	1.09	1.01	1.20	1.26	1.38	1.85	.96	1.75	1.22	1.16	1.29	b c
'Hetzii'/ 'Plumosa'	1.57	.98	.96	1.19	.97	2.09	.92	1.09	1.29	.83	1.19	b c
'Plumosa'/ 'Plumosa'	.97	1.09	1.04	.81	.98	1.36	1.83	1.01	.98	1.34	1.14	c
'Von Ehron'/ 'Von Ehron'	1.51	1.69	1.58	1.39	2.01	2.00	1.24	.95	1.17	2.70	1.62	a
MEANS	1.24	1.17	1.19	1.23	1.31	1.58	1.27	1.23	1.36	1.45	1.30	

*Note: Analysis of variance based upon arcsin transformed data; not table values. Means followed by identical letters not significantly different at the 1% level.

Table 3. Stock-scion ratios for magnesium content of juniper graft combinations sampled at regular intervals after grafting.*

COMBINATION	DATES 6/3	6/7	6/12	6/17	6/22	6/27	7/2	7/7	7/12	7/17	MEANS	DMR(5%)
'Hetzii'/ 'Von Ehron'	2.05	.78	1.52	1.57	1.57	1.28	1.48	1.15	1.25	1.29	1.39	a b
'Von Ehron'/ 'Hetzii'	.53	.56	.26	.60	.49	.52	.64	.71	.45	.47	.52	
'Plumosa'/ 'Von Ehron'	.63	.76	.71	.83	.83	.98	.75	.77	.48	1.49	.82	d e
'Von Ehron'/ 'Plumosa'	1.07	.86	.74	.82	.64	.98	.76	.85	.79	.78	.83	d e
'Hetzii'/ 'Hetzii'	.97	.94	1.04	1.43	.84	.83	.68	.77	1.11	1.61	1.02	c d e
'Plumosa'/ 'Hetzii'	.65	.72	.48	.85	.58	1.69	.75	.82	.86	.62	.80	e
'Hetzii'/ 'Plumosa'	1.82	.83	1.73	2.39	1.24	2.70	1.05	1.14	1.16	1.26	1.53	a
'Plumosa'/ 'Plumosa'	1.21	.83	1.31	1.11	1.19	1.47	.82	1.54	.87	1.33	1.17	b c
'Von Ehron'/ 'Von Ehron'	.82	.83	.76	.93	.92	1.33	.72	.74	.85	3.83	1.17	c d
MEANS	1.08	.79	.95	1.17	.92	1.31	.85	.94	.87	1.41	1.03	
DMR(5%)	a b c	c	b c	a b c	b c	a b	b c	b c	b c	a		

'Von Ehron and Hetzii'. Like calcium, potassium translocation was not significantly affected by understock used. Unlike calcium, all combinations accumulated potassium in the understock.

The mean of magnesium for all combinations (Table 3) was close to 1:00, suggesting that its movement through the union was relatively unimpeded. Four of the nine combinations accumulated magnesium in the scion; all others contained higher amounts in the rootstock. All self grafts distributed magnesium in similar fashion, having stock-scion ratios close to 1:00. Combinations involving 'Hetzii' as a scion had higher amounts of magnesium in rootstocks than other heterografts which tended to accumulate magnesium in the scion. 'Von Ehron' as a scion on 'Hetzii' rootstocks contained the highest concentration of magnesium when compared to other combinations. This constituted the lowest observed stock-scion ratio for all combinations and elements (0.52).

Examination of significant magnesium stock-scion means across dates revealed that 12 day grafts had more magnesium in the scion than at any other date. This was the only date that all graft combinations contained higher amounts of magnesium in scions than in stocks. This observation could be either a measure of rootstock depletion or the first date at which magnesium traversed the graft union.

A significant interaction between graft combinations and dates indicated that magnesium distribution varied among graft combinations at a given date. Greatest deviations occured in 'Hetzii' on 'Plumosa Compacta' grafts during the 1st, 3rd, 4th and 6th harvest dates when there was a high accumulation in the understock.

In certain interclonal combinations and their reciprocals magnesium concentration appeared to be associated with only one partner of the graft. This is evident with grafts of 'Hetzii' on 'Von Ehron' and, with exception of two dates, 'Plumosa Compacta' on 'Hetzii' (Table 3). 'Hetzii', being common to these combinations, may suggest a magnesium balance which is influenced by the nutritional status of this clone.

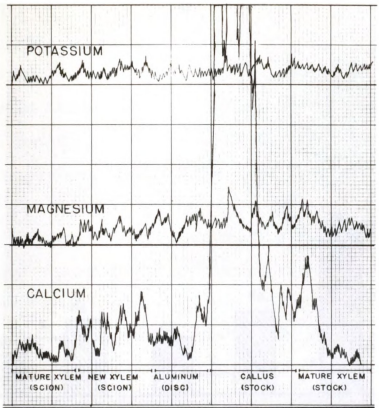
Comparison of grand means revealed that potassium and calcium accumulated in the understock, while magnesium appeared evenly distributed. The relatively slow mobility of calcium was also demonstrated by Biddulph et al. (1958) in studies of translocation in bean grafts where calcium was shown to be less mobile than sulphur or phosphorus.

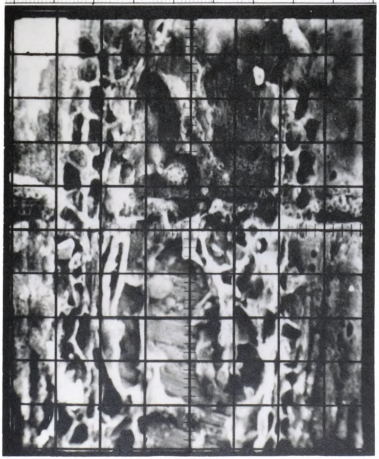
Experiment 2:

Data obtained from electron microprobe X-ray analysis for calcium, potassium and magnesium distribution was accumulated graphically and converted to numerical values based upon the area underneath the curves. Figure 1 illustrates a typical curve and oscillogram delineating the tissue through which the analysis was run. These data, although incomplete, provide certain significant information. Whereas, analytical techniques for Experiment 1 allowed for comparisons of mineral distribution between graft components, microprobe analysis made possible detection and distribution of minerals within individual tissues of the graft union.

If mineral content of the xylem is taken to represent transitory materials, stock accumulation (Figure 2) indicates that calcium passes least readily through the graft union. Potassium and magnesium appeared to move with relative ease as indicated by generally equal

Figure 1. Line profile analysis of a juniper graft union showing A) distribution of K. Mg and Ca; and B) secondary electron oscillogram with line scan from 1 to 2. Magnification X500.


POTASSIUM
MAGNESIUM
CALCIUM
MATURE XYLEM (SCION)
NEW XYLEM (SCION)
ALUMINUM (DISC)
CALLUS (STOCK)
MATURE XYLEM (STOCK)



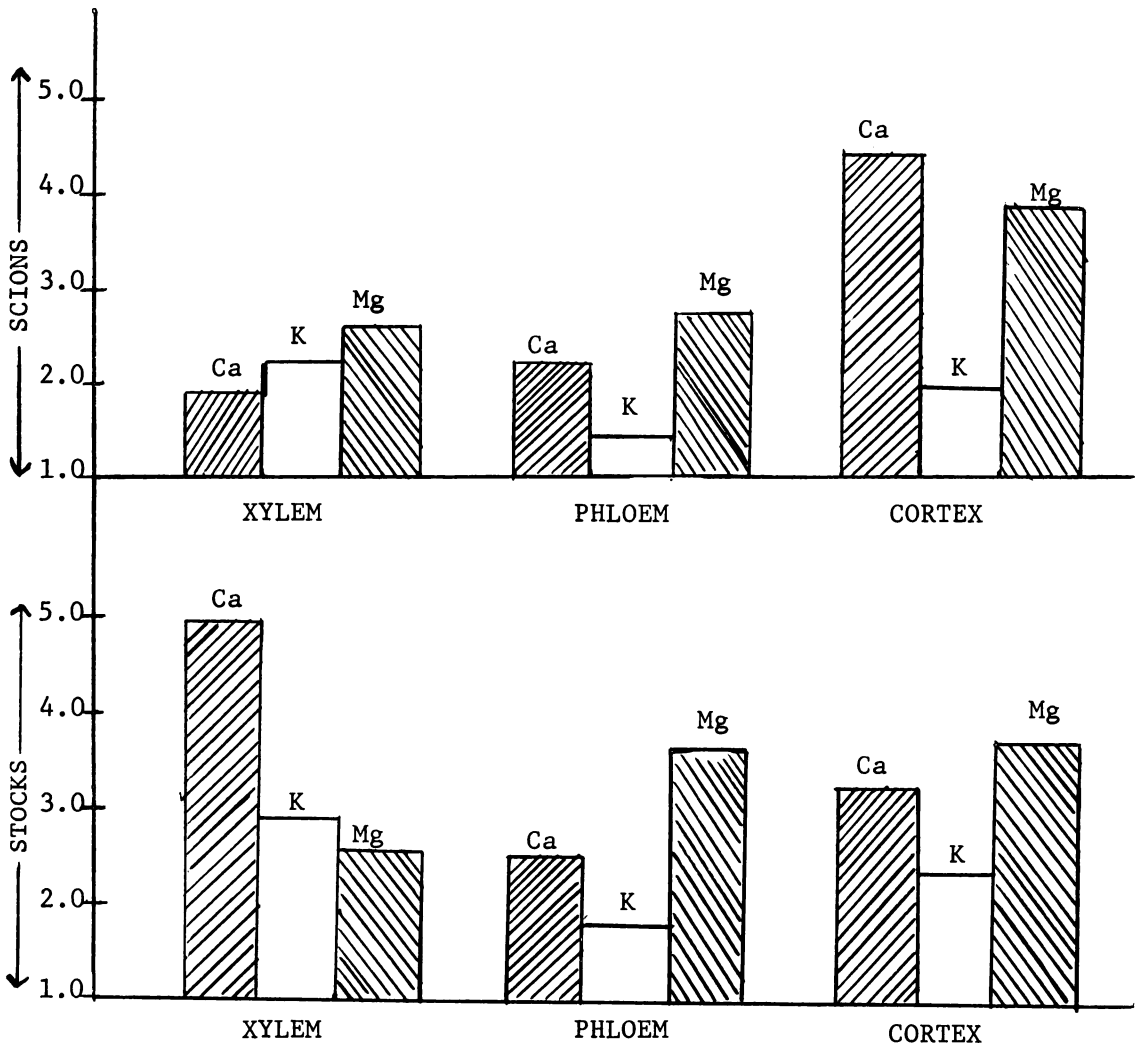


Figure 2. Mean calcium, potassium and magnesium content of interclonal juniper graft tissues within stock and scion as measured by electron microprobe X-ray analysis.

stock-scion balance in the xylem.

With the exception of magnesium, mineral distribution in the phloem was balanced between stock and scion and may represent base levels for this tissue. Magnesium content of the stock phloem was greater than that observed in xylem of the stock; the possible result of accumulation and lateral transport from the xylem.

In cortical tissue, calcium was present in greater amounts in the scion than in the stock, while potassium and magnesium were distributed equally between graft components. The excess amount of calcium present in the scion may have been 1) readily transported laterally in the stock to the cortex where it moved acropetally into the scion, or 2) readily translocated across the union in the xylem, then transported laterally to the cortex. The unrestricted translocation of this element observed by Bukovac et al. (1958) in apple grafts suggests the second hypothesis to be the more valid.

Unlike calcium, scion potassium levels were greater in xylem than in phloem or cortical tissue while magnesium levels were equivalent in xylem and phloem but much higher in the cortex. The observed cortical tissue levels of calcium and magnesium may represent differences in structural element levels for these tissues or storage resulting from "luxury consumption".

Summary

Calcium, potassium and magnesium content of interclonal juniper grafts were determined in two separate experiments, using ashing-photometry tests and electron microprobe X-ray analysis.

Plant Ashing and Photometry

Accumulation of solutes in understocks, expressed as stock-scion ratios obtained from separate ashing of entire stocks and scions, occurred in decreasing order; potassium, calcium, and magnesium.

'Hetzii', as a scion, accumulated more calcium than scions from other clones. While no difference was found for 'Von Ehron' or 'Hetzii' selfgrafts, those of 'Plumosa Compacta' accumulated small amounts of potassium in the understock. Grafts involving 'Hetzii' accumulated more calcium than potassium in the scion.

Mean stock-scion ratios of 1.00 for magnesium, suggested that this element moved relatively unrestricted across the graft union in comparison to calcium or potassium. More magnesium was found in understocks of grafts with Juniperus chinensis 'Hetzii' scions than in all other combinations. Juniperus sabina 'Von Ehron' as the scion, accumulated more magnesium than any other combination. All grafts after 12 days contained more magnesium in the scion than at any other time.

In graft combinations with Juniperus chinensis 'Hetzii' low magnesium levels were evident whether it was used as stock or scion. This suggests that a lower magnesium level may be in this clone.

The fact that more calcium and potassium accumulated in understocks than magnesium suggested that differences existed in translocation rates across the graft union.

Microprobe Analysis:

Considering xylem concentration as a measure of mobile elements, calcium appeared to accumulate in the understock while potassium and

magnesium moved readily through the graft union.

In phloem tissue, distribution was generally equal between stock and scion for all elements; magnesium being slightly greater in the stock.

Though potassium and magnesium were found equally distributed between stock and scion in the cortex, calcium accumulated in greater amounts in the scion. Because of its apparent blockage at the xylem union, excess scion calcium in the cortex was thought to result from lateral stock transport to the cortex where it was able to diffuse through the union; and accumulate in possible "luxury" amounts in the scion.

Though only general comparisons were possible, it was interesting to note that results obtained from ashing - photometry and from electron microprobe were quite different. Ashing and photometry data, a measure of entire stock or scion mineral composition, showed understock accumulation to be, in decreasing order, potassium, calcium and magnesium. Microprobe data, concerning the graft union only, indicated the apparent decreasing order of understock accumulation to be calcium, potassium, and magnesium. Thus, ashing data may represent element composition of the intact graft partners, while electron microprobe values reveal apparent rates of element movement through the graft union.

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SECTION IV

Serological Determination of Tissue Contribution from Stock and Scion in *Juniperus* L. Grafts

Introduction

Studies of mixed graft union tissue have been limited by an inability to identify cells derived from each of the graft components. Immunological technqies offer promising new ways to overcome this problem. This approach has been used by plant taxonomists to ascertain botanical relationships using protein similarity as a basis for separation of taxa. It has been possible, therefore, to suggest systematic changes in such woody perennial genera and families as *Quercus* and *Castanea* (13), *Magnoliaceae* (15), *Cornaceae* and *Nyssaceae* (8), and in *Solanum* (10).

Rives (21,22) used serological techniques to predict the affinity of one graft partner for another in studies of grape. In this case, immune precipitin reactions occurred in those varieties characteristically difficult to graft while positive reactions occurred in those varieties which successfully intergraft.

In similar studies, Green (12) studied serological relationships among species of six families and two sub-families known to successfully intergraft. He demonstrated positive precipitin reactions for typically successful interspecies grafts, and consistently negative reactions for non-successful grafts.

Kostoff (17) reported precipitin reactions in graft unions and concluded that plants were capable of acquiring immunity to foreign plant protein similar to that occurring in immunized animals. Chester (3,4) disputed this theory after immune response studies of extracts

from more than 30 species and varieties of woody plants failed to result in precipitin reactions. He suggested that the precipitation observed by Kostoff was calcium oxalate crystals.

Though antigen extracts used for plant studies have been prepared principally from dried seed (12,13,15), Rives (21,22) and Tucker (25) obtained favorable responses from shoot tip extracts.

Due to variation from animal to animal in response to injections, certain workers (1,9) are now using inbred strains of mice instead of rabbits. Using the Balb/C Jax strain of white mice, Fink and Quinn (9), found an intraperitoneal injection to yield the greatest amount of antisera; this has been confirmed by Anacker and Munoz (1).

In recent years a modified antibody technique using fluorescent labelling has played an important role in histological studies of animal tissue (5,26,11).

Very little information is available on fluorescent antibody techniques in plant histochemistry. Coons (5) described its use for microslide preparations of animal tissues. His procedure included cryostat sections of fresh frozen tissue; the slide preparations were incubated with labelled antibody and examined under the ultraviolet microscope.

The present study was designed to investigate the serological technique as a tool for studying developing graft unions. Procedures modified from those used for both plant taxonomy and animal tissue studies were used as the basis for the experimental technique.

Material and Methods

Graft Microslide Preparation

Three clonal juniper varieties, Juniperus horizontalis 'Fountain', Juniperus chinensis 'Hetzii', and Juniperus chinensis 'Pfitzeriana Kallay', were obtained in October 1966 from a commercial nursery source[1].

Upon arrival, the understock plants, measuring 1/8 and 3/16 inch diameter, were subjected to cold treatment for approximately 60 days to satisfy their cold requirements. Scion wood, obtained from the same nursery source, was placed in storage at 35° F. to assure continued dormancy. On December 10-11 understocks were potted and benched in a 68° to 70° F. night temperature greenhouse. Between January 15, and January 17, 1967 a number of the following graft combinations were prepared using a modified side graft.

	Stock	Scion
1.	J. horizontalis 'Fountain'	J. horizontalis 'Fountain'
2.	J. chinensis 'Hetzii'	J. chinensis 'Hetzii'
3.	J. chinensis 'Pfitzeriana Kallay'	J. chinensis 'Pfitzeriana Kallay'
4.	J. chinensis 'Pfitzeriana Kallay'	J. chinensis 'Hetzii'
5.	J. chinensis 'Hetzii'	J. chinensis 'Pfitzeriana Kallay'
6.	J. horizontalis 'Fountain'	J. chinensis 'Hetzii'
7.	J. chinensis 'Hetzii'	J. horizontalis 'Fountain'
8.	J. horizontalis 'Fountain'	J. chinensis 'Pfitzeriana Kallay'
9.	J. chinensis 'Pfitzeriana Kallay'	J. horizontalis 'Fountain'

All graft unions were covered with damp sphagnum moss, and the entire bench covered with 4 mil clear polyethylene supported 24 inches above the plants on a wooden frame.

Using standard propagation practices, the understock plants were cut back to one-half their mass 40 days after grafting; the remainder

[1]D. Hill Nursery Co., Dundee, Ill.

removed at the union 60 days after grafting.

For serological studies, stem segments encompassing the graft union were collected at random. Samples of each combination were collected from 10 to 60 days after grafting. The graft unions were placed in refrigerated storage (-22°C) in polyethylene bags to await sectioning.

The samples were evacuated and infiltrated for one hour in a 1.5% solution of Reten[2/] using a venturi type vacuum aspirator. Sections were cut at 10 microns using a Model CTD refrigerated microtome (crystat)[3/] at a cutting temperature of -15° C. Sections were picked up directly from the microtome blade using a thin film of 75% Reten on a microslide previously coated with ARG adhesive Jensen (14).

Antibody Preparation

Animal variation was minimized by using an inbred strain of mice, Balb/C Jax, for immunization. Three month old female mice were injected with antigen from each of the two junipers studied. Although sex had no effect, Fink and Quinn (9) reported that four to five month old animals produced more antibody than younger ones. The animals were reared in an air conditioned animal room under carefully controlled, sterile conditions[4/].

Antigen sera was prepared from leaf and succulent stem tissue. This material, upon removal from the plant, was freeze-dried in a

2/A catonic, water soluble polymer, product of Hercules Powder Co.
3/International Equipment Company.
4/Animals, material and related facilities provided for under National Science Foundation Grant Number G.B. 2714 supporting serological studies of Agropyron spp.

refrigerated lyophilizer and ground in a ballmill. Since differing species might elicit differing antibody responses, the tetraploid Juniperus chinensis 'Pfitzeriana Kallay' and the diploid Juniperus horizontalis 'Fountain' were chosen as antigen sources.

Protein was extracted from plant tissue powder by suspending 500 mg tissue in 4 ml of 70% ethanol for 4.5 hours at 68° C. After a 5 minute centrifugation at 3,000 r.p.m. in a clinical centrifuge, the supernatant was held at 4° C. overnight, then recentrifuged at 2600 g. The resulting supernatant was freeze-dried.

A micro-Kjeldahl test was used to determine nitrogen concentrations of protein extracts. Using a factor of 6.0 to convert total nitrogen values to total protein extracted, a yield of 0.84 mg protein/ml of solvent was established for Juniperus horizontalis 'Fountain' and 1.83 mg protein/ml solvent from Juniperus chinensis 'Pfitzeriana Kallay'. In preparation for immunization, lyophilized protein was adjusted with physiological saline to a concentration of 0.50 mg/0.25 cc injection. The antigen preparation was filtered through a Millipore filter of 0.65 micron pore size to remove contaminating bacteria.

Prior to injection the antigen was diluted with an equal volume of Freund's adjuvant to induce blockage of the lymphatic system; a procedure necessary to promote antibody accumulation containing ascites fluid in the body cavity. All injections were intraperitoneal using a 1 cc syringe and a 1 inch, 18 gauge disposable needle.

The animals were injected five times at weekly intervals

followed by two booster shots. Three animals were immunized for each antigen used. Failure to accumulate sufficient volume of peritoneal fluid after 12 weeks made it necessary to administer an additional 1/2 cc of Freund's adjuvant.

A microprecipitin titer test was employed to determine antibody levels of the antisera (2). This procedure involved the reaction of several antisera concentrations with the antigen used to immunize the animals. Dilutions of 4:1, 8:1, 16:1, 32:1, and 64:1 were prepared in a porcelain spot plate using a one ml microsyringe. The tests included antigen controls, antisera controls, and cross reactions with antigen from the other clone to measure antibody specificity. The resulting precipitin reaction was observed under a dissecting microscope, and the dishes photographed.

Ascites fluid was removed from test animals at several dates until the minimum necessary volume was obtained. The resulting fluid was frozen immediately upon extraction.

Antisera were prepared for conjugation using a slight modification of the procedure described by Goldman (11), as follows:

1. Removal of albumin
    a. To ascites fluid add an equal volume of 0.15 M NaCl.
    b. With mechanical stirring in a 4^{o} F. ice bath add slowly an equal volume of cold saturated $(NH_4)_2SO_4$.
    c. Continue to stir for 30 minutes.
    d. Centrifuge in the cold (4^{o}C.) and retain pellet. A table model Sorvall Clinical centrifuge at approximately 3500 r.p.m. was used in the present work.
    e. Wash, pellet once using cold 1/2 saturated $(NH_4)_2SO_4$.

f. Re-centrifuge and retain precipitate.

g. Dissolve precipitate in phosphate buffered saline (pH 7.2). Total volume approximately 1/3 that of the original ascetes fluid.

2. Removal of ammonium ions-$(NH_4)+$.

a. Dialyze against 1 liter phosphate buffered saline (PBS) in the cold ($4^oC.$) with mechanical stirring.

b. Change PBS at 1 hour and 5 hours, then continue dialysis over night.

Conjugation of antibody protein with fluorescein isothiocyanate employed the following simplified technique described by Jutila (16).

1. Determine protein content of immune globulin. In the present study the micro-Kjeldahl technique was used.

2. To an Erlenmeyer flask add with mechanical stirring:

a.	Physiological saline (0.85%)	10 ml.
b.	HCO_3-CO_3 buffer (0.5 M, pH 9.0)	3 ml.
c.	Acetone	2 ml.

3. Cool in acetone-dry ice bath until crystals form.

4. Add with stirring, 10 ml. diluted globulin (from 2b above).

5. Cool with stirring as in 3 above.

6. Add slowly 1.5 ml acetone-fluorescein isothiocyanate (0.05 mg/mg globulin protein).

7. Stir in the cold ($4^oC.$) for 18 hours.

8. Dialyze against phosphate buffered saline (0.01 N, pH 7.2) until saline no longer contains dye.

9. Filter through 1.5 micron Millipore Filter.

10. Store for short periods at $4^oC.$ or shell freeze and store at $-70^oC.$ for extended periods.

Prior to reaction of labelled antibody with microslide preparations, the conjugates were absorbed for 12 hours with Fluorescent

Antibody (FA) liver powder[5/] and centrifuged for 30 minutes in a refrigerated centrifuge[6/] at 22,500 X g; liver extract pellet being discarded.

Optimum conjugate concentration for microslide labelling was determined by testing with un-diluted conjugate and conjugate at 1:1 and 2:1 dilution (phosphate buffered saline: conjugate).

A limited quantity of labelled antibody made it possible to study only 4 of the 9 graft combinations prepared. Because 'Fountain' on 'Hetzii', 'Hetzii' on 'Fountain', 'Pfitzeriana Kallay' on 'Fountain' and 'Fountain' on 'Pfitzeriana Kallay' were highly successful grafts (7), they were chosen for study. In studying the latter two combinations, the preparations were allowed to absorb unlabelled antibody of one clone, then were incubated with conjugate of the other clone. This was done to eliminate as much non-specific labelling as possible, and was an adaptation of a procedure described by Redys et al. (20) for blocking cross reactions in Group A and Group B streptococci.

Labelling proceeded for 30 minutes in moist blotter lined Petri dishes, at 37° C. Excess antibody was carefully removed by syringing with phosphate buffered saline. Coverslips were mounted using a commercial fluorescent antibody mounting media[7/] and the preparations microscopically examined using a ultra violet light source. Photomicrographs were made of significant observations.

5/Difco Laboratories, Detroit, Michigan
6/International Equipment Co.
7/Difco Laboratories, Detroit, Michigan.

Results and Discussion

The results of this investigation indicated that the use of fluorescent antibody methods appeared to offer a promising histochemical tool for studies of plant graft union development.

An interesting observation, although only indirectly related to this study, was made relative to protein concentrations of the junipers used as antigen sources. Several micro-Kjeldahl determinations, used to arrive at the most efficient extraction technique for antigen, indicated that 'Fountain' contained a mean of 0.84 mg protein/ml of extractant as compared to 1.83 mg protein/mg extractant for 'Pfitzeriana Kallay'.

Prior to harvest of the antisera from animals, the sera was checked for titer level using a microprecipitin test. To check for suspected non-specificity of antibody production, cross reaction, using unrelated antigen, was included in the titer test. The resulting precipitin reactions are illustrated in Figures 1A for 'Fountain' and in 1B for 'Pfitzeriana Kallay'.

Represented in figures 1A and 1B are antigen controls (AC) and antisera controls (SC) in lower squares, antigen reacted with serially diluted (4:1, 8:1, 16:1, 32:1, 64:1 physiological saline: antisera) antisera in the middle squares, and serially diluted antisera reacted with antigen of the other juniper in the upper squares. The degree of "milkiness" of spots represents the amount of precipitation brought about by the antigen-antisera reaction.

Antigen and antisera controls appeared to be un-reacted except

for the former in 'Fountain'; this may have resulted from cross contamination. Precipitin reaction for 'Fountain' was more intense than 'Kallay' as indicated by the "milky" appearance. This was believed due to a higher titer level for 'Fountain'. The strong reactions were observed at a dilution of 1:64 in 'Fountain' while at 1:32 dilution 'Kallay' faded providing evidence of this difference in titer levels.

Of greatest significance was the observations that reactions occurred when antisera was combined with antigen of the juniper not used to obtain each antisera (top rows of squares, Figure 1A and 1B). Occurrence of cross reactions strongly suggested immune sera was not totally clonal specific. Greater specificity, however, was noted in 'Fountain' sera than in that of 'Pfitzeriana Kallay' when intensity of cross reactions were compared.

Background fluorescence or illumination was measured photographically with ultraviolet light, non-fluorescein labelled xylem and cortical tissues of clones used as antigen sources (Figure 2). A comparison of these photomicrographs revealed that even though background fluorescence was relatively high in both varieties it appeared to be greater in 'Fountain' than in 'Pfitzeriana Kallay'. The observed background fluorescence provided confidence that observed differences between fluorescein antibody labelled and non-specifically labelled tissues were real.

Figure 3 represents photomicrographic comparisons of fluorescence intensity between fluorescein labelled and non-labelled xylem, phloem, and cortical tissue. To obtain accurate comparative measures of

A

B

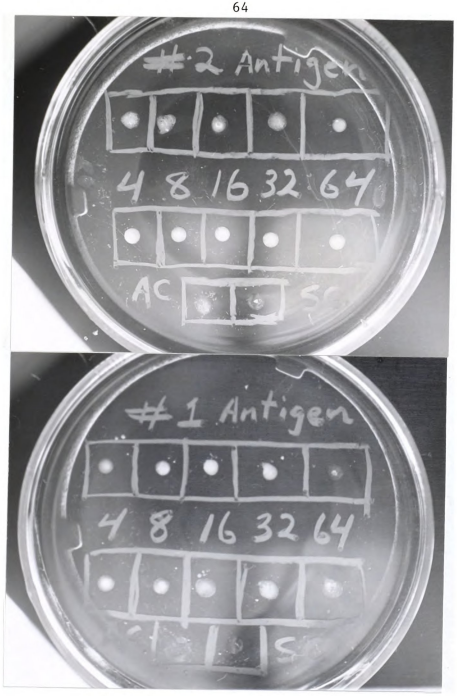
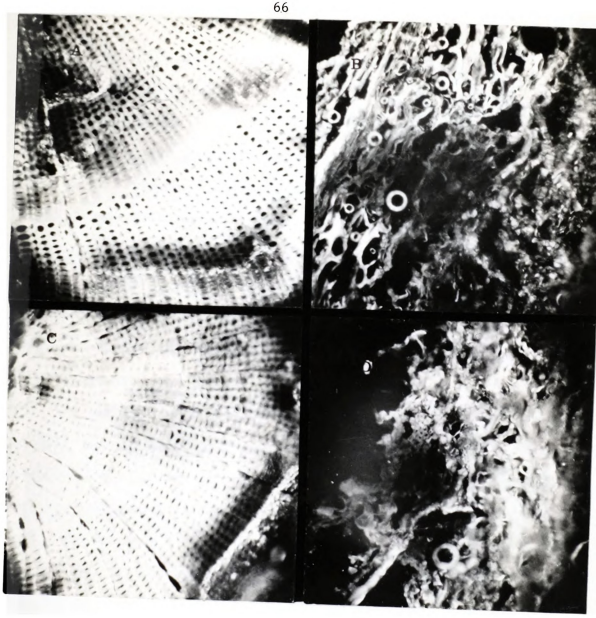


Figure 1 - Microprecipitin test plates for antisera of Juniperus horizontalis 'Fountain' (A) and Juniperus chinensis 'Pfitzeriana Kallay' (B). Lower squares: antigen controls (AC) and antisera controls (SC). Middle squares: diluted antigen-antibody reactions. Top squares: diluted cross-reactions, in A with 'Pfitzeriana Kallay' antigen, and in B with 'Fountain' antigen.

Figure 2 - Background fluorescence of non-fluorescein labelled xylem and cortex. (a) Juniperus horizontalis 'Fountain' xylem and (b) Cortex, (c) Juniperus chinensis 'Pfitzeriana Kallay' xylem (d) and cortex.


C
D

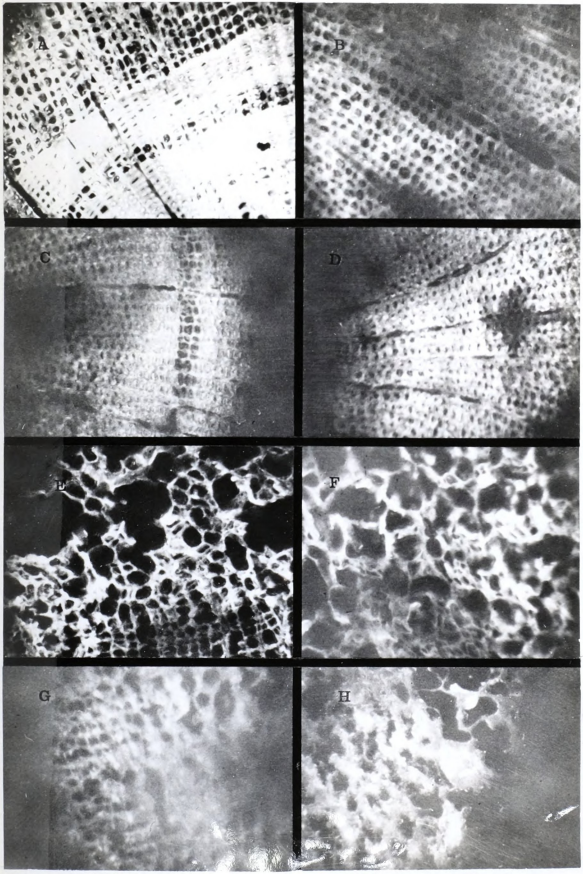
intensity, U.V. illumination level was left stable with only fields of observation varying.

Photomicrographs 3A and 3B represent flourescence of scion xylem and understock xylem respectively, of a 'Fountain' on 'Kallay' graft having scions labelled with antibody specific for the scion. Figures 3C and 3D represent serial sections of the same graft combinations, but reacted with 'Pfitzeriana Kallay' antibody. Examination of these photomicrographs revealed fairly distinct difference in labelling of xylem between the scion (Figure 3A) and stock (Figure 3B). When stock labelled, differences were again detected, although in reversed order; the stock fluorescence (Figure 3D) exceeded that of the scion (Figure 3C). Notable was the observation that degree of contrast between labelled and unlabelled xylem was much greater when using 'Fountain' antibody than with that of 'Pfitzeriana'. This, however, was due, in part, to the greater background fluorescence observed in unlabelled tissue of 'Fountain'. This was added evidence of greater specificity of reaction for 'Fountain' than for 'Pfitzeriana'; a condition first detected in microprecipitin test results (Figure 1).

Although intensity, both total and comparative, was lower in cortical tissue, differences were apparent between scion labelled 'Fountain' cells (Figure 3D) and understock cells (Figure 3F).

Figures 3G and 3H represent the same graft combinations labelled with 'Pfitzeriana' antibody. As noted for xylem, the expected reversal of fluorescence was observed. 'Fountain' antibody appeared to have less specificity in this tissue than in xylem, while that of 'Pfitzeriana' remained equivalent for both tissues.

Figure 3 - Photomicrographs of fluorescent antibody labelled stocks and scions of Juniperus horizontalis 'Fountain' on Juniperus chinensis 'Pfitzeriana Kallay' grafts. A. Xylem of 'Fountain' scion fluorescein labelled with 'Fountain' antibody. B. 'Pfitzeriana Kallay' understock of the same graft. C. Scion xylem of graft labelled with 'Pfitzeriana Kallay' antibody. D. Understock xylem of the same graft. E. Cortical tissue of 'Fountain' scion with fluorescein labelled 'Fountain' antibody. F. Understock cortex of the same graft. G. Scion cortex of graft labelled with 'Pfitzeriana Kallay' antibody. H. Understock cortex of the same graft.


A
B
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H

Typical examples of fluorescence patterns observed in mixed tissue of juniper graft unions are shown in Figure 4. These illustrations point to the potential usefulness of fluorescent antibody techniques for identifying tissue belonging to one component of heterogeneous graft unions.

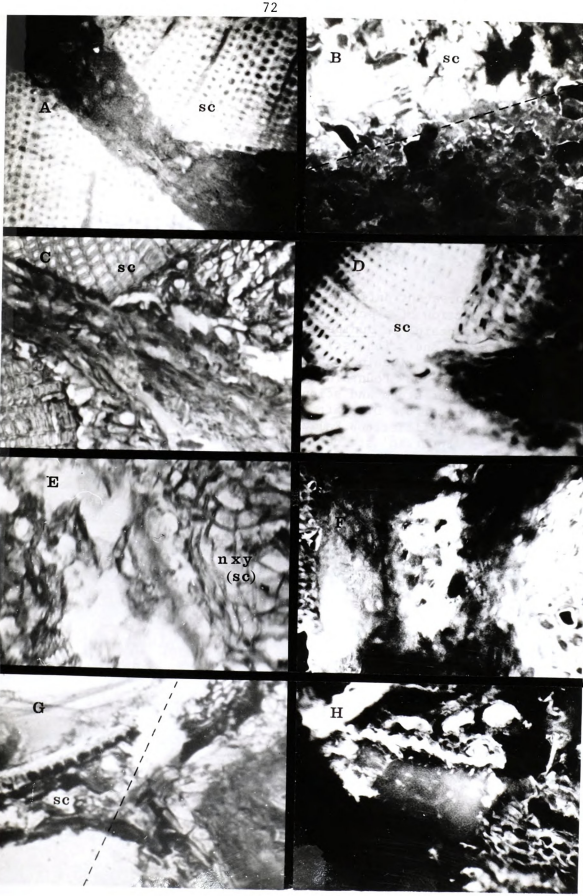
Figure 4A shows fluorescence labelling of mature stock and scion xylem tissue; the labelled 'Fountain' scion fluoresces more strongly than the Juniperus chinensis 'Hetzii' understock. The lack of fluorescence of the intervening cells suggested that they arose from the understock.

In the cortical tissue of a 'Fountain' on 'Hetzii' graft a more exacting separation of stock and scion tissue was noted. This is illustrated in Figure 4B. The broken line represents the approximate position of the original graft incision.

Figure 4C represents the stock-scion junction of a 'Pfitzeriana' on 'Fountain' graft stained with standard biological stains and viewed with a standard light source. The same graft combination when scion labelled and viewed under ultraviolet light is illustrated in Figure 4D, where, although the stock portion of the graft is out of plane, greater fluorescence was observed in the scion. Although much of the fluorescence appeared to be background or non-antibody, fairly distinct fluorescent spots were observed in the labelled scion phloem (upper right corner). Most of the tissue between the stock and scion appeared to arise from the understock.

Figure 4E orients the fluorescing tissue of the 'Pfitzeriana' on 'Fountain' graft shown in Figure 4F. Major labelling appeared in

Figure 4 - Fluorescent antibody labelling of graft unions. A. Xylem junction of a scion (sc) labelled Juniperus horizontalis 'Fountain' on Juniperus chinensis 'Hetzii' graft. B. Mixed stock and scion (sc) cortical tissue of graft described in A. Graft line indicated by the broken line. C. 'Pfitzeriana Kallay' on 'Fountain' graft showing point of cambial alignment of stock and scion (sc). Graft stained with safranin and Azur B. D. Graft described in C labelled with fluorescent 'Pfitzeriana Kallay' antibody. E. 'Pfitzeriana Kallay' on 'Fountain' stained with safranin and Azur B. Newly formed scion xylem nxy (sc) to the right side of the photomicrograph. F. Serial section of the same graft labelled with fluorescent antibody of scion. G. Mixed cortical and epidermal tissue of 'Pfitzeriana Kallay' on 'Fountain' graft stained with safranin and Azur B. Junction between stock and scion (sc) indicated by broken line. H. Same section as in G with fluorescein labelled 'Pfitzeriana Kallay' antibody.


A
sc
B
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(sc)
F
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H

the new scion xylem (right) and what appeared to be an island of crushed scion callus tissue near the middle of the photomicrograph; all other tissue having apparently arisen from the stock.

Figures 4G and 4H illustrate labelling patterns in cortical and cork tissues of a 'Pfitzeriana' on 'Fountain' graft; the dotted line delineating the original graft incision. Greater fluorescence was noted in the labelled scion tissue.

Though results using fluorescein labelled antibody were encouraging, a degree of refinement was deemed necessary to procure antibody of sufficient specificity to assure accurate separation of all tissues. The level of background fluorescence seen when unlabelled tissue was viewed under ultraviolet light (Figure 2) suggested that labelling was relatively low in certain tissues; especially xylem.

The fact that protein levels appeared to vary in direct proportion to ploidy level suggests that fluorescence due to protein specific antibody may be quite low and that any differences between graft tissue may have resulted from differing amounts of protein available for labelling rather than due to antisera specificity. Due to this possibility, techniques should be modified to utilize finite plant cell fractions as antigens rather than crude plant extract, as used in the present study.

Summary

Immunization of inbred white mice using Juniperus horizontalis 'Fountain' and Juniperus chinensis 'Pfitzeriana Kallay' as antigen sources produced species specific antibody which, when labelled with

fluorescein isothiocyanate aided in plant graft studies. Labelled microslide preparations of interclonal grafts examined under dark-field illumination and ultraviolet light source, provided good isolation of tissues derived from each graft component. Major objective of the study was to determine feasibility of this technique for ascertaining the contribution of the stock and scion to healing of the graft union.

Microprecipitin reactions of antigen with immune serum resulting from its introduction into animals revealed highest titer levels in 'Fountain' antisera; good reactions occurred at dilutions of 64:1, the highest tested. Cross titering of each antisera with antigen of the other clone indicated fairly high levels of cross reaction, taken as a measurement of non-specificity of the antibody produced. Because less intense cross titer reactions were observed using 'Fountain' antisera, greater antibody specificity was suspected for protein of this juniper; a supposition born out by the observed reaction when this antisera was applied to grafts involving 'Fountain' with other clones.

Examination, under ultraviolet light, of non-antibody treated microsections indicated the level of background fluorescence to be relatively high. Greater amounts were observed in 'Fountain' tissues. Although this observation suggested that clonal differences in fluorescence due to antibody labelling were quite small, known background fluorescence levels provided confidence that any observed differences in fluorescence between tissues of heterogeneous grafts were real and due to antibody labelling.

When grafts of 'Fountain' on 'Pfitzeriana' were incubated with 'Fountain' antibody, scion xylem and cortical cell fluorescence exceeded that for the understock with the most obvious differences occurring in the xylem. When this graft combination was treated with labelled antibody of the understock, tissues of this graft component exhibited more fluorescence. Unlike 'Fountain' antibody, specificity was equally apparent in xylem and cortical cells.

Interclonal graft unions studied by fluorescent antibody labelling of one graft component was generally found to result in clear separation of tissues belonging to labelled and unlabelled components of the graft. Specificity, however, was greater in the more parenchymatous tissue of the phloem, cortex and epidermis than in the xylem.

Though results were encouraging, it is felt that use of refined antigen rather than crude extract would result in antibody production of greater specificity.

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APPENDIX A

Cytological, Cultural and Histological Studies of the Progressive Development of Interclonal Juniperus L. Grafts

Introduction

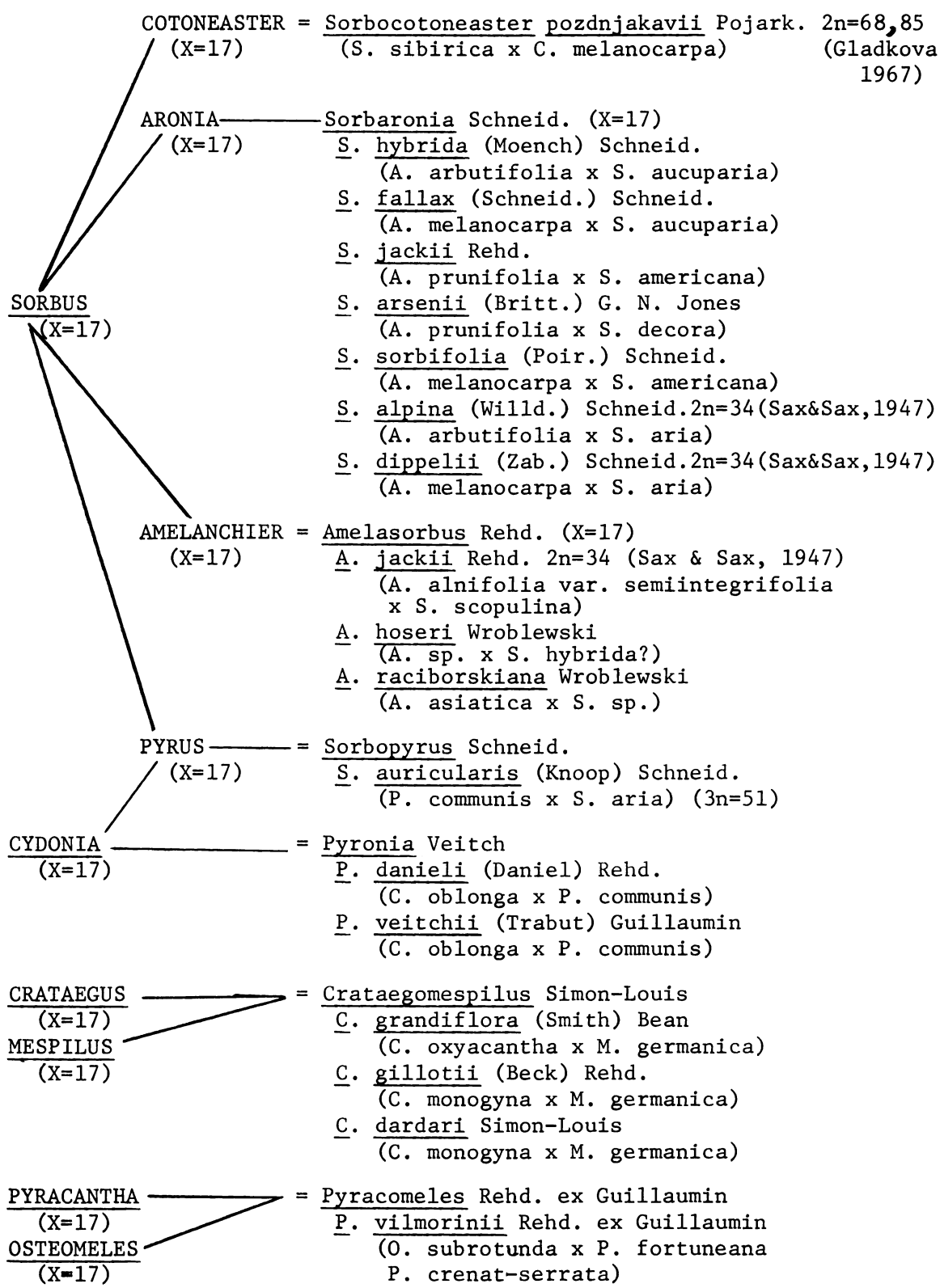
The horticulturally important practice of graftage has, for many years, been the object of considerable study with major emphasis being placed on graft compatibility. Many interesting questions have been raised concerning the developmental biology of the graft union, with only some of the questions adequately answered. Due to the commercial importance of fruit growing, most graft studies were on fruit tree species. The goals of this study were to analyze the influence of differences in cytology, histology and graft partner vigor upon ultimate success of the graft union.

Aside from the observation that as botanical relationship between graft partners becomes increasingly more removed, grafting success generally decreases; little study has been devoted to the effect of cytological relationships and their effect on graft success.

A number of cases of sexual "inter-generic" hybrids are known to occur between genera of the same family. The largest number occurs in the sub-family Pomoideae of the Rosaceae. These and their known species are listed in Table 1 (Cave, 1958-64; Darlington and Wylie, 1955).

Knowledge of the cytogentics of these inter-generic hybrids is provided principally in two papers by Sax (1929) and Sax and Sax (1947). They point out that the fact that Sorbus, Aronia, Amelanchier, and Pyrus can be crossed in certain combinations indicates a certain degree of intergeneric similarity, although the degree of sterility

Table 1. "INTERGENERIC" HYBRIDS IN THE ROSACEOUS SUBFAMILY POMOIDEAE Focke



displayed by the hybrids demonstrate the cytogenetic differentiation usually found in diverse species. It would be reasonable to suspect that such sterility might also be correlated with the grafting success between such species.

Examination of reported interspecific and intergeneric grafts indicates that in all successful grafts the base chromosome number is the same for stock and scion. This relationship parallels that found in sexual hybrids (Table 1).

Dodd and Gershoy (1943) studied interspecific grafts in the genus Viola, in the interest of establishing a relationship between the capacity for hybridization and capacity for graft success. They concluded that no such relationship existed; however, examination of the results (Table 2) casts some doubt on their interpretation.

Table 2. Results of grafting experiments in the genus Viola. (from Dodd and Gershoy, 1943)

Stocks	Scions				
	V. striata (X=10)	V. odorata (X=10)	V. papilionaceae (X=56)	V. canadensis (X=6)	V. tricolor (X=13)
V. striata (X=10)		X	0	S	0
V. odorata (X=10)	X		0	S	0
V. papilionacea (X=56)	S	S		S	S
V. canadensis (X=6)	X	X	X		X
V. tricolor (X=13)	0	X	X	S	

X indicates a successful graft. 0 indicates a failure not due to observed technical difficulties. S indicates a failure which may be due to technical difficulties.

According to the authors, it appeared that insufficient numbers of grafts were prepared to statistically establish grafting success. Furthermore they expressed some concern that faulty techniques may have entered into certain of their failures. It appears from these data that where base chromosome number of stock and scion deviated from X=10, greater success occurred where the deviate was used as the understock.

With consideration for graft component base chromosome numbers, a re-examination of data obtained by Evans, Watson and Davidson (1961) suggested that graft compatibility was generally better when such numbers are the same for stock and scion. Rosa multiflora, a species with 2n=14, and belonging to the subfamily Rosoideae; and Sorbus aucuparia, Chaenomeles japonica, Malus pumila and Pyracantha coccinea 'Lalandi' all from the subfamily Pomoideae, were chosen for study. Results of intergeneric grafts among these woody plants are given in Table 3.

Examination of the above results indicated that greatest success was obtained when members of the same sub-family were intergrafted. This may have been due, at least in part, to their all being diploids with the same absolute and basic chromosome numbers. When members of different subfamilies with differing basic and absolute chromosome numbers, were intergrafted, grafting results were highly unfavorable. The data from Pyracantha have been excluded in arriving at these hypothesis, since the plants were not in a favorable physiological condition for grafting. The success of self-grafts of the other test subjects attests to their favorable condition for grafting.

Table 3. Survival and average shoot elongation of 4 month old grafts. (from Evans, Watson and Davidson, 1961)

Scion	Understock	Percent Success	Shoot Elongation
Sorbus	Sorbus	100.0	42.3
Malus	Sorbus	100.0	39.3
Chaenomeles	Sorbus	52.4	19.9
Rosa	Sorbus	0.0	0.5
Pyracantha	Sorbus	0.0	0.0
Chaenomeles	Chaenomeles	85.7	39.0
Malus	Chaenomeles	95.2	25.8
Rosa	Chaenomeles	0.0	0.0
Pyracantha	Chaenomeles	0.0	0.0
Malus	Malus	80.0	21.5
Rosa	Malus	0.0	0.0
Pyracantha	Malus	0.0	0.0
Pyracantha	Pyracantha	0.0	0.0
Rosa	Rosa	76.2	88.0
Pyracantha	Rosa	0.0	0.0

In the genus Juniperus, the basic chromosome number is x=11, and all of the species for which chromosome counts have been recorded, except Juniperus chinensis, are diploids with 2n=22, or have diploid forms (Sax and Sax, 1933; Cave, 1958-1964; Darlington and Wylie, 1955). Juniperus chinensis is reported as being tetraploid, with 2n=44 (Darlington and Wylie, 1955). Van Sloun (1959) evaluated two closely related North American diploid junipers, Juniperus scopulorum and Juniperus virginiana, as understocks for four juniper clones--Juniperus chinensis pfitzeriana (an Asiatic tetraploid), Juniperus horizontalis 'Lividus', Juniperus scopuloum 'Montana No. 1' (both North American diploids), and Juniperus sabina tamariscifolia (an Eurasian diploid). Results of this work are given in Table 4.

Table 4. Surviving juniper stock/scion combinations on July 27, 1959. (from Van Sloun, 1959)

Scion/stock combinations	% survival
Diploid/diploid grafts	43
Lividus/virginiana	52
Savin/scopulorum	68
Savin/virginiana	43
Montana No. 1/scopulorum	21
Montana No. 1/virginiana	13
Tetraploid/diploid grafts	
Pfitzer/scopulorum	0
Pfitzer/virginiana	1

Van Sloun found no significant difference between Juniperus scopulorum and Juniperus virginiana as rootstocks for any given scion. From a cytogenetic viewpoint more survival was obtained when scion/stock combinations involved diploid/diploid grafts than when they

involved tetraploid/diploid grafts. This might suggest that grafting success may be partially attributable to differences in ploidy levels of stock and scion.

Ahlgren (1962) did work on interspecific pine grafting with a genus having a base chromosome number of x=12. The species studied included Pinus peuce, Pinus cembra, Pinus koraiensis (Santamour, 1960), Pinus resinosa and Pinus strobus (Saylor, 1961) all of which are diploids with 2n=24. Grafting success for the six combinations studied are listed in Table 5.

Table 5. Graft survival for six interspecific pine graft combinations. (from Ahlgren, 1962)

Combinations	Graft survival
P. peuce/P. strobus	100%
P. koriaensis/P. strobus	83%
P. cembra/P. strobus	77%
P. peuce/P. resinosa	70%
P. koriaensis/P. resinosa	68%
P. cembra/P. resinosa	81%

These data substantiate the grafting success when both base and absolute chromosome numbers of stock and scion are equivalent.

Among the questions arising out of observed incompatibilities in grafted plants is that of the possible role of biochemical vectors in control of graft success. In several cases toxic principles which were transported from one graft partner to another were assumed to be the causes for graft failures (Toxopeus, 1936; Gur, 1957; Nauriyal et al., 1958).

Toxopeus (1936) observed that when sweet orange was budded into sour orange understocks, graft failure was evident in two to three months with eventual death in 8 to 12 months. Because failures occurred only when sweet orange was the scion, it was concluded that a toxic substance was produced by the scion and translocated to the stock where it was fatal. Similar conclusions were reached by Nauriyal et al. (1958) concerning failures of Eureka lemon scions grafted upon Trifoliate orange understocks. Because external symptoms of girdled branches resembled those of the graft combinations they suggested a toxic substance produced in the scion caused injury to the conducting tissue of the stock.

To explain failures of pears grafted upon quince understock, Gur (1957) proposed that a cyanogenetic glucoside called prunasin, was produced in the stock phloem adjacent to the union where it decomposed liberating hydrocyanic acid. He further proposed that the HCN prevented cambial activity and destroyed the conducting tissue near the union.

Toxic substances in pear on quince grafts have also been postulated by Mosse and Scaramuzzi (1956) as the cause of graft failures. They suggested that breaks in woody tissue continuity occurred when these lethal substances accumulated in older phloem near the union and eventually reached the cambium.

In addition to lethal chemical agents viruses have also been reported to cause graft failures. In many instances the virus was shown to be the lethal substance by budding from a susceptible to a resistant strain, in which case fewer failures were observed.

In an effort to explain the dwarfing effect of EM IX understocks as compared to non-dwarfing EM XVI, Martin and Stahley (1967) studied the levels of phloridzin (a growth inhibitor), coumaric acid (decarboxylates IAA), and chlorogenic acid (prevents decarboxylation of IAA) in the bark of these understocks. Although phloridzin levels were quite high in both stocks, and could not explain growth controlling behavior, a different mode of metabolism was suggested as the cause for the differential growth response. Chlorogenic acid was found to be slightly more concentrated in EM XVI than in IX but coumaric acid was higher in the latter understock. The total amount of growth promoting substance, as measured by coleoptile tests, was higher and growth inhibiting compounds lower in EM XVI than in EM IX.

Studies of graft union structure have concentrated principally upon the morphological and gross anatomical level. Therefore, certain questions remain unanswered about stock-scion relationships, many of which can now be answered with more sophisticated and accurate instrumentation.

Tissue discontinuity at the graft union has long been considered an indicator of incompatible graft unions. Whether this weakness occurs in the xylem (Waugh, 1904; Bradford and Sitton, 1929a; Chester, 1931; Chang, 1937), or in the phloem (Bradford and Sitton, 1929a; Bradford and Sitton, 1929b), it is known to result in a structurally weak graft union (Booth, 1913-1914; Proebsting, 1926, 1928; Chang, 1937). Mosse (1962), pointed out that mechanical weakness may only be considered a valid criteria for rating compatibility of older trees since young unions are inherently weak due to lack of lignification of

tissue at the union. In fruit tree grafts, apparently normal mature bearing trees suddenly break at the union leaving a relatively smooth exposed surface, suggesting that the structural weakness results from discontinuity of vascular tissue (Hatton, 1936; Mosse, 1960, Mosse, 1962). One example of this has been reported in a self-graft of Abies concolor (Eames and Cox, 1945) in which no observable cambial union occurred between stock and scion and no parenchymatous tissue separated the graft components.

In extensive anatomical studies of incompatible graft unions of pear on apple, Bradford and Sitton (1929b) observed unions in which a break in cambial continuity occurred, presumably at the end of each season. This was followed by a re-graft the following spring. The condition may have resulted from differential growth rates, or staggered dates of growth cessation at the end of the season. However, studies on relatively young grafted plants (Sass, 1932; Herrero, 1951; Evans, Watson and Davidson, 1961), revealed that wood discontinuity of this type does not occur during the first year after grafting.

Proebsting (1928) described another form of graft abnormality which is manifested by a distortion of newly formed xylem elements resulting from uneven growth rates of stock and scion. Such distortion was not limited to incompatible combinations. Vessel elements in longitudinal sections of one-year old Sorbus aucuparia self grafts were observed by Evans, Watson and Davidson (1961) in both cross and longitudinal section at the point of union. This distortion was believed due to a reorienting of tissue during vascular bridging of the union.

Phloem discontinuity has been recognized as a symptom of graft incompatibility (Proebsting, 1928; Bradford and Sitton, 1929a; Herrero, 1951; Lapins, 1959). This symptom may be expressed as isolated patches of cork between stock and scion (Lapins, 1959), or as darkly stained, disorganized tissue bounded by rows of cells resembling the phellem of the periderm (Evans, Watson and Davidson, 1961). It seems plausible that such isolation could occur when graft partners were in insufficient contact for proper mixing of tissue. Under these conditions, healing might follow the pattern known for normal wound healing in which newly differentiated tissue tends to advance over the wounded surface in a rolling motion (Bradford and Sitton, 1929b). This would, in a loosely fitted graft, result in isolation of some periderm tissue.

Phloem discontinuity has indirectly been demonstrated by the presence of large accumulations of basipetally translocated starch, at the base of the scion (Booth, 1913-1914; Kostoff, 1928; Proebsting, 1928; Kac, 1931; Roberts, 1934; Chang, 1937; Mosse and Herrero, 1950; Herrero, 1951). More recently, radioactive tracers have shown blockage of basipetally translocated phosphorus in scion phloem of interstock grafted apple trees (Dickenson and Samuels, 1956) and in ungrafted plants in which a ring of bark was deleted from the stem (Colwell, 1942). In the latter case, accumulated phosphorus was found in both xylem and phloem at the scion base, suggesting lateral translocation at the point of tissue discontinuity.

The possibility exists that the ability of two plants to form a successful graft union may be controlled to a degree by the inherent

similarity or dissimilarity of their anatomy. Such a hypothesis would seem feasible if one considered that differences, i.e. between size and/or number of cells of the transport tissue between stock and scion, could result in a lack of vessel continuity during early stages of graft healing. This condition could affect early graft survival as well as exerting a long term effect on plant growth. As an example, it is known that upward translocation through dwarfing apple rootstocks is slower than through equivalent tissue of a non-dwarfing understock (Scholz, 1958). This may be due to the fact that vessel elements of dwarfing understocks are smaller in cross-sectional area (Beakbane, 1941, 1956; Komarofske, 1947). Matubara (1931) reported a positive correlation between number of vessels per unit area of stem and graft survival.

Although investigations of graft union ultrastructure are relatively new, use of the electron microscope, radio isotopes, histochemical reagents and other techniques are showing promise as tools for enlightening our knowledge of cell wall structure.

Buchloh (1960) dealt specifically with cell wall histochemistry of graft unions. With the electron microscope, he studied the relationship of lignification to graft compatibility. He reported that upon contact of stock and scion cells, a middle lamella was formed between cells of the graft components. If the presently accepted mode of middle lamella formation is correct, lamella establishment must occur by atypical means between heterogeneous cells of the graft union. When a cell undergoes division, the middle lamella forms in the cell plate, expands laterally to the sidewalls, and is subsequently

overlain by a deposition of secondary wall contributed by both daughter cells. This mode of pectin deposition would seem improbable under conditions found in grafted tissue.

Formation of pectic substances between cells of the graft union may be analogous to that for its formation in outer cell walls of epidermal cells. Priestly (1943) reported the presence of alternating lamellar layers of pectin and cutinized cellulose. Because cutin content of these cellulose lamellae increased in a peripheral direction, Esau (1960) suggested that this substance may be secreted toward the surface from epidermal and more deeply seated cells. This explanation may also apply to the origin of the alternating pectin lamellae, and, in turn, help to explain middle lamella deposition in graft unions.

The structure of pectic substances has been a subject of some controversy. Traditionally ruthenium red has been used as a biological stain for these substances, but it has been shown that this stain is quite unspecific (Bonner, 1936; Hock, 1942; Kertesz and Loconti, 1944; Kertesz, 1951). Bonner (1936) explained the lack of specificity as an affinity for carboxyl groups of the 6th carbon atom, therefore, cellulose, when subjected to mild oxidation, stains with ruthenium red, as do other compounds having such groups. The degree of specificity is apparently related to the effect of acidity upon the carboxyl groups of the pectic substance. Bonner (1936) further pointed out that ruthenium red does not differentiate between protopectin, pectin, pectic acid, or calcium pectate.

Basic hydoxylamine has been shown (Cornaz and Deuel, 1954; Albersheim, Muhlethaler and Frey-Wyssling, 1960) to be more specific

for pectin than ruthenium red. It reacts with the esterified carboxyl groups of pectin to produce hydroxamic acid which forms an insoluble complex with ferric ions.

The middle lamella is composed primarily of calcium pectate (Mangin, 1888-1893; Bonner, 1936; Kertesz, 1951). However, Ginzburg (1958, 1961) maintained that cells were cemented together by a gel of protein and non-cellulosic substances, including pectin. Conversely Setterfield and Bayley (1957, 1961) suggested that the middle lamella was a polysaccharide matrix where cellulose was absent rather than a region of pectate localization. Preston (1964) contended that pectin was a portion of the cellulose matrix which existed as an adcrusting substance between the microfibrils. Such a distribution of pectin could mask the stain reaction of the middle lamella and reduce the specificity of ruthenium red.

Lignification of cells of the graft union and formation of a mutual middle lamella between components of pear-quince grafts were considered as essential to development of structurally sound unions by Buchloh (1960). He proposed that a secondary chemical differentiation occurred in the cell wall by which the pectic substance of the middle lamella disappeared and was gradually replaced by lignin with all cells but parenchyma subsequently dying. He also pointed out that compatible grafts contained a lignin concentration in cell walls at the graft as high as in those some distance away with highest amounts in the middle lamella.

The present study was undertaken with the following as principal goals:

1. By cultural, anatomical, and histochemical means determine the success of, and changes occurring during, progressive healing of successful graft combinations.

2. Determine possible differences in events of objective (1) as they may occur in interclonal grafts between plants of different ploidy levels.

3. Ascertain the relative contributions of tissue from stock and scion to the healing of the graft union.

Materials and Methods

Genetically stable Juniperus chinensis 'Hetzii', Juniperus horizontalis 'Fountain', and Juniperus chinensis 'Pfitzeriana Kallay' were used in this study; all are hereafter referred to by their cultivar names.

On October 24, 1966, the plant material was obtained from a wholesale nursery firm[1/], and following a cold treatment in a lathhouse, were potted December 10 in 4 inch clay pots. The plants were then benched in a 68°F. night temperature greenhouse. Scion wood, obtained from the same source, was stored at 35°F. to break rest and to assure continued dormancy. Stock and scion wood were approximately one year from cuttings; stem diameters being 1/8 to 3/16 inches.

Between January 15 and January 17, 1967, the following graft combinations were prepared using a modified side graft:

Stock	Scion
1. J. horizontalis 'Fountain'	J. horizontalis 'Fountain'
2. J. chinensis 'Hetzii'	J. chinensis 'Hetzii'

1/ D. Hill Nursery Co., Dundee, Ill.

3. J. chinensis 'Pfitzeriana Kallay'	J. chinensis 'Pfitzeriana Kallay'
4. J. chinensis 'Pfitzeriana Kallay'	J. chinensis 'Hetzii'
5. J. chinensis 'Hetzii'	J. chinensis 'Pfitzeriana Kallay'
6. J. horizontalis 'Fountain'	J. chinensis 'Hetzii'
7. J. chinensis 'Hetzii'	J. horizontalis 'Fountain'
8. J. horizontalis 'Fountain'	J. chinensis 'Pfitzeriana Kallay'
9. J. chinensis 'Pfitzeriana Kallay'	J. horizontalis 'Fountain'

The completed grafts were covered with damp sphagnum moss, and the entire bench covered with 4 mil clear polyethylene supported on a wooden frame 24 inches above the plants.

Using standard propagation practices, the understock plants were cut back to one-half their mass 40 days after grafting; the remainder being removed at the union 60 days after grafting. Graft survival was recorded 5 months after grafting.

To study the effect of foreign protein on graft development, tissue homogenates were made from Juniperus horizontalis 'Fountain', Juniperus chinensis 'Hetzii', and Juniperus chinensis 'Pfitzeriana Kallay' leaf and stem tissue. The tissue was prepared by maceration in a Sorvall Omni Mixer[2/] with enough distilled water to create a preparation of pasty consistancy. On January 17, 1967, the following treatments were prepared by applying the tissue homogenate to the graft incision prior to completion of the graft:

Graft Combination	Extract Used
1. 'Fountain' on 'Fountain'	'Hetzii'

[2/]Ivan Sorvall, Inc., Norwalk, Conn.

2. 'Fountain' on 'Fountain' 'Pfitzeriana Kallay'

3. 'Hetzii' on 'Hetzii' 'Fountain'

4. 'Hetzii' on 'Hetzii' 'Pfitzeriana Kallay'

5. 'Pfitzeriana Kallay' on 'Pfitzeriana Kallay' 'Fountain'

6. 'Pfitzeriana Kallay' on 'Pfitzeriana Kallay' 'Hetzii'

Five months after grafting, survival data was recorded.

At six 10 day intervals following grafting, 3/4 inch stem segments containing the graft union were collected for each combination. Upon harvest, the graft unions were immediately frozen in polyethylene bags to await sectioning.

Samples were evacuated and infiltrated for one hour in a 1.5% solution of Reten[3/] using a venturi type vaccuum aspirator. Sections were cut at 10 microns using a refrigerated microtome (Cryostat)[4/] with a cutting temperature of -15°C. Sections were picked up directly from the microtome blade on glass slides coated with a thin film of 0.75% Reten.

Pectin distribution, especially in the middle lamella was studied on a series of preparations from all graft combinations at 10, 20, 30, 40, 50, and 60 days after grafting. The sections were stained with ruthenium red (1: 5000 aqueous solution) for 12 hours at room temperature and the cover slip mounted with glycerine jelly.

To investigate tissue lignification in the region of the graft union, a series of sections from all combinations and dates were stained with Azure B for one hour at room temperature. This was a

3/A cationic, water soluble polymer; Hercules Powder Co.

4/International Equipment Co. (Model CTD)

slight modification of the procedure suggested by Jensen (1962).

Following Jensen (1962), cell walls were studied by a stepwise extraction of each component after which sections were stained with the non-specific stain, periodic acid-Schiff's reagent. The sections were stained for 30 minutes in periodic acid followed by 15 minutes in Schiff's reagent. Color photomicrographs of the stained sections were prepared and compared for reduction in stain intensity; a measure of the amount of each cell wall constituent removed at each extraction.

Wilson (1961) reported difficulty in maintaining adhesion of woody plant sections to microslides during extraction in strong base. He was able, however, to overcome the problem during cell wall extraction by using a thin layer of fiber glass over the sections. This was secured by a second microslide held to the first by aluminum wire to form a "sandwich".

This did not appear feasible in this study since the fiber glass and wiring might disrupt the fragile callus tissue of the graft union. For this reason, several combinations of materials were used to overcome the loss of sections during extraction. One combination was a piece of green saran shade cloth which was overlain with ¼ inch hardware cloth and secured with paper clips. Another combination used saran shade cloth overlain with fine stainless steel mesh which was held together with hair clips.

All stained preparations, with the exception of those stained with ruthenium red were mounted permanently in Permount[5/], and stored until microscopic examination.

[5/]Fisher Chemical Co.

For anatomical study, sections were stained with safranin (15 minutes) and counterstained with aniline blue (1 minute); a stain combination considered to be ideal for gymnosperms (Johansen, 1940; Jensen, 1962). The staining procedure was altered from that described by Jensen (1962) by the inclusion of a stepped alcohol dehydration series between safranin and aniline blue. Poor preparations resulted when slides entered the clove oil-absolute alcohol-xylene solution unless this was done.

The possible correlation between anatomical differences of stock and scion and graft success was studied using an eyepiece micrometer to determine mean cell size for vessel element and ray cell diameters and lengths in ungrafted wood of each clone. Data on diameter of newly formed, wound induced vessel elements found between faces of the graft incision was also recorded. Newly formed vessel elements were measured in 60 day old grafts only.

As a measure of relative vigor, counts of normal tracheids and those stimulated by wounding during grafting and produced subsequently, were made for all combinations of junipers studied and at the six dates following grafting.

Figure 1 serves to illustrate sectors of the grafted plant hereafter referred to as "Normal" tracheids and "Wound" tracheids in reference to cell count and cell measurement data.

Results and Discussion

The graft survival data (Table 6) revealed that general graft success was 58% to 100% for all graft combinations at the end of

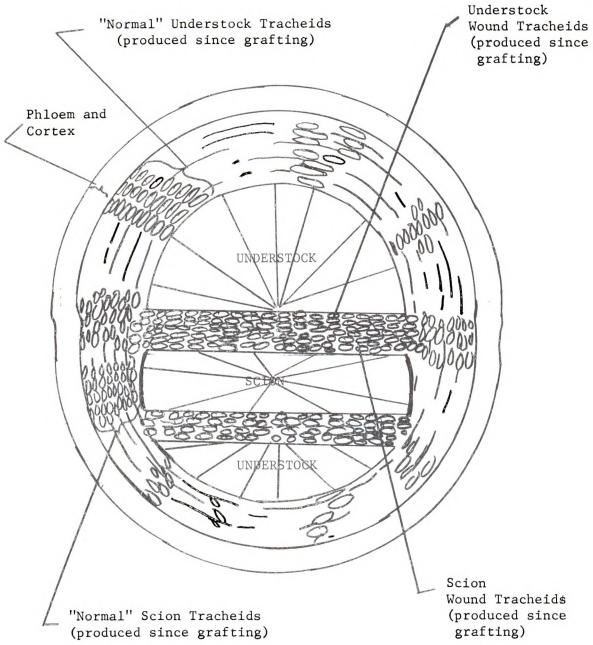


Figure 1. Diagramatic representation of a typical 60 day old graft union viewed in cross-section. Points are indicated at which cell counts and cell measurement data were taken.

Table 7. Survival counts and visual observations of 5 month old tissue extract treated self grafts based on 5 plants per combination.

Self-Graft	Extract Applied	Survival 6-13-67	Observations 5-4-67
1. J. horizontalis 'Fountain'	J. chinensis 'Pfitzeriana Kallay'	4	Less vigor than non-treated graft.
2. J. horizontalis 'Fountain'	J. chinensis 'Hetzii'	4	More vigor than #1
3. J. chinensis 'Pfitzeriana Kallay'	J. horizontalis 'Fountain'	4	Nearly equal vigor to untreated self graft
4. J. chinensis 'Pfitzeriana Kallay'	J. chinensis 'Hetzii'	1	Equal vigor to non-treated self; Less vigor than #3
5. J. chinensis 'Hetzii'	J. chinensis 'Pfitzeriana'	1	Same vigor as non-treated self graft
6. J. chinensis 'Hetzii'	J. horizontalis 'Fountain'	0	

5 months. Self-grafted control plants, the diploid and triploid junipers, Juniperus horizontalis 'Fountain' (2n=22) and Juniperus chinensis 'Hetzii' (3n=33) (Section 1, Thesis) were highly successful while an extremely low success was observed for Juniperus chinensis 'Pfitzeriana Kallay' (4n=44). The success obtained on 'Fountain' and 'Hetzii' junipers signified that adequate grafting techniques were used for these clones.

Intergeneric grafts fell into two distinct groups. The most successful combinations were 'Hetzii' on 'Fountain', 'Fountain' on 'Hetzii' and 'Fountain' on 'Pfitzeriana Kallay', in which all 12 plants survived. Least successful combinations were 'Hetzii' on 'Pfitzeriana Kallay', 'Pfitzeriana Kallay' on 'Hetzii' and 'Pfitzeriana Kallay' on 'Fountain'.

Almost all highly successful grafts contained 'Fountain' as a component, conversely, all less successful grafts had 'Pfitzeriana Kallay' as one partner. This suggested the possibility that 'Fountain' may have exerted a stimulating effect on graft healing while 'Pfitzeriana Kallay' suppressed or failed to contribute to graft union development. Such a condition might arise from insufficient growth factors or to the presence of inhibitors. This supposition is supported by the low success observed for self grafts of Juniperus chinensis 'Pfitzeriana Kallay' and by its poor performance observed when used as a scion for Juniperus virginiana by Van Sloun (1959). 'Hetzii' being involved in a large number of successful grafts, suggests that it may exert a beneficial influence upon graft union formation.

Results of preliminary studies using tissue extract treated self-grafts suggested pursual of this research to be desirable. Because extract treated grafts were found to respond differently from those of non-treated controls it appeared that applied extracts produced a marked influence upon graft success (Table 7).

Application of 'Hetzii' or 'Pfitzeriana Kallay' extract to grafted surfaces of 'Fountain' juniper resulted in no apparent effect upon graft success, but reduced scion vigor was observed when 'Pfitzeriana Kallay' extract was used and increased vigor with the former extract.

Extracts of 'Fountain' when applied to 'Pfitzeriana Kallay' grafts resulted in a significant increase in graft success. Whereas untreated self-grafts of 'Pfitzeriana Kallay' (Table 6) were only 25% successful, these grafts with extract applied were 80% successful. A marked increase in vigor accompanied the observed grafting success (Figure 2).

These results suggest the presence of a growth factor in Juniperus horizontalis 'Fountain', which when applied to the graft union stimulates healing of the 'Pfitzeriana Kallay' graft union. This hypothesis is supported by the results of the previous experiment, where in most successful grafts, 'Fountain' juniper was a component. The ease of rooting add circumstantial evidence of the presence of such a substance. Lanphear and Meahl (1966) reported 100% rooting of Juniperus horizontalis plumosa under growth chamber conditions which also supports this hypothesis. Chromatograms of tissue extracts from this juniper yielded a substance (rf=0.8) which exhibited strong root producing capacity in the mung bean bio assay.

Figure 2 - The effect of application of leaf and stem extract to graft surfaces of Juniperus chinensis 'Pfitzeriana Kallay' self grafts. No extract (center), 'Fountain' extract (right), and 'Hetzii' extract (left).

Table 6. Survival counts of 5 month old juniper grafts based on 12 plants per combination.

Combinations	Rep. I	Rep. II	Rep. III	Total 6/13/67
1. J. horizontalis 'Fountain'/ J. horizontalis 'Fountain'	4	3	4	11
2. J. chinensis 'Pfitzeriana Kallay'/ J. chinensis 'Pfitzeriana Kallay'	3	0	0	3
3. J. chinensis 'Hetzii'/ J. chinensis 'Hetzii'	4	4	3	11
4. J. chinensis 'Hetzii'/ J. horizontalis 'Fountain'	4	4	4	12
5. J. horizontalis 'Fountain'/ J. chinensis 'Hetzii'	4	4	4	12
6. J. chinensis 'Hetzii'/ J. chinensis 'Pfitzeriana Kallay'	2	3	2	7
7. J. chinensis 'Pfitzeriana Kallay'/ J. chinensis 'Hetzii'	2	4	2	8
8. J. horizontalis 'Fountain'/ J. 'Pfitzeriana Kallay'	4	4	4	12
9. J. chinensis 'Pftizeriana Kallay'/ J. horizontalis "Fountain'	3	2	3	8

Earlier work (Section 2 of thesis) indicated that initiation of callus tissue production in self grafts of Juniperus chinensis 'Pfitzeriana Kallay' was 20 days behind the other junipers studied. Speculatively 'Fountain' juniper may stimulate tissue production in the 'Pfitzeriana Kallay' graft union. The necessity of callus tissue production to grafting success has been well established among propagators, and was reported by Harmon and Weinberger (1967) to correlate positively with successful grafting of grapes.

Extract of 'Hetzii' on 'Pfitzeriana Kallay' self grafts resulted in no observable difference in graft success when compared to untreated controls, and ultimate vigor was less pronounced than when 'Fountain' was the extract source. This observation might suggest a lack, in 'Hetzii', of the beneficial substance possessed by 'Fountain'.

Because 'Hetzii' self grafts were highly successful in control grafts, it was difficult to explain the poor results obtained when 'Pfitzeriana Kallay' extracts were applied to these graft unions. Although observed vigor was equivalent to control grafts, total graft success was markedly suppressed by application of 'Pfitzeriana Kallay' extract. One might speculate that, because it is quite unsuccessful as a self graft, extracts of 'Pfitzeriana Kallay' may interfere with and have a suppressive effect upon the 'Hetzii' self grafts. Although a high degree of success was obtained when 'Pfitzeriana Kallay' extract was applied to 'Fountain', some reduction of growth was noted. In this case, any suppression of graft success by 'Pfitzeriana Kallay' may have been countered by the opposing effect of the 'Fountain' growth factor.

The reason for failure with 'Fountain' self grafts treated with 'Hetzii' is unknown.

Comparative measurements of ray cells and tracheids produced some interesting relationships which may help explain structurally some of the differential responses in interclonial grafts of Juniperus horizontalis 'Fountain', Juniperus chinensis 'Hetzii' and Juniperus chinensis 'Pfitzeriana Kallay'. Cell measurements are presented in Table 8.

Ray cell dimensions revealed significant differences between 'Pfitzeriana Kallay', 'Fountain' and 'Hetzii' in length and cross-sectional area; the latter two not differing significantly from each other.

Although significant differences did not exist between all means, a direct relationship was noted between ray cell measurement and ploidy level of the junipers studied ('Fountain' 2n=22, 'Hetzii' 3n=33, and 'Pfitzeriana Kallay' 4n=44). This relationship was positive for ray cell tangential diameter and negative for ray cell length. 'Pfitzeriana Kallay' ray cells were significantly shorter and thicker than those of the other taxa. Therefore, this juniper should possess a greater number of tangential walls per increment of radial distance; a condition which might influence lateral translocation in the plant. Esau (1953) points out that ray cell length increases with plant vigor.

Ray cell dimensions observed by Bailey (1920) in one year old stems of Pinus strobus differed markedly with similar measurements for juniper. Measurement of 50 ray cell initials revealed a length of 17.8 microns with a tangential height of 22.9 microns and a tangential

Table 8. Mean tracheid and ray cell measurements (n=50), coefficients of variability, and Duncan's multiple range tests for data from two-year-old stems.

	Ray Cell Tangential Diameter (microns)			Ray Cell Radial Length (microns)		
	'Kallay'	'Hetzii'	'Fountain'	'Kallay'	'Hetzii'	'Fountain'
C.V.	13.30%	11.30%	13.30%	19.80%	17.30%	27.40%
Means[1/]	6.91[a]	6.53[b]	5.52[b]	21.14[a]	36.40[b]	36.93[b]

	Tracheid Diameter (microns)			Tracheid Length (microns)		
	'Kallay'	'Hetzii'	'Fountain'	'Kallay'	'Hetzii'	'Fountain'
C.V.	21.40%	17.40%	20.62%	20.50%	19.60%	18.10%
Means	3.70[a]	3.99[a]	3.94[a]	213.73[a]	344.73[b]	205.05[a]

Diameters of newly Formed Tracheid Elements Produced at the Graft Incision of the Stock (microns)

	'Kallay'	'Hetzii'	'Fountain'
C.V.	39.90%	30.30%	28.20%
Means	9.13[a]	7.84[b]	9.84[a]

[1/]Means followed by like letters not significantly different.

width of 13.8 microns. All but the latter measurement was larger than that observed for junipers. Because ray initials are typically flattened in a radial direction, tangential width in the pine would be expected to exceed that observed for Juniperus L. for mature cambial derivitives.

No significant difference in tracheid diameters was observed. However, 'Hetzii' had a significantly greater tracheid length.

Even though no differences were detected among clones with respect to mature tracheid diameter, 'Hetzii' differed significantly from the other junipers in diameter of new tracheids in the graft incision. Coefficients of variation for these means indicated a high degree of variability when compared to the other cells.

Tracheid length of 'Fountain' and 'Pfitzeriana Kallay' did not differ significantly, but both had shorter tracheids than 'Hetzii'. Measurements were considerably smaller than those reported for tracheids of the arborescent Juniperus virginiana reported by Bailey and Tupper (1918).

The size of new tracheids produced in the graft union were two or three times the diameter of those produced by non-wounded tissue; an observation also reported by Block (1952) in wound meristems, thus suggesting that graft union formation is basically a form of wound healing.

The comparative vigor of the nine interclonal juniper grafts and their component parts during the 60 days following grafting was measured by cell counts of all new differentiated tracheids arising during that period. The raw data for these counts are given in

Appendix Tables B1 and B2 respectively and condensed values are given in Tables 9 and 10 of the text.

Analyses of variance for wound tracheids and normal tracheids were computed independently on the assumption that no interaction existed between cell counts of each. When plotted (Figure 3) the parallelism between curves for the two classes of cell counts verified that interaction was absent.

Concommitantly this plot indicates that cell production in the stock and scion are similar. The slight depression in scion cell production in 30 day old normal wood was due to a general cessation of growth during the period when greater metabolic activity was occurring at sites of injury. Because wound tissue cell size exceeded that of normal cambial derivitives (Table 8), supression in normal wood production was not alleviated by an equal increase in cell numbers in the injured scion wood.

A significant F value for date means of wound tracheids and normal tracheids (Tables 9 and 10) signified that cell production at each date differed statistically from other dates.

The mean cell counts for all graft combinations revealed that fewer cell numbers were observed in Juniperus horizontalis 'Fountain' and Juniperus chinensis 'Pfitzeriana Kallay' self grafts in both normal and wound induced tracheids (Tables 9 and 10). The greater number of normal cells suggested that Juniperus chinensis 'Hetzii' self grafts were the most vigorous of the clones studied. If 'Hetzii', a triploid juniper (section I of thesis), originated from diploid and tetraploid parents; on the basis of hybrid vigor, it might be expected to produce

TABLE 9. RADIAL CELL COUNT AND STOCK-SCION MEANS FOR UN-WOUNDED XYLEM IN INTERCLONAL JUNIPERUS L. GRAFT PARTNERS AT 5 POSTGRAFT DATES. (N=18)

DAYS FROM GRAFTING	'FOUNTAIN'/ 'FOUNTAIN'		'KALLAY'/ 'KALLAY'		'HETZII'/ 'HETZII'		'HETZII'/ 'FOUNTAIN'		'KALLAY'/ 'FOUNTAIN'		'HETZII'/ 'KALLAY'		'FOUNTAIN'/ 'KALLAY'		'FOUNTAIN'/ 'HETZII'		'KALLAY'/ 'HETZII'	
	STOCK	SCION	STOCK	SCION	STOCK	SCION	STOCK	SCION	STOCK	SCION	STOCK	SCION	STOCK	SCION	STOCK	SCION	STOCK	SCION
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	3.7	0.0	0.0	0.0	0.0	0.0	0.0	2.2	1.9
30	6.6	2.8	0.0	0.0	9.7	1.7	7.2	3.8	9.5	4.5	7.8	6.3	6.1	3.9	6.9	3.4	7.7	3.0
40	6.3	4.6	7.3	9.4	9.5	10.0	12.9	6.0	15.7	7.4	10.2	11.4	11.9	8.5	11.9	7.8	15.6	11.5
50	13.2	10.4	14.7	0.0	22.2	19.8	15.3	11.3	14.7	11.6	21.2	18.0	14.7	9.7	17.0	13.5	17.6	12.3
60	18.0	10.8	19.2	19.3	21.6	18.6	19.7	17.3	20.3	27.5	17.3	28.8	35.9	18.6	24.7	15.2	22.7	17.6
MEANS	8.8	5.7	8.2	5.7	12.6	10.0	11.0	7.7	13.0	10.9	11.3	12.9	13.7	8.1	12.1	8.0	13.2	9.3
COMB MEANS	7.27		7.01		11.33		9.36		11.98		12.10		10.93		10.03		11.20	
DMR (1%) COMB	E		E		B C		D		A B		A		C		D		C	

ANALYSIS OF VARIANCE

SOURCE	DF.	S.S.	M.S.	F
DATES	4	26,398.0195	6,599.5039	3,303.8716**
GRAFT COMBINATIONS	8	1,744.6133	218.0767	109.1745**
GRAFT PARTNERS	1	1,093.6484	1,093.6484	547.5068**
DATES X COMBINATIONS	32	2,250.6367	70.3324	35.2101**
DATES X PARTNERS	4	315.6641	78.9160	39.5073**
COMBINATIONS X PARTNERS	8	458.2500	57.2813	28.6764**
COMBINATIONS X PARTNERS X DATES	32	2,094.8125	65.4629	32.7723**
ERROR	445	888.8906	1.9975	
TOTAL	539	35,259.0820		

+ MEANS FOLLOWED BY DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT.

** SIGNIFICANT AT 1% LEVEL.

TABLE 10. RADIAL CELL COUNT AND STOCK-SCION MEANS FOR WOUNDED XYLEM IN INTERCLONAL *JUNIPERUS* L. GRAFT PARTNERS AT 5 POSTGRAFT DATES. (N=18)

DAYS FROM	'FOUNTAIN'/ 'FOUNTAIN'		'KALLAY'/ 'KALLAY'		'HETZII'/ 'HETZII'		'HETZII'/ 'FOUNTAIN'		'KALLAY'/ 'FOUNTAIN'		'HETZII'/ 'KALLAY'		'FOUNTAIN'/ 'KALLAY'		'FOUNTAIN'/ 'HETZII'		'KALLAY'/ 'HETZII'	
GRAFTING	STOCK	SCION	STOCK	SCION	STOCK	SCION	STOCK	SCION	STOCK	SCION	STOCK	SCION	STOCK	SCION	STOCK	SCION	STOCK	SCION
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	1.3	0.0	0.0	0.0	0.0	0.0	0.0	1.9	1.0
30	2.7	1.6	0.0	0.0	3.1	2.3	3.7	1.9	2.7	1.8	3.6	3.1	5.0	2.6	4.7	2.1	1.6	2.1
40	3.7	3.0	5.4	6.0	9.7	5.8	6.7	4.4	7.2	5.2	10.6	6.7	7.6	3.6	6.6	5.4	11.2	6.8
50	7.8	5.8	9.1	0.0	9.1	6.1	11.6	7.3	7.6	8.0	12.1	10.9	9.0	6.7	12.3	12.4	14.1	7.2
60	7.4	8.9	16.6	12.3	13.8	11.8	11.4	8.0	14.6	18.1	21.4	17.3	21.9	15.2	13.1	14.8	11.6	10.3
MEANS	4.3	3.9	6.2	3.7	7.1	5.2	6.7	4.3	7.0	6.9	9.5	7.6	8.7	5.6	7.3	6.9	8.1	5.5
COMB MEANS	4.08		4.94		6.17		5.50		6.91		8.56		7.16		7.12		6.76	
DMR (1%) COMB	F		E		C D		D E		B		A		B		B		B C	

ANALYSIS OF VARIANCE

SOURCE	DF.	S.S.	M.S.	F
DATES	4	12,052.4375	3,013.1094	1,522.7893 **
GRAFT COMBINATIONS	8	871.5000	108.9375	55.0557 **
GRAFT PARTNERS	1	345.4297	345.4297	199.8454 **
DATES X COMBINATIONS	32	1,607.9570	50.2487	25.3951 **
DATES X PARTNERS	4	137.7305	34.4326	17.4018 **
COMBINATIONS X PARTNERS	8	149.0156	18.6270	9.4138 **
COMBINATIONS X PARTNERS X DATES	32	467.6172	14.6130	7.3853 **
ERROR	445	880.5117	1.9787	
TOTAL	539	16,571.6680		

+ MEANS FOLLOWED BY DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT.

** SIGNIFICANT AT 1% LEVEL.

a greater number of cells than either 'Fountain' (2n=22) or 'Pfitzeriana Kallay' (4n=44).

Cell counts showed 'Hetzii' on 'Pfitzeriana Kallay' grafts to have the greatest vigor in normal and wound induced tissue. 'Fountain' or 'Kallay' on the other understocks produced statistically equivalent numbers of cells in injured wood but all values were significantly greater than their self grafts. In normal wood 'Hetzii' self grafts, 'Kallay' on 'Hetzii' and 'Fountain' on 'Kallay' all responded equally but with significantly larger numbers of cells, than the other self grafts, 'Fountain' on 'Hetzii', or its reciprocal.

In both wounded and normal wood, 'Fountain' on 'Hetzii' grafts produced a significantly greater number of cells than their reciprocals which may be due to the differences in vigor of the self grafts of these clones. A comparison of the production of 'Fountain' versus 'Kallay' on 'Hetzii' in normal wood revealed significantly higher values for 'Kallay' than for 'Fountain'. Thus, the influence of stock on scion may be related to ploidy level. Wound tracheid numbers were not significantly different.

Significant differences among graft partners of both classes of wood (Tables 9 and 10) was interpreted as representing the differences between grand stock and scion means with the scions producing less cells than understocks. This relationship is illustrated in Figure 3.

The interaction between combinations and dates was found to be significant for both wound and normal tracheids. This relationship may have resulted from the failure of each graft combination to produce like numbers of cell at any given date. The most obvious example of

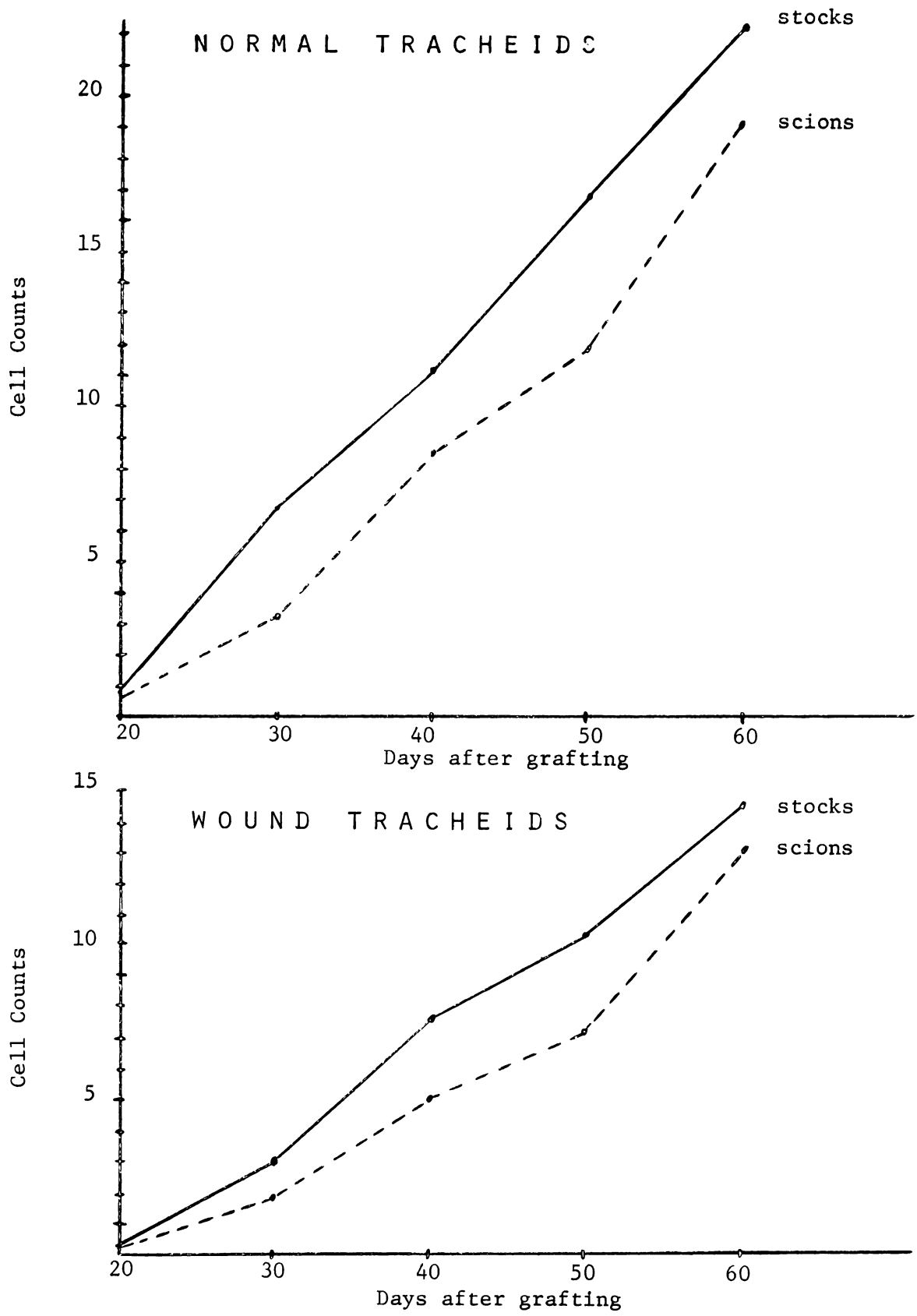


Figure 3 - Mean cell counts at wounded and un-wounded sites of stocks and scions of interclonal juniper grafts at five dates after grafting.

this interaction was the lack of cell division in 'Kallay' self grafts during the initial 30 days after grafting. By 40 days, however, these grafts were producing nearly equivalent numbers of cells. A similar but not as dramatic lag was observed in 'Fountain' self grafts in both wounded and normal wood during the same period.

'Hetzii' self grafts also exemplified the date by combination interaction. It was noted that in normal wood, cell counts at 50 and 60 days were equivalent, thus indicating a leveling off of cell division by that time. The wound induced tracheids of this graft had a similar plateau between 40 and 50 days after which activity was resumed to 60 days. A leveling off of cell production in wounded tissue was also noted in 'Kallay' on 'Hetzii' grafts between 40 and 50 days following grafting, but this behavior was not clearly evident in normal wood.

As a result of a failure of graft partners to respond in like fashion at any given date, a significant interaction was detected between partners and dates. Contributing to this interaction was the fact (Figure 3) that scions, in both wound induced and normal wood, produced a quantity of cells at 50 days equal to that produced by stocks at 40 days. These dates also show cell production to be equal in 20 day old grafts in both wounded and un-wounded wood; an observation interpreted as a lack of new tracheid production by either graft component. The convergence of the lines in Figure 3 at 60 days signified that healing and associated balance of cell production was nearing completion.

The significant interaction observed between graft combinations

and graft partners (Table 9 and 10) appeared to result from a failure of each clone to respond equally well when used as a stock and when used as a scion for the other clones. These relationships are presented in Table 11.

In combinations of 'Fountain' on 'Kallay' significantly greater numbers of cells were produced in normal wood of both stock and scion than in other combinations with 'Fountain' as a scion. When reciprocal graft means were examined for normal tissue, the same relationship held. This observation indicated the enhancing effect of 'Fountain' on 'Kallay' self grafts in the plant extract experiment results reported earlier (Table 7).

When wound tracheid numbers were compared with 'Fountain' as the scion, response was greater in 'Fountain' on 'Hetzii' than in other combinations; this being considered a probable reflection of the effect of 'Hetzii's' greater vigor on the low vigored 'Fountain'. The influence of 'Fountain' scions on 'Kallay' stocks was again apparent when stock counts were made. As an understock, 'Fountain' also had a significant influence upon cell counts of 'Kallay' scions, but no significant differences were observable between 'Fountain' understocks when using 'Hetzii' and 'Kallay' as scions.

Examination of data for 'Hetzii' scions indicated that in both wound induced and normal wood, cell production increased with increasing ploidy level of the stock in the order: 'Fountain', 'Hetzii' and 'Kallay'. This relationship held for understock cell production for wound tracheids only, the 'Hetzii' stock values were significantly greater than those for 'Kallay' in normal wood. As an

Table 11 - Comparative mean radial cell counts for wound induced and normal stock and scion tissue produced after grafting in nine interclonal juniper graft combinations.

NORMAL TRACHEIDS

stock \ scion	'Fountain'	'Hetzii'	'Kallay'	stock means	scion means
'Fountain'	5.7 / 8.8	7.7 / 11.0	10.9 / 13.0	10.9	8.1
'Hetzii'	8.0 / 12.1	10.0 / 12.6	9.3 / 13.2	12.6	9.1
'Kallay'	8.1 / 13.7	12.9 / 11.3	5.7 / 8.2	11.1	8.9
scion means	7.3	10.2	8.6		8.7
stock means	11.5	11.6	11.5	11.5	

WOUND TRACHEIDS

stock \ scion	'Fountain'	'Hetzii'	'Kallay'	stock means	scion means
'Fountain'	3.9 / 4.3	4.3 / 6.7	6.9 / 7.0	6.0	5.0
'Hetzii'	6.9 / 7.3	5.2 / 7.1	5.5 / 8.1	7.5	5.9
'Kallay'	5.6 / 8.7	7.6 / 9.5	3.7 / 6.2	8.1	5.6
scion means	5.5	5.7	5.4		5.5
stock means	6.8	7.8	7.1	7.2	

understock, 'Hetzii' did not follow the pattern observed for scions. Although significantly greater numbers of cells were produced with 'Kallay' as the scion in both classes of wood, self grafts exceeded 'Fountain' in cell production; the reverse was observed for wound tracheids.

In contrast to the generally observed tendency for scion cell production to increase directly with ploidy level of the understock, 'Kallay' scions varied inversely with ploidy level; largest counts being observed where 'Fountain' was the understock. In understocks this trend was broken by the larger numbers of cells produced by 'Hetzii' understocks. In reciprocal grafts 'Hetzii' scions also produced the largest cell numbers when combined with 'Hetzii' scions, while in normal wood, it produced significantly more cells when 'Fountain' was the scion.

The major problem in adapting the cell extraction procedure to graft unions was the maintenance of the structural integrity of the grafted tissue throughout the extraction procedure. A procedure developed in this study was satisfactory for securing the tissues to the slide, however, some of the parenchymatous callus was still lost during extraction. The use of silicone rubber to adhere the sections, as described by Holmes and Brown (1967), appeared to offer promise for overcoming problems encountered, but this was published after the work was completed.

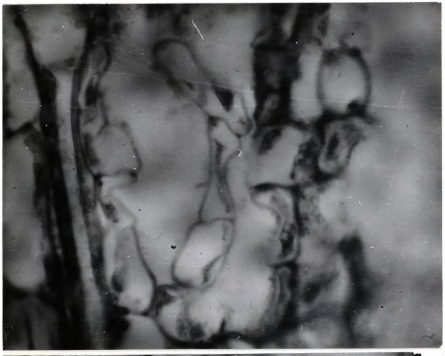
To study pectic substances in common walls of mixed stock and scion cells, a series of sections from graft combinations were stained with ruthenium red.

Although reported to lack specificity for pectins (Hock, 1942; Kertesz and Loconti, 1944; Kertesz, 1951; Bonner, 1936), ruthenium red clearly delineated the middle lamellas of mature tracheids. In callus cells, however, the stain was diffused throughout the entire cell wall and masked the middle lamella. This precluded the possibility of distinguishing stock callus from scion callus on the basis of presence or absence of middle lamella.

Localization of ruthenium red in typical graft union tissue in longitudinal section is illustrated in Figure 4A. The lack of a clearly defined middle lamella is shown in the photomicrograph. The lack of definition may be an indication that this structure is comprised of other substances as well as calcium pectate. If composed of a protein gel (Ginzburg, 1958, 1961), or cellulose adcrusted with pectin (Preston, 1964) its presence might be quite ill defined.

The pectin localization in longitudinal section of wood ray cells at the xylem-pith junction is illustrated in Figure 4B. Heavy pectin localization was evident in the tangential walls (arrows) when compared to the staining of the radial cell walls. Correspondingly there were more pits in the tangential walls.

A



B

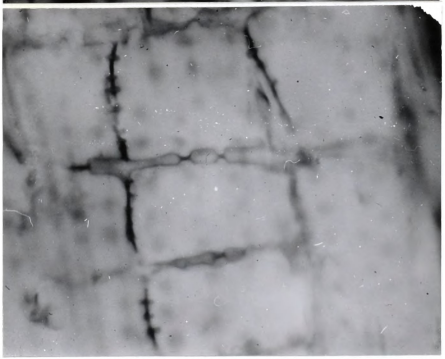


Figure 4 - Pectic substance localization in juniper graft unions and in medullary ray cell walls. A. The diffuse nature of ruthenium red staining substances in graft callus cells. B. Typically heavy concentration of pectic substance in tangential walls of ray cells and characteristic heavy pitting in these walls.

Summary

Interploidy grafts of Juniperus horizontalis 'Fountain' (2n=22), Juniperus chinensis 'Hetzii' (3n=33), and Juniperus chinensis 'Pfitzeriana Kallay', were prepared to study relationships between ploidy level and graft success. Self grafts of the diploid and triploid junipers were highly successful while those of the tetraploid Juniperus chinensis 'Pfitzeriana Kallay' were unsuccessful. Since most unsuccessful graft combinations contained 'Pfitzeriana Kallay' as one partner it was postulated that an insufficient level of a growth substance or growth inhibitor may have been responsible.

Most successful interclonal grafts contained the diploid juniper Juniperus horizontalis 'Fountain', thus suggesting the presence of a substance capable of improving graft union development.

Self grafts were prepared and tissue extracts from one of the other junipers was applied to the cut surfaces in an attempt to detect species limited substances which might influence graft union development. The effect of plant extracts upon graft formation and development was an alteration of the percentage of graft successes in the difficult to graft combinations.

Application of 'Hetzii' or 'Pfitzeriana Kallay' extract to the cut surfaces of the 'Fountain' juniper produced no changes in total success, but 'Pfitzeriana Kallay' extract produced less visible scion vigor.

The most marked effect of tissue extract on graft success was the application of 'Fountain' extract to cut surfaces of 'Pfitzeriana Kallay'. This treatment resulted in 80% grafting success as compared

to 25% when un-treated. Ultimate vigor of this treatment was superior to self grafted controls and the other treated grafts.

The presence of a growth stimulating substance in Juniperus horizontalis 'Fountain' was postulated, and absence of, or reduced levels in Juniperus chinensis 'Pfitzeriana Kallay' were suggested for the differential response of the extracts on graft success and vigor.

The 20 day delay in callus tissue production in self grafts of 'Pfitzeriana Kallay' as compared to 'Hetzii' or 'Fountain' suggests that the failure and lack of scion vigor in grafts involving 'Pfitzeriana Kallay' may result from this condition.

Self grafts of Juniperus chinensis 'Hetzii' responded very poorly when treated with 'Pfitzeriana Kallay' extract. This suggests that this extract may interfere with normal callus production and graft development. The possible suppressing effect of this extract and the total lack of success of 'Fountain' treated 'Hetzii' self grafts were impossible to explain and await further study.

Results of ray cell measurements revealed that the cells of 'Pfitzeriana Kallay' were significantly shorter and larger in diameter than those of the other two junipers. No statistical difference was observed between 'Hetzii' and 'Fountain' junipers. However, the tracheid cells of 'Hetzii' were significantly longer. Diameter of 'Hetzii' wound tracheids was significantly smaller than those of 'Pfitzeriana Kallay' or 'Fountain', with no difference between 'Pfitzeriana Kallay' and 'Fountain'. Wound tracheid cells were two to three times the diameter of normal mature cells.

Cell counts of new tracheids, both in normal wood and in tissue

arising at graft surfaces, revealed that the contribution of the stock was greater than the scions. The lowest numbers of cells were in the self grafts of Juniperus horizontalis 'Fountain' and Juniperus chinensis 'Pfitzeriana Kallay'. The most vigorous combination was 'Hetzii' on 'Pfitzeriana Kallay'. The highest cell counts among self grafts were in 'Hetzii'. This may be a possible "hybrid vigor" of this triploid juniper over the diploid 'Fountain' and tetraploid 'Pfitzeriana Kallay'.

In both wound induced and normal tissue, 'Pfitzeriana Kallay' self grafts failed to initiate new tracheal cells during the first 30 days after grafting. A similar but shorter lag in cell production was found in 'Fountain' self grafts. In normal wood of 'Hetzii' self grafts a plateau in cell production was reached between 50 and 60 days, while in wounded wood, the plateau was reached between 40 and 50 days. The fact that stock and scion cell counts had approximated one another by the 60th day following grafting was considered to be indication that graft healing was near completion by that date.

With the exception of 'Fountain' on 'Hetzii' grafts, when used as scions, cell counts of these clones increased significantly with increasing ploidy level of the understock on which they were grafted. With 'Pfitzeriana Kallay' as a scion, however, the inverse relationship was observed.

Of the three clones used as scions, highest mean cell counts were observed with 'Hetzii', but as an understock, greater counts were observed in 'Pfitzeriana Kallay'. 'Hetzii' scions produced the largest cell numbers in normal wood while 'Pfitzeriana Kallay' numbers were

greater in wounded wood.

In all grafts involving 'Fountain' and 'Pfitzeriana Kallay', cell counts of 'Pfitzeriana Kallay' were significantly higher than the self grafts of each clone. This substantiated the effect observed when tissue extracts of 'Fountain' juniper was applied to 'Pfitzeriana Kallay' resulting in increased graft success.

Due to heterogeneity and structural weakness of the tissue, a cell wall extraction procedure for studying cell wall constituents during graft healing was not successfully adapted to this investigation.

Staining with ruthenium red was found to be rather diffuse throughout primary cell walls rather than being limited to a well defined lamellar area thus eliminating this as an identification of cellular contribution by the stock and scion. Observations were made, however, on the highly pectinaceous nature and heavy pitting of xylem ray cell tangential walls as compared to their radial walls.

Of greatest significance among results of this study was the effect of certain juniper clones on grafting success. The beneficial effect of Juniperus horizontalis 'Fountain' upon graft success and cell production of Juniperus chinensis 'Pfitzeriana Kallay', whether applied as an extract to self grafts or as a graft component, was a prime example. Such findings justify further investigation into the nature of the effects observed in the present study.

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Summary

Using Juniperus horizontalis 'Plumosa Compacta', Juniperus horizontalis 'Fountain', Juniperus sabina 'Von Ehron', Juniperus chinensis 'Hetzii', and Juniperus chinensis 'Pfitzeriana Kallay', a series of related experiments were conducted to study the cytological, anatomical, nutritional, immunological, and cultural aspects of self and interclonal graft unions during their development.

Because of possible cytological implications in graft union behavior, somatic chromosome counts were made for three previously uncounted juniper clones. An unexpected chromosome number of 3n=33 was obtained for Juniperus chinensis 'Hetzii', and counts of 2n=22 were noted for Juniperus horizontalis 'Plumosa Compacta' and Juniperus sabina 'Von Ehron'.

Comparative anatomical studies of Juniperus horizontalis 'Fountain', Juniperus chinensis 'Hetzii', and Juniperus chinensis 'Pfitzeriana Kallay' revealed that tangential medullary ray cell diameter increased with increasing ploidy level.

Anatomical study of interclonal juniper grafts showed no basic difference in developmental sequence; only differences in timing of each stage.

Filling of voids between stock and scion with callus from phloem and cortex was the first observable event in healing. This stage was reached by the end of 20 days and was first observed in grafts with 'Fountain' as a component, and last in those containing 'Pfitzeriana Kallay'.

After 20 to 30 days, isodiametric cells appeared from uninjured cambial tissue adjacent to the graft incision, which by tangential and radial divisions overwalled the injured graft surfaces. This did not occur in 'Pfitzeriana Kallay' until the period 30 to 40 days after grafting.

By radial divisions, overwalling cells filled the area between stock and scion with well organized tissue and crushed intervening callus by the end of 40 to 50 days.

In 50 to 60 day old grafts, a "cambial bridge", composed of typical elongated tracheids, formed between cambial rings of stock and scion. From the new cambial bridge, new xylem began to form. By this date, new tissue contribution was equivalent from stock and scion.

Study of mixed graft tissue revealed that common walls of heterogeneous cell pairs appeared as double wall structures, each with middle lamellas. Abnormally large numbers of pits, and occasional blind pits, believed due to unaligned pit pairs, were also observed.

Mineral distribution of interclonal juniper grafts, studied by ashing of entire graft components, showed 'Hetzii' scions to accumulated more calcium and potassium than in understocks. 'Plumosa Compacta' self grafts accumulated potassium in the understock while, in other self grafts, this element was equally distributed.

Data obtained from ashing and photometry indicated that rate of translocation of elements studied was, in decreasing order, potassium, calcium, and magnesium; the latter was relatively unrestricted at the

graft union.

Electron microprobe X-ray analysis showed that calcium tended to accumulate in the understock xylem of typical interclonal juniper grafts, while potassium and magnesium appeared to move readily across the graft union.

Although elements were equally distributed between phloem and cortex of stock and scion, slight magnesium accumulated was found in the phloem and significant amounts of calcium were observed in the scion cortex. Accumulation of this element in understock xylem led to a postulated lateral movement to the understock cortex where diffusion through the union occurred.

Microprobe analysis indicated the rate of element translocation at the union to be, in decreasing order; magnesium, potassium and calcium.

To determine the feasibility of serological techniques for graft union study, interclonal graft union microslide preparations were treated with fluorescein labelled antibody preparations. Immunization of inbred white mice with leaf and stem extract of Juniperus horizontalis 'Fountain' and Juniperus chinensis 'Pfitzeriana Kallay' resulted in strong, titer levels as measured by microprecipitin reactions. Cross reactions showed 'Fountain' to have greatest antisera specificity.

In 'Fountain' on 'Pfitzeriana Kallay' grafts incubated with 'Fountain' antibody, fluorescence of the scion exceeded that of the stock. Labelling with 'Pfitzeriana Kallay' antibody resulted in major fluorescence in the stock.

Though antibody specificity was generally greatest in parenchymatous tissue, 'Fountain' antibody fluoresced more in the xylem.

During the course of serological study, micro Kjeldahl determinations showed 'Pfitzeriana Kallay', a tetraploid with 4n=44, to contain slightly more than twice the amount of protein nitrogen than the diploid 'Fountain' (2n=22); suggesting that differences in fluorescence levels may have been partly due to amount of protein available for labelling.

Among the self grafts, 'Hetzii' and 'Fountain' were found to be successful, while 'Pfitzeriana Kallay' was unsuccessful. Degree of success was accordingly affected in interclonal grafts involving each of these clones as components.

Studies with self grafts in which the cut surfaces had been treated with a leaf extract from another clone at time of grafting showed 'Fountain' to improve graft success of 'Pfitzeriana Kallay'; self grafts of which were not successful in control grafts.

When applied to 'Fountain' self grafts, 'Pfitzeriana Kallay' caused no change in graft success but a reduction in scion vigor was noted. 'Hetzii' self grafts, however, responded very poorly to application of this extract.

Cell measurements indicated 'Pfitzeriana Kallay' to have the shortest, thickest ray cells of the junipers studied, while 'Hetzii' tracheids were found to be the longest. Tracheids formed subsequent to wounding were larger than those in normal tissue.

Cell counts revealed that greatest contribution of new tracheids arose from the understock of juniper grafts. Among self grafts,

'Hetzii' scions produced largest numbers of cells while 'Pfitzeriana Kallay', used as a scion for interclonal grafts, produced greatest cell numbers.

'Pfitzeriana Kallay' self grafts failed to produce new tracheal cells during the first 30 days after grafting. A similar but shorter delay was also found in 'Fountain' self grafts. In 'Hetzii' self grafts, a plateau in cell production between 50 and 60 days in normal wood, and between 40 and 50 days in wound induced wood, was observed.

In most instances, scion cell counts of all clones increased significantly with increasing ploidy level of the understock; the reverse occurred when 'Pfitzeriana Kallay' was the scion.

In most graft unions 'Hetzii' scions and understocks produced greatest cell numbers. In wound tissue, however, 'Pfitzeriana Kallay' understocks produced greatest numbers of cells.

In 'Fountain' on 'Pfitzeriana Kallay' grafts, cell numbers were significantly higher than those of either self graft; this thought to be an expression of the effect observed when 'Fountain' extract was applied to 'Pfitzeriana Kallay' self grafts.

Ruthenium red staining revealed that pectic substances were concentrated in the middle lamella of mature tracheids but very diffuse in undifferentiated tissue.

In addition to adding to our basic knowledge of the cytology, histology, and sequence of events occurring during graft healing of certain clones of Juniperus L., new procedures showing promise for future studies were tested, and certain new avenues of approach in graft studies were brought to light.

APPENDIX B

Appendix Table B4. Analyses of variance for stock-scion mineral content ratios of juniper grafts sampled at regular intervals after grafting*

Calcium

SOURCE	D.F.	S.S.	M.S.	F.
Total	269	6,034.86		
Dates	9	118.13	13.13	.75 N.S.
Combinations	8	1,066.51	133.31	7.61**
D X C	72	1,695.01	23.54	1.34 N.S.
Error	180	3,155.21	17.53	

Potassium

SOURCE	D.F.	S.S.	M.S.	F.
Total	269	5,979.06		
Dates	9	172.43	19.16	.919 N.S.
Combinations	8	637.22	79.65	3.820**
D X C	72	1,416.30	19.67	.940 N.S.
Error	180	3,753.11	20.85	

Magnesium

SOURCE	D.F.	S.S.	M.S.	F.
Total	269	8,017.15		
Dates	9	510.93	56.77	3.21**
Combinations	8	2,096.13	262.02	14.80**
D X C	72	2,222.25	30.87	1.74**
Error	180	3,187.84	17.71	

*Analysis of variance based upon arcsin transformed data.