ROOT GRAFTING IN PINUS RADIATA D. DON.

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by
John Page Wood
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This statement is to certify that the experiments described in this thesis were my own original work.

John Page Wood.
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SUMMARY

1. Root grafting, as deduced from the number of living stumps in thinned stands, was widespread in *Pinus radiata* D.Don. stands in the A.C.T. It commenced only when the stands were 10 years old but then increased rapidly with increasing stand age up to at least 25 years.

2. The connection of live stumps to live trees, either directly or indirectly through another living stump, was established by excavation of some root systems.

3. There was no evidence that silvicultural treatment, such as initial spacing of the trees or thinning, influenced the incidence of root grafting. Shallow soils appeared to inhibit root grafting and/or survival of living stumps.

4. The radioisotope, $^{86}\text{Rb}$, and the dye, acid fuchsin, were used successfully to trace movement through fused root systems. Both tracers moved from living stumps to associated living trees at rates of 2 to 10 inches per hour, but no movement in the opposite direction was detected. Limitations in the field application of these techniques are discussed.
5. There was no evidence that living stumps influenced the growth of associated trees.

6. Techniques for grafting seedling roots, and preliminary experiments on the movement of substances through such grafts, are described.
CHAPTER 1

INTRODUCTION

Forest authorities in Australia have become increasingly aware of the importance of forests as a renewable national asset and have consequently undertaken a greatly intensified re-forestation scheme to meet anticipated forest demands. *Pinus radiata* D. Don, because of its adaptability and exceptional growth, has become the major re-forestation species in southeastern Australia, and in 1967 covered over 500,000 acres with an additional 50,000 acres being planted annually (F. & T.B. 1967). The last decade has seen a correspondingly increased interest in those factors which affect the growth and development of radiata pine, yet little attention has been given to the underground portions of the tree, which are just as vital as the above ground parts to its continued well-being and survival.

Root grafting is a natural phenomenon common to a great number of tree species and is found frequently in radiata pine. Although it has been reported that root fusion in other species may have considerable ecological, pathological and
silvicultural implications, there is little quantitative information available as to its incidence or significance in *Pinus radiata*.

For these reasons, this study of root grafting in *Pinus radiata* was undertaken to determine:—

(1) the age at which root grafting begins, and its incidence throughout the life of a stand;

and (2) the possible significance and function of root grafts within forest stands.

LITERATURE REVIEW

1.1 **Natural occurrence of root grafts**

Root grafts occur widely in nature and are of three basic types: self grafts, intraspecific grafts, and interspecific grafts. Self grafts form between roots of the one tree, whilst intraspecific grafts are found between roots from different individuals of the one species. Interspecific root grafts, involving roots from two different species, are uncommon (Beddie 1941). Each type of root graft may be further classified according to its shape or orientation; for example, intersecting grafts, longitudinal grafts and bridge grafts (Graham 1959). However, it is considered that the terms polar, antipolar and right-angular root grafts (Armson and Driessche 1959) are more meaningful as they convey an idea
of the orientation and physiological alignment of the roots taking part in the fusion.

Graham and Bormann (1966) reviewed the reported incidence of root grafts in various species throughout the world. These will not be reiterated here, but some further references, including those reported in Australia, are given in Appendix A.

1.2 Mechanisms of root graft formation

Root fusions may form in various ways. Specialized mechanisms are involved in the positive attraction of some parasitic tree roots to their hosts (Iyengar 1965), and in the ready fusion of the aerial roots of Ficus globosa (Rao 1966). For most species, however, the mechanisms by which roots graft are best described by Graham and Bormann (1966) who used the roots of Pinus strobus to illustrate the process. Two roots growing parallel to, or across, each other establish a point of contact. Since both roots are firmly supported by the soil on either side of the point of contact, subsequent radial growth causes a pressure to develop. As this pressure increases, ridges of tissue are formed, the intervening bark breaks down, and eventually, vascular continuity is established. Bormann (1966) stresses, however, that vascular development may not proceed equally in all areas of
the contact zone. Where the tissues are anatomically and physiologically aligned, normal tissue growth occurs and an efficient union is established, but where tissues within the two roots are oppositely aligned, irregular and highly convoluted grain develops. This led Bormann to propose that effective root fusion can only occur between tissues with normal anatomical and physiological alignment. Whilst some others support this view (Yli-Vakkuri 1953), work done with artificially induced root grafts suggests that functional antipolar grafts can be formed (Fischer et al 1960).

Tissue growth around the graft union tends to be distorted for two or three years before continuous growth rings are produced, enabling the age of the root graft, and the roots concerned, to be estimated from cross-sections. Normally, root grafts can only be verified by checking for vascular continuity; this necessitates partial bark stripping or even sectioning the suspected graft to ensure that xylem tissue is continuous and not separated by firmly enclosed bark.

1.3 Factors modifying root graft incidence

Root graft formation is modified by a complexity of factors, all of which interact in nature. For convenience, these factors
will be treated separately and have been grouped under "tree", "physiological", "environmental", and "silvicultural" factors.

(i) **Tree factors:** The rooting habit of tree species may be such as to give rise to a greater or smaller number of root contacts (La Rue 1934). For example, trees with a predominantly vertical, as opposed to a horizontal, root spread could be expected to form root grafts less frequently; this may explain some of the differences in root graft incidence between species.

Tree age, *per se*, seems to have very little influence on root grafting. Seedlings of various species, one to five years old, form root grafts when grown at close spacings either in nurseries or in the field (Berchenko 1959, Graham and Bormann 1966, Schultz and Woods 1967). There are indications however, that very young roots must be woody (Beddie 1941) and be above a minimum size (Fischer *et al.* 1960) before fusion will occur. Conversely, trees up to 65 years old have formed functional root grafts (Stout 1956).

As a tree grows, its roots spread radially outwards from its base. The rate of spread for a particular tree and its surrounding neighbours will determine, for a given spacing, when roots will overlap and be likely to fuse. Initially, root contact is possibly
avoided through root deflection to evade the zone of exhausted soil moisture immediately surrounding active roots (Wendelken 1955). As root density increases, this mechanism breaks down and contacts are established. A number of workers have described the root spread of various species using isotope and excavation techniques (Stout 1956, Curtis 1964, Hough et al. 1965; Ferrill and Woods 1966, Brown and Woods 1968, Grose 1968). Grose's results are particularly interesting as he was able to construct a table showing the average root spread of trees of different crown classes with time. With similar quantitative data for other species, it should be possible to predict when and where root grafts are most likely to form.

Since the root spread of a tree is related to its crown class (in the root studies of a number of species referred to above, mean lateral root spread was approximately four to five times the crown radius) a higher incidence of root fusion may be expected in trees of the better crown classes. Grose (1968), in fact, found a relationship between crown class and root graft incidence. No root fusions were observed between suppressed, or between suppressed and intermediate class trees, due possibly to the limited root spread of these trees and/or to the low rate of
diameter increment of the roots. Pawsey (1962) and Yli-Vakkuri (1953) found relationships similar to those described, but Lanner (1961) and De Byle (1964) failed to find any correlation between crown class and root graft incidence.

(ii) Physiological factors: Incompatibility of stem grafts is well recorded in the literature and could be expected to occur on a similar scale in roots, yet little reference has been made to it. Grose (1968), working with white spruce, observed many instances where considerable growth malformation had resulted from roots exerting pressure on each other without root fusion taking place. He suggested that this indicated root incompatibility. This could be a further cause of the infrequency of root grafts in some tree species.

Interspecific root grafts are sometimes found in nature (Beddie 1941, Jones and Partridge 1961), indicating that root tissues can unite across species boundaries. It would be extremely interesting to observe the tissue anatomy at such a root graft interface, and to determine whether interspecific root grafts are capable of supporting the life of stumps. Interspecific root grafts occur infrequently and although the root systems of different species may be so interlocked that they cannot be separated intact,
fusion rarely takes place (Hellmers et al. 1955). Whenever root grafting (other than self grafts) occurs it is likely that the roots involved are genotypically unlike. Such grafts must show varying degrees of compatibility, so it would seem logical to expect differences in the mechanism and function of root grafts on this basis (Graham 1960).

(iii) **Environmental factors:** La Rue (1934) and Bormann and Graham (1959) considered that the soil plays an important part in root graft formation, even though some tree species appear to be particularly adaptable and will form root grafts in any soil type. For most tree species, excavation studies have shown the majority of root grafts to be formed in the top 12-18 inches of soil where roots are normally concentrated (Armson and Driessche 1959, Bormann and Graham 1959, Schultz and Woods 1967). However, in situations where impervious clay layers, bed rock, or high water tables confine roots to a shallow soil layer, increased root density results in increased root fusion (Stout 1956, Fayl 1965, Fraser and Gardiner 1967, McCavish 1967). Rock-ines within the soil profile also appears to increase the incidence of root grafting by enforcing intimate root contact through confinement and channelling of roots as they pass around obstructions.

There is only limited information on the influence of site quality on root grafting. It could be predicted from studies of the relationship between tree vigour and root spread that the radial root development of trees on a high quality site would be much greater than the root spread of a similarly aged stand on a lower quality site, so that the chances of roots contacting and fusing would be greatest on the higher quality sites. This proposition is confirmed, in part, by trends found in Pinus radiata stands (Pawsey 1962), and by De Byle (1964) who observed frequent root fusions in high quality stands of Populus tremuloides but none in low quality stands of the same age.

(iv) Silvicultural practices: Silvicultural practices influence root grafting in forest stands by altering the opportunity for root contact. Site preparation before planting may confine root development to localized regions and thus induce greater root fusion. For example, plough layers (Armson and Driessche 1959), mounding of soil in poorly drained areas, and deep row ripping in shallow soils, all have the effect of confining and concentrating roots.
Different planting techniques modify the incidence of grafted roots. For instance, the cluster method employed by the Russians promotes early root fusion (Berchenko 1959, Lanner 1963), whereas the roots of regularly spaced seedlings (6' x 6' to 9' x 9') in plantations cannot be expected to fuse until sufficient time has elapsed to allow overlapping of root systems. Closer spacings will produce greater root overlap at an earlier age and will result in greater root graft incidence. The chances of roots fusing in uniformly spaced plantations are considered fewer than in natural stands where individual trees are commonly more closely spaced (Schultz and Woods 1967). Thinning practices also alter root graft and living stump incidence. A thinning from below, where suppressed and intermediate trees are removed, will result in a higher percentage of the remaining living trees being root grafted with a low incidence of living stumps; a thinning from above will increase the numbers of living stumps, but may decrease the percentage of trees root grafted (Armson and Driessche 1959, Schultz 1962). With time, the number of root grafts present in a stand will decrease because of increasing death of root systems through subsequent thinnings. The use of chemicals to de-bark or thin forest trees drastically reduces root
graft incidence, as it eliminates the possibility of the treated root systems remaining alive, and may even result in the death of associated untreated trees.

1.4 Root graft function

The fact that living stumps survive and produce annual growth rings indicates that root grafts do function and allow foods and hormones to flow across the root graft interface. Very little is known about how or why root grafts function as they do - yet this information is vital to the full understanding of the role of root grafts. Movement appears to occur within the phloem and xylem but as different mechanisms operate in each case they will be considered separately.

Organic substances within the phloem have a marked capacity for lateral movement, and lateral transfer across the root graft interface appears to occur whenever favourable gradients exist. Hence, following loss of vigour by one member of a grafted pair (by suppression or crown removal), a food gradient may be established enabling continuing growth of the lower bole and/or root system of the non-vigorous grafted member (Bormann 1966). Little else is known about organic movement across the root graft interface or what factors may influence the
direction of flow.

Translocation studies, using isotopes and dyes as tracers, have led to a much better understanding of the factors controlling xylem movement across the root graft interface. Whilst some of the results are conflicting, this only reflects an inability to control the complexity of factors influencing the function of root grafts in the natural environment. Graham (1960) considered that dye uptake through injected living stumps reflected the physiological responses of associated intact trees, as well as the number and size of grafts involved, the degrees of anatomical or biophysical compatibility, and microenvironmental conditions influencing transport through individual roots. He showed that dye uptake was increased on days of high transpiration but failed to obtain any patterns of periodicity. However, Schultz and Woods (1967) found extreme diurnal fluctuation in half of the 20 living stumps they injected with $^{32}\text{P}$. The intensity of insolation was closely correlated with $^{32}\text{P}$ transfer to the associated living tree, suggesting that many root grafts only function during times of high water stress. De Byle (1964) considered that solute concentration gradients induce some additional movement. The failure of dyes and isotopes to move into and across all known
root grafts, even during times of high water stress, would seem to indicate that other physiological and environmental factors affect root graft function. The degree of functionality of a particular graft is thought by Bormann and Graham (1966) to be modified by changes in polarity and tissue distribution patterns resulting from the orientation of the graft. However this does not explain the failure of some grafts to function. As this knowledge is of prime importance in disease control and the proper use of chemicals in the forest, much more research into this aspect of root grafts is warranted.

Not all root grafted stumps remain alive after a thinning; survival depends on the size, number, and effectiveness of root grafts already formed, together with the vigour of the associated living tree (Lanner 1961). Of trees that do survive, the majority produce annual growth rings with food supplied by the host tree. Usually ring width decreases with increasing age of the living stump, but instances where, for a short period, ring width immediately after thinning was greater than before, have been reported (Lanner 1961, Satoo 1964). This can probably be attributed to the use of stored foods within the living stump for new xylem production, and it may explain why
the width of the first three to five growth rings in *Chamaecyparis obtusa* living stumps was correlated with the vigour of the tree prior to felling (Satoo 1964).

As a living stump ages, annual growth normally becomes confined to areas above grafted roots, giving rise to discontinuous growth rings. This decrease in wood production implies a diminishing food supply from the host tree (Schultz and Woods 1967), as a result, perhaps, of declining root graft function. However, as many living stumps have survived for extended periods with continued production (Davidson 1963) senescence does not appear to be the only factor limiting food supply. Studies of the root systems of living stumps reveal that a large proportion of small feeder roots die soon after the tree's crown is removed (Bormann and Graham 1959, Pawsey 1962, Bormann 1966), and in a quantitative study of the root system of white spruce (*Picea glauca*), Grose (1968) found that the average living stump seven years after thinning carried only 5.2 per cent of the weight of fine roots on an average living tree. This indicates that very little of the food transported across a root graft is invested into feeder root maintenance or primary root growth. Whilst there are exceptions to this overall trend, the
absence of fine roots could explain the gradual decline of living stumps, for they would tend to become moribund and die.

1.5 Practical implications of root grafts.

Root fusion is so profuse in many tree species that traditional ideas of competition should be modified (Kuntz and Riker 1956, Cooper 1961, De Byle 1964). Bormann (1966) considered that "the development of a naturally occurring white pine stand is shaped by two contrasting ecological factors (1) competition and (2) non-competitive forces governed by inter-tree food translocation. Competition operates to reduce the number of individuals and to increase the volume of the environment occupied by each individual. The non-competitive force, on the other hand, counteracts the effects of competition by delaying the death of individuals. Competition is the more important force". In another paper however, Bormann (1962) indicated that hormone transfer from a host tree could seriously disrupt the physiology of an associated non-vigorous grafted tree, thus contributing to the eventual demise of the non-vigorous tree's crown.

In times of very severe stress it has been suggested that a root grafted union could act as a unit and transport substances from stem to stem along tension and diffusion gradients (De Byle 1964, Bormann 1966). While isotope and dye translocation studies
indicate such movement to be very slow, work done with inter-
 twined root systems (Bormann 1959), and with artificially root
grafted seedlings (Fischer et al. 1960) under controlled conditions,
demonstrates that water moves from a non-stressed plant to a
stressed plant in sufficient quantity to delay or even prevent death
of the stressed plant. Similar transference of substances within
root grafted unions could explain why groups containing dominant,
intermediate, and suppressed trees persist in native and untreated
forest stands, as normal competitive factors would be expected to
disrupt any such patterns by eliminating the non-vigorous trees
present (Laessle 1965, Bormann 1966).

Few studies on the role of root fusion in interspecific
competition have been carried out, so it is difficult to say
whether it is beneficial, detrimental, or neither. The belief that
root fusion does aid interspecific competition has led many
Russian authors to recommend nest planting as a standard
silvicultural practice, but Lanner (1963), while reviewing pertinent
Russian literature, explained the political, rather than the
scientific reasons for this belief, and concluded that the method
had not fulfilled its expectations.

It has long been thought that root fusion increases the
stability of trees normally subjected to wind throw (Rigg and Harrar 1931). In a study to determine the resistance of trees to lateral stress on three different sites, McCavish (1967) concluded that grafting and interwining of root systems was responsible for the increased stability observed on drier sites, and not deeper and wider root spread as was previously thought. Hence, where wind throw is likely to be an important factor, planting techniques which encourage root fusion should be practised (Graham and Bormann 1966).

Graham and Bormann (1966) have comprehensively reviewed the literature on the pathological implications of root grafting so that, for the sake of completeness, only broad considerations will be covered here. Some serious tree diseases are spread by root grafts and/or root contacts e.g., Dutch elm disease (Ceratostomella ulmi), oak wilt (Chalara quercina), root rot (Fomes annosus), and shoestring fungus (Armellaria mellea). An awareness of the role root grafts play in the transmission of these diseases is essential before effective control and eradication measures can be taken. After a thinning, dead stumps provide an ideal substrate for Fomes annosus prior to attack on living root systems. Lanner (1961) and Bormann (1961) suggested that since living stumps
appear to be resistant to fungal and insect attack, their formation should be encouraged. As this is not always possible Boyce (1966) proposed an alternative method involving natural antibiosis. If a stand is innoculated with *Peniophora gigantea* prior to thinning, the fungus will compete successfully with *Fomes annosus* for available colonization sites and thus restrict the spread of *Fomes*. Whilst living stumps may provide some immunity in this particular instance, it should be realized that in many vascular diseases, connections with other root systems spread the disease, so it would be equally important to discourage root fusion or to sever any present, if the disease is localized.

Where lightning strikes have caused a group of trees to die in quick succession, this was thought to be due to a root grafted union reacting as a physiological entity (Wichmann 1925). However, Minko (1966) proposed a mechanism of horizontal spatial discharge where the electric charge is dissipated throughout the crown canopy of the trees involved. Consequently, he does not consider that root grafts transmit the charge within the group.

There has been considerable speculation about the role of living stumps and root grafts within a forest stand without enough
factual data. It was once thought that root grafted living stumps could explain the immediate response of some species to thinning (Adams 1940, Lanner 1961), but more recent quantitative data indicate that living stumps fail to influence the height and radial increment of the tree partner (Schultz 1962, Holmsgaard and Scharff 1963, Bormann 1966). Where growth responses have been obtained after thinning, they can usually be attributed to an improvement of environmental conditions resulting from the removal of a nearby competitor (Bormann 1966).

1.6 Methods of assessment of root grafts.

(a) Root excavation is still considered the most efficient means of locating and estimating the number of root grafts within a forest stand (Miller and Woods 1965). This advantage is offset by the cost and time involved in any sizeable study, as well as the great deal of damage caused by picks and shovels to the minor roots. In an effort to overcome root damage Miller and Woods (1965) used a garden trowel and hand cultivator in addition to an air compressor to blow soil away from fine roots, but this method was slow and inefficient in anything but fairly dry, sandy soil.

Hydraulic excavation of root systems appears to have
greatest potential. On suitable sites, this method requires about a third of the time taken by hand methods, it reduces labour costs, and preserves a greater proportion of the fine roots (Singer and Hutnik 1965). Wherever drainage was adequate, Schultz and Woods (1967) used water at 75 psi to excavate roots of loblolly pine. They found heavy soils needed loosening before they could be efficiently and effectively removed by water. A variation of this method is suggested by Hellmers et al. (1955) who used high and low pressure nozzles, the former for removing bulk soil and the latter for removing soil from around plant roots.

(b) In a thinned forest, **living stumps** provide visible evidence of root grafted unions and are frequently used to assess root graft incidence. Living stumps are impossible to distinguish from dying stumps immediately after felling so an adequate time lapse, which will vary from species to species and from location to location, is required before differences become apparent (Bormann 1961, Schultz and Woods 1967). Generally, methods of assessment ensure the presence of living sapwood by observing callus tissue formation (Schultz 1962), or by making a series of axe cuts around the base of the stump (Pawsey 1962). Care must be taken to check the whole stump circumference, as narrow
strips of living sapwood may otherwise be missed (Bormann 1961).

(c) **Tracer techniques** involving the use of isotopes and/or dyes have been extremely valuable in detecting root grafts and in determining how they function. When the results of such studies are interpreted however, three considerations must be borne in mind. (1) The method by which tracers are injected may alter the translocation pattern within a union. (2) It is assumed that the tracer will move through any existing root graft whereas some or all of the fusions may be nonfunctional at the time of the experiment. Thus a negative result does not necessarily mean that root grafts are absent. (3) The detection of tracers in receptor trees is not conclusive evidence of root graft transfer, for tracers have been shown to move between adjacent nonfused roots, through soil solution, or even through mutually shared fungal hyphae.

Dyes commonly used in root graft studies include acid fuchsin, eosin, safranin O, and fluorochromium (Kuntz and Riker 1956, Bormann and Graham 1959, Fischer et al. 1960). No comparative studies to determine the best dye for this type of work have been carried out, but acid fuchsin has been used successfully in a variety of species.
Isotopes suitable for tracer work in root grafted unions are limited to those which are readily transported within the sapwood; they must produce radiations detectable through several inches of wood and bark, and they must pose a minimal health hazard by having a short half life. Isotope tracers most commonly used are $^{32}\text{P}$ and $^{86}\text{Rb}$, while $^{131}\text{I}$, $^{45}\text{Ca}$ and $^{82}\text{Br}$ have also been used. Graham (1959) found that $^{131}\text{I}$ failed to move in root grafted white pine stumps when $^{86}\text{Rb}$ did, suggesting that $^{131}\text{I}$ may not be suitable for use in pine species.

Isotopes can be detected non-destructively, and therefore have a marked advantage over dyes but this is offset by the sophisticated equipment needed for isotope detection. Dyes leave a permanent, visible record of their translocation path which may be of importance when the actual patterns of sapwood movement are being studied. Perhaps the best technique incorporates both methods. The comparative efficiency of isotopes and dyes in determining the presence of root grafts appears to vary with the species treated. Bormann and Graham (1959) found both types of tracers equally efficient in detecting major root grafts, but isotopes more efficient in detecting minor root grafts in white pine, whereas De Byle (1964) reached
opposite conclusions using similar tracers in *Populus tremuloides*.

Injection of a donor stump or tree involves cutting the living sapwood and leaving the tracer in contact with it for a length of time to allow adequate absorption and transmission. Injection methods differ widely. De Byle (1964) and Miller and Woods (1965) consider that incision of the living sapwood should be carried out under liquid in order to avoid air embolism, but Bormann and Graham (1959) found that, provided the period between incision and injection is not too long, there is no significant difference between the treatments. When maximum sapwood absorption is desired, the tracer may be applied to the surface of a recently cut stump (Bormann and Graham 1959) or into a metal collar constructed over several sapwood incisions made around the circumference of a living stump (Miller and Woods 1965). If localized tracer applications are required, aluminium injection tubes with attached reservoirs seem ideal (Kozlowski et al. 1967), although the insertion of small lateral roots into vials containing the tracer can also be quite effective (Kunz and Riker 1956, Owston and Smith 1968).

(d) Where *silvicides* are used to thin and/or debark a forest stand, valuable information on the incidence of root
grafting can be obtained by observing the amount of backflash which occurs. This method assumes that transfer from one tree to another takes place through root fusions— which is usually the case (Bormann and Graham 1959). Injection methods are less refined than isotope or dye injection and consist of applying silvicide to a number of axe cuts made around the circumference of the tree. Chemicals which move across root grafts include various arsenite preparations (Himelich and Neely 1962, De Byle 1964), 2, 4, 5-T butyl ester and picloram (Kimber 1967), ammonium sulphamate (Bormann and Graham 1959), and copper sulphate (Jones and Partridge 1961). It was previously thought that some organic compounds (e.g., phenoxyacetic compounds) were not transferred across root grafts (Bisset and Shaw 1954) but more recent work suggests that in some species at least movement does occur (Fenton 1965, Kimber 1967).

(e) There is an assortment of techniques used to illustrate root graft function and presence which can be broadly classified as biological methods because of the substances or methods of detection employed. For example, fungal spores which are readily carried in the translocation stream of some trees may be tagged with isotopes before injection; their subsequent
presence in nearby trees indicates the presence of at least one active root graft. While this technique is very limited in its application, because of the specific nature of tree diseases, Kuntz and Riker (1956) showed conclusively that oak wilt spores tagged with $^{110}$Ag $^{131}$I were transferred through root grafts to uninfected trees.

A biological method incorporating a different principle was used successfully by Fischer et al. (1960). They injected streptomycin into one member of a pair of artificially root grafted seedlings and confirmed transfer of the antibiotic through the root graft by carrying out a Bacillus subtilis test on segments of the receptor tree. An untried biological method with practical implications involves the use of systemic insecticides. Insecticides such as Rogor 40 and Ekatin, which are rapidly dispersed throughout a tree in the xylem and phloem streams, could be tested for, in non-injected trees, by insect assay techniques. As there would be obvious difficulties in assessing the death of insects in large trees, this method may only have experimental value under glasshouse conditions.

1.7 Methods of expressing the incidence of root grafts.

The number of parameters used to express root graft
incidence lessens the value of many studies as quantitative comparisons within and between species cannot be made. This could be overcome if authors included with their method of expression certain basic information about the stand being studied. For example, the age, spacing, stocking, area, soil type and site index of the sample plot, together with its past silvicultural history are required for full evaluation of the data. In the following section, the advantages and disadvantages of indices most commonly employed will be discussed. Reasons for the index used in the experimental part of this study will be given in Chapter 2.

(a) The percentage of trees with root grafts: the exact number of root grafts present in a forest stand can only be determined accurately by complete site excavation. In most cases, the cost and time involved is unwarranted as it is sufficient to know the percentage of living trees grafted to other root systems (be they of living trees or living stumps). Theoretically, this information is best obtained by using tracer techniques applied to a sample of randomly selected trees throughout a stand. If the reliability of this assessment method improves, there is no doubt that the percentage of
trees with root grafts would be the best index to use. In the meantime, however, other more easily obtained and more reliable indices are being used.

(b) **Number of living stumps:** in thinned forests, living stumps provide visible evidence of root grafted systems, so that counts of living stumps have been incorporated into a number of indices expressing root graft incidence. All these have limitations which must be fully understood before the data can be interpreted. They do not take into account, (1) multiple grafts between living stumps and living trees, (2) single or multiple grafts between living stumps, or (3) single or multiple grafts between living trees. For these reasons indices based on counts of living stumps normally underestimate the amount of root fusion actually present.

One index of root fusion, using living stumps, is the percentage of all stumps which are alive. Some idea of the percentage of living trees root grafted, may be obtained with this index in lightly thinned stands, but in medium to heavily thinned stands the total number of stumps increases rapidly, giving greatly reduced percentages which bear no relationship to the living trees still standing. It can be used to indicate quantitative differences between stands of the same or different species.
provided that all plot information is included. Similar limitations apply when the number of living stumps per unit area is determined, but this index can be used meaningfully to illustrate the influence of site factors on living stump incidence (and possibly root graft incidence) in similarly treated stands of the same species.

The number of living stumps expressed as a percentage of the number of living trees relates the number of known fused root systems (living stumps) to the existing living trees and is therefore more meaningful than those indices which do not. In lightly thinned stands, the index will underestimate the incidence of root system fusions as suppressed and intermediate trees are normally removed and these crown classes tend to be infrequently root grafted. Also, the total number of stumps is few relative to the total number of trees. Over the intermediate range of thinnings this index should give a good estimate of root system fusion. In very heavily thinned stands the index may rise above 100 per cent indicating that some living trees have more than one associated living stump. It should be realised though, that even when this index is over 100 per cent it does not mean that all living trees on a plot are fused to other root systems.
CHAPTER 2

THE INCIDENCE OF ROOT GRAFTING IN STANDS
OF PINUS RADIATA IN THE A.C.T.

INTRODUCTION

Root grafting is prevalent in radiata pine forests (Adams 1940, Pawsey 1962, Will 1966, Hollingworth 1967). In general, the incidence of root fusion appears to be higher in radiata pine than in most other tree species, and, in some cases, is quite exceptional. For instance, Will (1966) estimated that all trees in a nine year old, site quality I stand, planted at 6' x 6', had at least one intraspecific root graft.

Although Pawsey (1962) studied the incidence of living stumps in radiata pine aged from 8 to 18 years, and found positive correlations between root fusion and dominance, site quality, stocking density, and stand age, little data are available on the age at which root grafting begins, its occurrence throughout a normal rotation, and factors influencing root graft incidence. In this section, some information on these questions is presented.
MATERIALS AND METHODS

Twenty one plots, covering a range in stand age from 8 to 40 years, were examined in the Stromlo and Uriarra forests, Australian Capital Territory. The 8- to 19-year-old stands in Compartments 49 and 50, Stromlo Forest, were maintained by the A.C.T. Forests Section, Department of Interior. The stand history of these plots is vague, as they have been given routine treatments since the time of establishment when they were planted at a spacing of \(5\frac{1}{2}' \times 5\frac{1}{2}'\) or \(6' \times 6'\). It is suspected that they have been unmerchantably thinned several times (possibly at ages 7 and 11 years) with the first commercial thinning at age 17 years, when the standing basal area was reduced to 110-120 square feet per acre. The remaining plots were maintained by the Forest Research Institute, Canberra, and possessed complete stand histories dating back to 1948. A summary of pertinent plot data is given in Appendix B.

Living stumps were detected by making several axe cuts around the circumference of a stump, to determine the presence of living sapwood. In young stands, living stumps could be detected with reasonable certainty some 6 to 8 months after felling, whilst in older stands 1 to 1\(\frac{1}{2}\) years was required before
drying sapwood and decay enabled a positive distinction to be made. All stumps with any living sapwood were classed as live, regardless of the proportion of living sapwood present. However, the location maps identifying all living trees and stumps on each plot (Appendix C), show the proportion and orientation of live sapwood on all stumps.

Three common indices used to express root graft incidence in forest stands, namely, the percentage of all stumps alive, the number of live stumps as a percentage of the number of live trees, and the number of living stumps per acre, have been calculated for all plots.

RESULTS

Data obtained from the field plots are summarised in Table 2.1, and living stump incidence, expressed by three indices, is plotted in Figure 2.1.

Living stumps were not found in any stands younger than 10 years. In older stands, the incidence of living stumps varied considerably within and between plots, although distinct relationships with stand age were apparent. Clearly the method of expressing living stump incidence alters the pattern observed.
TABLE 2.1

Summary of data from all plots used to determine incidence of root grafting from living stumps (complete data given in Appendices B and C).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Plot Number</th>
<th>Initial Spacing</th>
<th>S.Q.</th>
<th>Area (acres)</th>
<th>LTS (1968)</th>
<th>LSs (1968)</th>
<th>TSs</th>
<th>% Trees Removed</th>
<th>LTs</th>
<th>LSs</th>
<th>TSs</th>
<th>LS/acre</th>
<th>Standing B.A. 1967 (sq. ft.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>50-g</td>
<td>5½'x5½'</td>
<td>VI-VII</td>
<td>1.5</td>
<td>950</td>
<td>1</td>
<td>1221</td>
<td>56</td>
<td>0.1</td>
<td>0.08</td>
<td>0.68</td>
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<td></td>
</tr>
<tr>
<td>9</td>
<td>49-f</td>
<td>6' x 6'</td>
<td>&quot;</td>
<td>694</td>
<td>0</td>
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<td>62</td>
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<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>50-f</td>
<td>5½'x5½'</td>
<td>&quot;</td>
<td>955</td>
<td>35</td>
<td>1022</td>
<td>52</td>
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<td>0.0</td>
<td>3.4</td>
<td>0.0</td>
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</tr>
<tr>
<td>11</td>
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<td>6' x 6'</td>
<td>&quot;</td>
<td>491</td>
<td>17</td>
<td>1327</td>
<td>73</td>
<td>3.5</td>
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<td>1.28</td>
<td>11.3</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>5½'x5½'</td>
<td>&quot;</td>
<td>552</td>
<td>34</td>
<td>1619</td>
<td>74</td>
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<td>0.0</td>
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<td>&quot;</td>
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<td>241</td>
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<td>611</td>
<td>72</td>
<td>12.0</td>
<td>4.6</td>
<td>47.5</td>
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<tr>
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<td>&quot;</td>
<td>1.5</td>
<td>675</td>
<td>72</td>
<td>1496</td>
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<td>4.8</td>
<td>48.0</td>
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<td></td>
</tr>
<tr>
<td>15</td>
<td>49-c</td>
<td>&quot;</td>
<td>&quot;</td>
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<td>675</td>
<td>72</td>
<td>1496</td>
<td>69</td>
<td>11.0</td>
<td>4.8</td>
<td>48.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>49-b</td>
<td>6' x 6'</td>
<td>&quot;</td>
<td>0.89</td>
<td>272</td>
<td>24</td>
<td>736</td>
<td>73</td>
<td>12.0</td>
<td>3.3</td>
<td>27.0</td>
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<td></td>
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<tr>
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<td>&quot;</td>
<td>&quot;</td>
<td>1.5</td>
<td>490</td>
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<td>1328</td>
<td>73</td>
<td>26.0</td>
<td>8.9</td>
<td>78.6</td>
<td></td>
<td></td>
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<tr>
<td>25</td>
<td>140-1</td>
<td>8' x 8'</td>
<td>IV</td>
<td>0.275</td>
<td>54</td>
<td>51</td>
<td>132</td>
<td>71</td>
<td>94</td>
<td>38.7</td>
<td>185</td>
<td>160</td>
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<tr>
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<td>140-3</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.242</td>
<td>61</td>
<td>24</td>
<td>100</td>
<td>62</td>
<td>39</td>
<td>24.0</td>
<td>99.4</td>
<td>212</td>
<td></td>
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<tr>
<td>&quot;</td>
<td>140-4</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.217</td>
<td>66</td>
<td>23</td>
<td>92</td>
<td>58</td>
<td>35</td>
<td>25.0</td>
<td>106</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>132-f</td>
<td>6' x 6'</td>
<td>II-III</td>
<td>0.208</td>
<td>42</td>
<td>34</td>
<td>206</td>
<td>83</td>
<td>81</td>
<td>16.5</td>
<td>163</td>
<td>209</td>
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</tr>
<tr>
<td>&quot;</td>
<td>132-g</td>
<td>9' x 9'</td>
<td>&quot;</td>
<td>0.212</td>
<td>43</td>
<td>25</td>
<td>79</td>
<td>65</td>
<td>58</td>
<td>31.7</td>
<td>118</td>
<td>221</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>51-a</td>
<td>9' x 9'</td>
<td>V</td>
<td>0.397</td>
<td>48</td>
<td>47</td>
<td>120</td>
<td>71</td>
<td>98</td>
<td>39.1</td>
<td>118</td>
<td>167</td>
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</tr>
<tr>
<td>&quot;</td>
<td>51-b</td>
<td>&quot;</td>
<td></td>
<td>0.397</td>
<td>41</td>
<td>31</td>
<td>121</td>
<td>75</td>
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<td>65</td>
<td>271</td>
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<td>112</td>
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<td></td>
<td>1.0</td>
<td>72</td>
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<td>&quot;</td>
<td>48-2*</td>
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<td>24.4</td>
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<td>&quot;</td>
<td>48-3</td>
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<td>&quot;</td>
<td>0.97</td>
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<td>&quot;</td>
<td>48-4**</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.485</td>
<td>41</td>
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<td>126</td>
<td>80</td>
<td>95</td>
<td>32.0</td>
<td>80.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = figures corrected omitting area of shallow soil
** = figures corrected omitting area occupied by drainage path
SQ = site quality
LTS = living trees
LSs = living stumps
TSs = total cut stumps
FIGURE 2-1  INCIDENCE OF LIVING STUMPS WITH STAND AGE. LIVING STUMPS PLOTTED AS A PERCENTAGE OF TOTAL STUMPS (a), AS A PERCENTAGE OF THE NUMBER OF LIVE TREES (b), AND AS THE NUMBER OF LIVING STUMPS PER ACRE (c).
When expressed as a percentage of the total number of stumps on a plot, the living stumps show an exponential increase from their initiation at age 10 years and reach a plateau at about age 25 years (Figure 2.1(a)). When expressed as a percentage of the number of living trees on the plot, the living stumps continue to increase exponentially up to at least 30 years of age (Figure 2.1(b)). When expressed on a unit area basis, the number of living stumps shows a marked optimum at about age 25 to 30 years.

DISCUSSION

Despite the abundance of living stumps in *Pinus radiata* stands throughout the A.C.T., difficulty was experienced in expressing this incidence in a meaningful manner (Section 1.7). The three common parameters used in this study showed different relationships over the age range studied and, if viewed uncritically, could lead to vastly different conclusions regarding the frequency of grafted root systems in a given plot. If an index is to have value in ecological and silvicultural work, it must bear some relationship to the living trees present in a stand.

On this basis, the number of living stumps expressed as a percentage of the number of living trees may seem the best index.
However, in the early years of stand development, when the total number of stumps is small relative to the number of living trees, this index will almost certainly underestimate the incidence of root grafting. In such a situation, the most realistic estimate of root grafting, as deduced from living stumps, is likely to be the number of living stumps expressed as a percentage of total stumps. The incidence of root grafting, determined in this way would, however, be influenced by the crown classes of the trees removed as root grafting is likely to be less in the suppressed and intermediate crown classes (Section 1.3). As the stand develops, and more trees are removed in thinnings, the total number of stumps, relative to the retained trees, increases and the number of live stumps expressed as a percentage of total stumps would underestimate the incidence of root grafting. At this time, the most realistic estimate of root grafting would be given by the number of living stumps expressed as a percentage of the number of live trees.

In this study, the number of living stumps as a percentage of living trees was never less than when expressed as a percentage of total stumps, even in the youngest stands (Figs. 2.1 (a) and (b)). This was almost certainly due to the fact that, in all stands
examined, at least 50 per cent of the trees had been removed in thinnings (Table 2.1). Hence, throughout this study, the most realistic estimate of root grafting is probably the number of living stumps expressed as a percentage of the number of living trees.

All of the indices used showed an increase in root grafting with increasing age of the stands, at least up to age 25 years. This increase in root grafting with stand age is considered to be a real phenomenon but it has not been proved unequivocally by the data presented. Direct proof requires checking the age of root grafts from ring counts. This was not done, but indirect evidence that root grafting increased with stand age is provided by the fact that in the younger stands (8 to 19 years old), in which up to 74 per cent of the original trees were removed in thinnings, the amount of root grafting, as estimated from all three indices, was markedly less than in older stands (Table 2.1).

These results indicate that, under the prevailing environmental conditions in the stands examined, root grafting did not occur until the trees were 10 years old and then increased markedly until at least age 25 years. In contrast to this, Will (1966) found all radiata pine trees in a 9-year-old SQ I stand in
New Zealand had intraspecific root fusions.

In the plots examined, living stump incidence varied greatly within and between plots of the same or similar ages. In an attempt to find the reasons for this, stand histories were examined, but it was impossible to draw consistent relationships between living stump incidence and imposed silvicultural treatments (e.g., initial spacing, thinning). For this reason it is considered that the factors affecting the occurrence of living stumps in these stands are environmental (e.g., site quality, soil factors) and physiological (vigour, extent of root spread) in nature. Differences in site quality, as reflected by the total volume of wood produced in a plot, may have contributed slightly to the within and between plot variation. Larger differences can be attributed to variations in the soil profile, for in Plot 4, Compartment 48 Stromlo (Appendix C), the line of demarcation between soils supporting living stumps and those not, corresponded with a drainage area passing through the centre of the plot. Soil profiles observed across the area revealed that the drainage path had a very shallow profile with a clay-rich horizon at a depth of 13 inches. On either side of the drainage path, where the soils were deeper and better drained, living stumps occurred profusely.
In a number of other plots the distinction was not so clear, but invariably areas where few or no living stumps occurred had shallow soils. It would seem, therefore, that in radiata pine, shallow soils inhibit living stump survival. As these results conflict with those reported in the literature (Section 1.3(iii)), it is felt that this warrants further investigation as it may mean that root grafts are formed but the living trees are not able to support associated living stumps when the conditions become adverse.
CHAPTER 3

VERIFICATION OF ROOT GRAFTS AND MOVEMENT OF SUBSTANCES THROUGH GRAFTED UNIONS

INTRODUCTION

Relatively little work has been done on the root systems of *Pinus radiata*. However, the majority of roots are known to be concentrated within 18 inches of the soil surface (Lindsay 1932, Bowen 1964, Will 1966, Raupach 1967); but where soils are suitable, tap roots may penetrate to depths of 10 to 12 feet producing systems of fine roots at varying depths (Pryor 1937, Will 1966). These deep roots contribute a significant proportion of water and nutrients to the tree only in times of stress in the upper soil layers (Tiller 1957, Bowen 1964). Normal vertical root development is altered by chemical or physical soil barriers such as high salinity, poor aeration, or compacted clay layers (Atkinson 1959, Bowen 1964, Raupach 1967).

With this type of rooting habit, it is not surprising that the majority of root fusions occur in the top 18 inches of soil (Adams
1940, Will 1966). In the work described here, several radiata pine root systems were excavated to observe the patterns of root distribution, the occurrence of root grafts, and to verify that living stumps are grafted to living trees.

In addition, tracer substances were injected into exposed and unexposed root grafted systems in order to study the patterns of tracer movement, the factors which modify these patterns, and to ascertain the effectiveness of tracers in detecting the presence of root grafts in the field.

MATERIALS AND METHODS

The area excavated was located in Compartment 62, Stromlo Forest, A.C.T. It was planted at an 8' x 8' spacing in 1941 (27-years-old at time of study) on a sandy alluvial soil with a compacted layer of sand, coffee rock, and clay between 18 and 24 inches depth. The stand was classed as site quality V and had been lightly thinned four times (in years 53/54, 55/56, 56/57, 59/60) to its present stocking of 178 stems per acre. The study was carried out during September and October 1968, when temperatures ranged between a maximum of 82.4°F and a minimum of 28.8°F with a mean maximum of 59.5°F and a mean minimum of
35.8°F. A total of 2.65 inches of rain fell during the study period, and while the total rainfall for the year was below average, the drought stress throughout the study was slight.

The litter layer and topsoil were removed using a rake and shovel until roots were encountered; the soil was then loosened with a garden fork and removed by water pumped at 75 to 100 psi from a fire tanker. This treatment partially exposed the root systems of three living trees and four associated living stumps to an average depth of 18 inches (Figure 3.1). All horizontal roots down to a diameter of 2mm were preserved. Although tap and sinker roots proceeded below the depth excavated (passing through the compacted soil horizon) these were neglected as it was considered that the chance of their forming intraspecific root grafts was slight.

In order to observe the translocation patterns of isotopes within root grafted systems, six injection points in and around the excavation site were selected. $^{86}$Rb was chosen as a suitable tracer because it is readily transported in the sapwood of trees, it is a strong emitter of gamma rays, and it has a short half life of 18.7 days (Graham 1959).

Prior to injection, a metal cup was firmly attached to
FIGURE 3.1

MAP OF ROOT EXCAVATION SITE

⊙=living tree  ●= living stump  ⊕= dead stump

area unexcavated

0  10  feet  NORTH
a shaved region near the base of a tree or stump and sealed with a waterproof caulking compound. When the injection was into the bole of a live tree, two saw cuts were made a short distance above the injection point to reduce the transpirational pull upwards. The cup was then filled with 1% KCl solution under which a half inch chisel cut was made into the sapwood, before adding 0.5 milli-curies of \(^{86}\)Rb. As soon as the solution had been absorbed, a reservoir containing 1% KCl carrier solution was set up to cover the cut with solution continually (Plate 3.1).

Portable transistorized equipment, consisting of an A.A.E.C. ratemeter Type 59A with a lead shielded NaI scintillation probe, an A.A.E.C. scaler Type 60, and a three inch graphic recorder, was used to monitor the radiation at varying distances from each injection point to determine background radiation, and to assess isotope distribution in time and space.

For comparison, a solution of acid fuchsin (1% w/v) was injected into three additional living stumps. At two of the isotope injection points, the KCl solution was replaced by acid fuchsin several days after injection in the hope that the dye would trace out a similar path to that of \(^{86}\)Rb, and would thus allow a direct comparison of the two techniques within the one
Plate 3-1.

System used for injection and monitoring of isotope 86Rb. 0.5 mCi 86Rb injected into a 1% KCl solution in cut (c). Cut continually covered with KCl solution from reservoir (r). Saw cuts (s) above injection point to reduce upward movement. Scintillation probe (p) covered with lead shield (k) used for monitoring.
root system. As dye detection necessitated destruction of the roots involved, assessment was left until isotope monitoring and description of the root systems were complete.

RESULTS

(i) Root distribution:

All major laterals and associated fine roots within a radius of three to four feet around each stump were confined to the surface 0–12 inches. Beyond this distance, the laterals tapered off and penetrated to greater depths (to the top of the compacted layer in some cases), and the small roots became more evenly distributed throughout the upper part of the soil profile. The radial spread of any of the root systems was not determined as most roots extended beyond the excavated area. Each root system possessed between one and four tap roots as well as several smaller sinker roots originating from major laterals. Some of these vertical roots passed through the compacted layer but the depth to which they penetrated was not determined. All the living stumps in the excavated area had living tissue around their circumferences even though several minor laterals on each stump had died.
(ii) **Root grafts:**

The exposed root systems revealed many self grafts and ten intraspecific root grafts, five of which were associated with the one living stump (Figure 3.2 shows nine of these grafts). Each living stump had at least one root grafted to a living tree, whereas dead stumps were not observed to have any grafts to living trees. The soil depths at which grafts occurred varied from 1 to 14 inches (mean depth 9 inches), whilst the diameters of fused roots ranged from 0.3 inches to 3.5 inches (mean diameter 1.2 inches). The grafted roots were never comparable in size, and six of the grafts were fused directly to the butt of a living stump or living tree. The root grafts were not formed preferentially on the side of the stump facing the associated root system.

(iii) **Anatomy of root grafts and living stumps:**

Several grafts obtained from the excavation site were sectioned to observe the vascular development within natural root grafts. Continuous tracheid development was best formed between those regions of the roots which were aligned roughly initially. Rough initial alignment can be found even in roots which cross at right angles, and in the graft illustrated in
FIGURE 3.2 DETAILED MAP OF THE EXCAVATED ROOT SYSTEMS SHOWING MAJOR ROOTS, ROOT GRAFTS (►), INJECTION POINTS (←○), AND MONITORING POSITIONS (2B and 2D). SECTIONS MADE AT POINTS 2A → 2E (see PLATE 3.2).
Plate 3.3(b), tracheid continuity is best developed on the top left hand side and bottom right hand side. Within this continuous tissue between the two roots, growth rings may be seen. While such rings might give the exact age and date of formation of root grafts, it is felt that caution should be exercised until it is shown conclusively that new tissue is laid down each year. In a number of the grafts, a region of included bark was found at the contact zone between the two roots. Plate 3.3(b) shows a small portion of included bark at the contact zone. The presence of bark at these regions tends to support Graham and Bormann's (1966) belief that root grafts are not initiated after wind movement has abraded the bark between contiguous roots (Cook and Welch 1957).

The anatomy of *Pinus radiata* living stumps was similar to that of stumps of *Pinus strobus* as described by Bormann (1966). The latest formed tissues were comprised of disorganised short tracheids laid down in swirl-like patterns. Only in a few instances were continuous or discontinuous growth rings discernible as most of the newly formed tissue was of uniform texture. This prevented an accurate visual estimation of the living stump's age.
(iv) Movement of isotopes and dyes:

(a) From live tree to living stumps via an exposed graft. $^{86}\text{Rb}$ was injected into the bole of live tree 1 (Figure 3.2), which was connected to living stump 5 via an exposed root graft, to determine whether the tracer would move into and across the graft. Some downward movement into the grafted root did occur but ceased at a distance of about 3 feet from the base of the tree. No tracer reached the graft union.

In spite of the saw cuts in the bole above the injection point, detectable amounts of isotope moved up the bole.

(b) From living stumps to live trees. One isotope injection was made into a stump possessing grafts to two live trees (Stump 2, Figure 3.2). One of these trees (Tree 1, Figure 3.2) was a vigorous dominant, whilst the other (Tree 3), although large, was in poor health. Tracer moved into both root grafted systems and was followed to the base of both live trees. No movement up either stem was detected. Initial translocation rates of isotope were measured as 9.8 inches per hour (after five hours) along the root leading to tree 1, and 5.4 inches per hour (after 12 hours) along the root leading to tree 3 (Figure 3.3).

Eight days after the initial isotope injection, the KC1
FIGURE 3.3. TRANSLOCATION OF $^{86}$Rb PAST TWO MONITORING POSITIONS (2B and 2D) LOCATED EITHER SIDE OF INJECTION POINT 2 (see FIGURE 3-2). ISOTOPE INJECTED 10:15AM ON 17/9/1968.

- - - - = count rates monitored at 2D, 48 inches from the injection point.
+-----+ = count rates monitored at 2B, 60 inches from the injection point.

mn = midnight
PLATE 3·2 DYE PATTERN IN ROOTS ON EITHER SIDE OF LIVING STUMP (2) IN FIGURE (3·2). ARROWS DENOTE UPPER SIDE OF ROOT. DYE INKED OVER FOR ILLUSTRATION.
carrier solution was replaced by a continuous supply of acid fuchsin, and the whole root system was destructively sampled after a further four weeks. Dye moved only into the two roots known to be grafted, and the dye patterns, at increasing distances on both sides of the injection point, are shown in Plate 3.2. In both cases, the dye tended to concentrate on the lower side of the larger roots. This portion of the larger roots was growing more actively as evidenced by the asymmetric growth rings. In the smaller parts of the roots more distant from the injection point, both growth and dye movement appeared to be more evenly distributed.

Acid fuchsin was also injected into living stump 5 (Figure 3.2) directly above the root fused to the root system of living tree 1. When the roots were examined 72 hours later, dye had moved through the root graft into the live tree at an average velocity of at least 1.7 inches per hour. The dye pattern at the contact zone between the two roots is shown in Plate 3.3.

Isotope was injected into a living stump (5A, Figure 3.4) in an unexcavated portion of the stand in anticipation of its movement to the closest living tree 5B. After nine days, a marked rise in radioactivity of living stump 5F (Figure 3.4) was
FIGURE 3.4. PATH OF TRACER MOVEMENT FROM LIVING STUMP 5A TO LIVING TREE 5G VIA LIVING STUMP 5F.

○ = LIVING TREE. ● = LIVING STUMP.

⊕ = DEAD STUMP. ○→ = INJECTION POINT.

▲ = ROOT GRAFT. 5f, 5g = MONITORING POINTS.
FIGURE 3-5 TRANSLOCATION OF $^{86}$Rb AT TWO MONITORING POSITIONS (5f and 5g, see FIGURE 3-4). ISOTOPE INJECTED 12:30PM ON 17/9/1968.

+——+ = count rates monitored at 5f, 9.5 feet from the injection point.

•——• = count rates monitored at 5g, 19 feet from the injection point.
PLATE 3.3  DYE PATTERN (b) AT CONTACT ZONE OF A LARGE ROOT GRAFT (a). INJECTION MADE 3.5 FEET FROM GRAFT INTO ROOT RUNNING FROM THE TOP OF THE PLATE. SECTION INDICATED BY DOTTED LINE. DYE PATTERN INKED IN FOR ILLUSTRATION.
observed (Figure 3.5) suggesting that stump 5A was grafted to living tree 5G via stump 5F. The base of living tree 5G was continuously monitored for 35 days without showing a significant increase over the background radiation. It was only following injection of acid fuchsin eight days after the initial isotope injection, and excavation of the roots 38 days later, that the path of movement from stump 5A via stump 5F to tree 5G was established conclusively. The dye streak entering the base of tree 5G was deeply embedded in the bole and this may have been the cause of failure to detect the isotope.

Living stump 7 (Figure 3.2) was injected with acid fuchsin on the side facing stump 5 with the expectation of movement across the graft connecting the two stumps. Roots were sectioned 29 days after the start of treatment and, although dye had moved into a number of laterals, including that connecting the two stumps, no dye moved across the graft. This situation differed from that described previously even though stump 7 was connected via stump 5 to living tree 1. In this instance, stump 7 was also grafted to living tree 4 and the main movement of dye was around the base of the stump into a root grafted to tree 4.

(c) From live tree to live tree. Injections of isotope
were made into four live trees (two dominant and two suppressed) with unexposed root systems. All surrounding live trees and stumps were monitored for 35 days but, in no case, was movement of isotope detected.

(d) Uptake and movement through a severed root.

The ability of living tree 1 (Figure 3.2) to draw on the whole root system of stump 5 was examined by severing a small root eight feet from the base of stump 5 on the side furthest from tree 1. The severed root was placed immediately in a solution of acid fuchsin. After 72 hours, dye had moved at least 16 feet (average velocity 2.7 inches per hour) into the base of living tree 1.

DISCUSSION

Despite abundant root overlap in the excavation site, the number of intraspecific root grafts detected was fewer than was anticipated. This paucity of root fusion may be attributed either to root incompatibility or to root tip avoidance of other roots (Wendelken 1955). As there was only one instance where roots were intimately enmeshed but not fused, it would seem that incompatibility is not the reason for the lack of fusion on this
Therefore, root tip avoidance resulting from soil water exhaustion and/or root exudates would be the most plausible explanation until subsequent radial growth allowed the roots to make contact. As the highest concentration of radially expanding roots was immediately around the butt of a tree, it is not surprising that this was the region in which the incidence of root fusion was greatest.

The results of the tracer studies show that $^{86}$Rb and acid fuchsin will move across root grafts, and can be valuable tools in studies of root grafting. However, serious limitations in the use of these substances were also apparent.

Where living stumps were injected with isotope or dye, the tracer moved from the donor stump to the receptive live tree, demonstrating the ability of live trees to draw on the root systems of connected living stumps. This movement occurred over considerable distances, through grafts of various sizes, and in one instance, via an intermediary stump. However, isotope could not be detected in all roots into which it had passed, at least with the levels of isotope activity and the equipment used here.

Movement of tracer from a living stump to a live tree
has been attributed to the transpirational pull of the receptor tree (Schultz and Woods 1967). High translocation rates can only be explained by this theory, but it is felt that where tracers move slowly, and particularly where they pass through two or more living stump root systems, root pressure may account for a significant proportion of the translocated material.

In no case were tracers detected moving from a live tree to a living stump, or from a live tree to another live tree. In some of these cases root grafts were known to be present and functional; in others their presence was only inferred, but their complete absence was unlikely. Given the presence of root grafts, lack of movement could be due to either or both the following causes.

It may be that movement across the root graft under the conditions prevailing in this study required a total reliance on the transpirational pull from one tree, as is the case where a living stump is grafted to a live tree. Where the transpirational pull is divided between two trees, the receptor tree cannot overcome the requirement of the injected tree (Bormann 1966). Possibly under different conditions as, for example, at times of greater environmental stress, this situation may not hold, but
there was no evidence of one tree parasitizing another in this study.

Alternatively, the apparent lack of movement from live tree to either a living stump or another living tree may result from the type of tracer injected. $^{86}$Rb, an inorganic element, would be expected to move in the xylem and be controlled by transpiration. To obtain downward movement, it may be necessary to use a tracer which would move readily in the phloem, such as a labelled organic molecule.

The translocation rates observed in this study ranged from 2 to 10 inches per hour, falling within the lower range of translocation rates observed by Schultz and Woods (1967) in *Pinus taeda*. Differences in rate may be controlled, at least to some extent, by environmental factors at the time of study. The results do show the ability of one tree to utilize the root system of another stump and suggest that, under conditions of stress, the importance of root grafts may be enhanced.

There are similarities between the translocation patterns observed in this study (Figure 3.3) and those described by Schultz and Woods (1967). In both cases, a
rapid rise in translocated tracer occurred in the first few hours and was followed by a prolonged drop in activity. Figure 3.5 illustrates another pattern in which detectable isotope increased steadily over a prolonged period. Schultz and Woods (1967) were able to establish relationships between the diurnal fluctuations in tracer movement, solar radiation, and high water stress. Similar fluctuations were observed in this study and although changes in temperature, relative humidity and cloud coverage were noted, no correlations were apparent.

A comparison of the distances over which \(^{86}\text{Rb}\) and acid fuchsin were detected showed that both tracers were valuable in assessing the activity of living stumps with partially exposed root systems. Acid fuchsin was detected visually at distances and concentrations at which \(^{86}\text{Rb}\) could not be detected above the background radiation. The need to destroy the root system to determine the dye's presence, however, was sufficient disadvantage to offset this benefit. In any event, the use of isotopic elements and a dye such as acid fuchsin appears to have limited application in the detection of root grafts in undisturbed forest stands. Nevertheless, these tracers are useful in determining movement patterns and the
degree to which living trees can utilize the root systems of living stumps through root grafts.
INTRODUCTION

The high frequency of living stumps in radiata pine stands, together with their ability to conduct substances to living trees, has been illustrated and discussed. This makes feasible Adams' (1940) suggestion that living stumps could contribute to the immediate growth response shown by thinned stands. No assessment of this possibility has yet been made in radiata pine stands. In the following study, the effects of living stumps on the growth of trees assumed to be attached to those stumps are examined.

MATERIALS AND METHODS

Compartment 140 Uriarra (Appendix C) was selected for the study as the growth history of each tree was available from the time of first thinning. This enabled the
age of each living stump to be determined. All trees were assigned living stumps on the basis of their proximity to, and orientation with, the living stumps present.

One hundred and five trees on a total of three plots were examined; but, for a particular set of comparisons, control trees (those without living stumps) and treated trees (those with living stumps) were selected from the one plot in order to minimise the effects of differences in treatment and/or environment.

Two methods were used to compare the increment data of each plot. Firstly, the cumulative diameter increments over a period of sixteen years of similarly-sized trees were compared graphically. Secondly, a two-way analysis of variance (for unequal replicates) was carried out on the increment data of the trees over the period two years before and three years after each thinning. Trees were grouped according to the number of living stumps they possessed at the particular thinning date, and their diameter increments expressed as a percentage of their diameters at the beginning of the period under consideration. In this way, it was hoped to compensate for the more rapid diameter growth of the vigorous
trees. Because the variance of the different treatments did not differ significantly, the percentage diameter increments were analysed directly without transformation.

RESULTS

As the results from the three plots were identical, only data from plot 1 are presented. The cumulative diameters of trees from similar size classes were plotted (Figure 4.1) and examined to see if association with newly created living stumps altered the slope of the growth curves, particularly in the year immediately after thinning when maximum benefit from an attached root system might be expected.

The formation of living stumps appeared to have no effect on the growth of trees even though some trees had as many as four associated living stumps by 1968. Similar results were obtained when cumulative basal areas were compared.

An analysis of variance (Table 4.1) of diameter increments in different years shows no significant difference between treatments or in the treatment x period interaction, confirming that the growth response of trees was not affected
**FIGURE 4-1. CUMULATIVE DIAMETER INCREMENT OF TREES WITH DIFFERENT LIVING STUMP ASSOCIATIONS. DATA FROM PLOT 1 COMPARTMENT 40 URIARRA, THINNED IN 1952, 1957, 1962, AND 1967.**

<table>
<thead>
<tr>
<th>Tree no</th>
<th>Year living stump formed</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>169</td>
<td></td>
<td>▼</td>
</tr>
<tr>
<td>90</td>
<td>X</td>
<td>....</td>
</tr>
<tr>
<td>59</td>
<td>X</td>
<td>△</td>
</tr>
<tr>
<td>22</td>
<td>X X</td>
<td>+ + +</td>
</tr>
<tr>
<td>140</td>
<td>X XX X</td>
<td>....</td>
</tr>
</tbody>
</table>

**Table:**

- **Tree no:** List of tree numbers.
- **Year living stump formed:** Years in which the stump was formed.
- **Symbol:** Various symbols represent different living stump associations.

**Diagram:**

- X: Year in which the stump was formed.
- △: Symbol for a specific living stump association.
- +: Symbol for another specific living stump association.

**Axes:**

- **Diameter (inches):** Y-axis.
- **Year:** X-axis.
### TABLE 4.1

Analysis of variance of the diameter increments of trees with different numbers of associated living stumps (Treatment) over a period of five consecutive years (Period) during which a thinning was effected.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squared Deviations</th>
<th>Mean Square Deviation</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>4</td>
<td>7.9</td>
<td>1.97</td>
<td>1.79 &lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Period</td>
<td>4</td>
<td>144.8</td>
<td>36.21</td>
<td>32.89 &lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Treatment x Period</td>
<td>16</td>
<td>8.6</td>
<td>0.53</td>
<td>0.48 &lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Error</td>
<td>130</td>
<td>143.1</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>154</td>
<td>304.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** Highly significant (P < 0.01)

NS Not significant
by the number of living stumps associated with them. A significant difference was obtained between periods due to one year being a very severe drought year, but as there was no significant difference between treatments or in the treatment x period interaction, one may conclude that trees with living stumps did not benefit from the potentially increased absorptive root system even in drought years.

DISCUSSION

Before interpreting the results further, it is necessary to point out some limitations in the data. The allocation of living stumps to the nearest tree may be justified in some species (Schultz and Woods 1967), but evidence from the limited excavation study described in Chapter 3 suggests that this is not always valid in *Pinus radiata*. As root connections were not confirmed, it cannot be said that the trees definitely supported the living stumps allocated to them. In addition, the growth response from a thinning treatment might be expected to override any minor growth effects produced by a living stump, particularly as the diameter measurements were taken once yearly. It is suggested that
more sensitive growth measurements at more frequent intervals are necessary to detect any such growth effects.

Despite the limitations mentioned, the results are similar to those of Schultz (1962), Holmsgaard and Scharff (1963), and Bormann (1966). These workers found in a variety of tree species that living stumps had no demonstrable effect on the increment of partner trees. Thus, in radiata pine, as in other species, it appears that living stumps do not play a major role in the normal growth of the stand.
CHAPTER 5

MANIPULATED ROOT GRAFTS

INTRODUCTION

The real significance of root fusion to the growth of forest trees can only be determined when root graft function is fully understood. This requires a knowledge of all plant and environmental factors influencing movement of substances through root grafts. It is suspected that relative tree dominance, food and nutrient gradients, water stress, anatomical and biophysical compatibility, soil water availability, light, and temperature, all influence root graft function (Graham 1960, De Byle 1964, Graham and Bormann 1966, Schultz and Woods 1967). In the field, it is almost impossible to isolate particular factors and to examine their influence on root graft function. If, however, root grafts could be created artificially between seedlings to allow investigations under controlled conditions, the study of root graft function would be facilitated.

This study was undertaken to determine the feasibility
of creating artificial root grafts between seedlings and to examine, briefly, the potential of such material in studying root graft function.

MATERIALS AND METHODS

Two-year-old radiata pine cuttings possessing well-developed lateral roots, ideal for root grafting, were obtained from the Forest Research Institute, Canberra, A.C.T. Each cutting was planted in a 1:1 mixture of perlite and peat moss in five inch diameter pots, and held for six weeks in a glasshouse equipped with heaters and coolers to avoid temperature extremes. The photoperiod was sixteen hours (normal day length supplemented with fluorescent and incandescent lights). The plants were watered daily and a complete nutrient solution was added twice weekly.

Prior to grafting, the roots of the plants were washed clean and graded to enable the pairing of cuttings of similar root size. Each plant pair was laid on a flat board and held down firmly using rubber bands. One root from each seedling was selected and the two were aligned alongside one another for grafting. The surfaces of the roots were blotted dry, bark was removed, and feeder roots were detached. Three types of graft
(approach, cleft, and reverse cleft - Plate 5.1) were attempted by making continuous cuts into each root and carefully joining the two cut surfaces. The graft union was bound immediately with small strips of parafilm which gave a waterproof seal and covered and supported the grafted roots. The seedling pairs were then repotted. Special care was taken to reduce root desiccation and damage during the grafting process.

It was thought that the "take" of the graft might be aided by the use of growth substances to stimulate callus development. To test this, a concurrent study was made to examine the effects of indole acetic acid (IAA) and gibberellic acid (GA) on callus formation on cut root surfaces. The hormones in a lanolin base were smeared onto, and kept in intimate contact with, cut root surfaces by clipping lengths of small diameter plastic tubing over the cuts before sealing with gauze and grafting mastic. Each treatment was applied to five roots.

Roots from both experiments were sampled at regular intervals for macroscopic and microscopic examination. All microscopic sections were stained with a 0.05% aqueous solution of toluidine blue.

Additional cuttings, grafted in the manner described,
PLATE 5.1(a) MANIPULATED ROOT GRAFTS IN RADIATA SEEDLINGS. FROM LEFT TO RIGHT, APPROACH, CLEFT, AND REVERSE CLEFT GRAFTS.

PLATE 5.1(b) SECTION OF A MANIPULATED ROOT GRAFT (cleft graft) SHOWING FUSION AND DIFFERENTIATION OF CALLUS BETWEEN STOCK (a) AND SCION (b). ORIGINAL CUT SURFACES SHOWN BY DOTTED LINES.
were used in a pilot study of the movement of $^{32}$P through root grafts. Twenty weeks after the grafts were made the seedling pairs were removed from their pots and the roots washed clean. The individual root systems of each seedling pair, joined only by the grafted root, were separated carefully and placed in adjacent pots containing $\frac{1}{40}$ Hoagland's solution (Curtis and Clark 1950). The seedlings were allowed to adjust to the nutrient medium for two weeks before treatments were applied.

The effects of shading, and of removal, of seedling shoots on movement of $^{32}$P through the root graft were examined. The shaded shoots were preconditioned for two weeks before the isotope (0.5 mCi $^{32}$P) was added to each donor nutrient solution. Shoot removal was carried out immediately before adding the isotope. Shading and shoot removal treatments were applied to both donor and receptor seedlings, and each treatment was duplicated.

The shoots of donor trees, and the shoots and roots of receptor trees, were monitored for eight days after the isotope was added using a Geiger-Muller tube attached to the monitoring equipment described earlier (Plate 5.2). The plant parts being monitored were shielded from the isotope source with plywood.
PLATE 5.2 METHOD USED FOR MONITORING MOVEMENT OF ISOTOPE (\(^{32}\text{P}\)) THROUGH MANIPULATED ROOT GRAFTS IN SEEDLINGS.
Every precaution was taken to ensure that the only contact between the seedlings of each seedling pair was via the manipulated root graft.

Eight days after isotope injection, the seedlings were harvested, pressed, and oven dried at 85°C for 24 hours. The dried plants were then placed in contact with X-ray film in the dark from 4 to 96 hours (depending on activity) after which the films were developed.

RESULTS

(1) **Root callus development.**

Effects of the plant hormones on root callus development were assessed objectively by determining the proportion of the original cut surface producing callus tissue. These proportions were measured microscopically using cross sections cut from roots receiving each treatment (Table 5.1, Plates 5.3 and 5.4). No consistent trends were observed and, overall, callus development on the control segments was as good as that on any other segment at any one sampling time. The vast majority of roots less than 3 mm in diameter died.

The time course of callus formation may yield some
Progressive callus development resulting from various IAA and GA applications to cut root surfaces. (One root sampled from each treatment on each occasion).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ratio of callus width to width of cut surface</th>
<th>Weeks after hormone application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>IAA ppm</td>
<td>GA ppm</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0.43</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0.27</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>0.56</td>
</tr>
<tr>
<td>450</td>
<td>0</td>
<td>0.16</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>d</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0.41</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>0.18</td>
</tr>
<tr>
<td>450</td>
<td>5</td>
<td>0.34</td>
</tr>
<tr>
<td>0</td>
<td>50</td>
<td>0.53</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>0.33</td>
</tr>
<tr>
<td>50</td>
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</tr>
<tr>
<td>5</td>
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<td>d</td>
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<td>500</td>
<td>0.30</td>
</tr>
<tr>
<td>450</td>
<td>500</td>
<td>0.34</td>
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</table>

d indicates treated root died
PLATE 5.3
CALLUS FORMATION ON TREATED ROOTS
SHOWING MACROSCOPIC VIEW OF CALLUS (a),
AND PHOTOMICROGRAPHS OF SECTIONS
THROUGH ROOTS (b and c). CALLUS
RATIO 0 (b) AND 0.16 (c).
PHOTOMICROGRAPHS OF SECTIONS THROUGH TREATED ROOTS. CALLUS RATIOS 0.61 (d), 0.90 (e), 1.0 (f). NOTE CALLUS DEVELOPMENT FROM CAMBIUM, EPITHELIAL CELLS AND PITH PARENCHYMA.
information on the manipulated root grafting process. No callus formed until three weeks after treatment, although areas of increased cell activity, as evidenced by small cells with large nuclei, were apparent earlier. Not all callus was initiated from the outer cambial layers, as pith parenchyma cells, epithelial cells, and isolated cortical parenchyma, all produced callus tissue on some roots (Plates 5.3 and 5.4). After three weeks, callus development became more profuse until, at the end of twelve weeks, callus tissue completely covered the cut surface on the majority of roots (Plate 5.4). Differentiation within this newly developed tissue was observed in only a few samples during the experiment.

(2) Manipulated root grafts.

Difficulty was experienced initially in getting the two roots of a grafted pair to fuse. Examination of the roots concerned, together with information gained from the hormone study, indicated that root size was the limiting factor. A root diameter of 3 mm appeared to be critical. Few roots below this diameter grafted successfully in contrast to the success achieved with roots of diameter greater than 3 mm.

Under the conditions of this study, the time required
for certain root fusion was thirteen weeks (Plate 5.1(a)). By this time, differentiation of the callus tissue had established a continuous xylem band between the roots concerned (Plate 5.1(b)). The three grafting techniques tried were equally successful suggesting that, initially, the physiological polarity of tissue had no effect on root fusion.

(3) **Root graft function.**

Isotope detection, using both the Geiger-Muller tube and autoradiography of seedlings, showed movement of isotope through root grafts of some seedling pairs. The results summarized in Table 5.2 show movement of $^{32}$P into four of the ten paired receptors but the patterns of movement observed are inconsistent. An autoradiograph of one of the seedling pairs is shown in Plate 5.5.

### TABLE 5.2

Summary of results of isotope movement through manipulated root grafts from donor to receptor seedlings.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Donor</th>
<th>Receptor</th>
<th>Number of Replicates</th>
<th>Positive Movement to Receptor</th>
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<tbody>
<tr>
<td>Shade</td>
<td>Sun</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Sun</td>
<td>Shade</td>
<td>2</td>
<td>1</td>
<td></td>
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<td>Topped</td>
<td>Untopped</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
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<td>1</td>
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</tr>
<tr>
<td>Untreated</td>
<td>Untreated</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

These experiments show that the formation of artificial root grafts in *Pinus radiata* seedlings is possible, and that when roots 3 mm or more in diameter are used, the chances of success are high. Fischer *et al.* (1960) have demonstrated the possibility of manipulating root grafts in seedlings of *Picea abies*. This technique offers considerable promise in studying the factors affecting movement through root grafts.

The polarity of tissues in the graft union did not appear important as far as the graft itself was concerned, but no work was done to determine whether or not polarity was important in movement of substances through root grafts (Section 1.2).

There was no evidence that application of the growth hormones IAA and GA stimulated callus development on cut root surfaces and, by inference, root grafting. Stem grafting is sometimes aided by growth hormones (Cook 1968), and it is possible that, at times, they may aid the formation of manipulated root grafts. Torrey and Loomis (1967) induced the differentiation of vascular tissues in radish roots by a mixture of growth hormones, the essential components of which were auxin and cytokinin.
PLATE 5-5 MOVEMENT OF $^{32}$P FROM DONOR SEEDLING (on left) TO RECEPTOR (on right) VIA MANIPULATED ROOT GRAFT SHOWN BY ARROW. MOUNTED SEEDLINGS ABOVE, AUTORADI.Graphics BELOW.
Possibly, use of a cytokinin would have stimulated callus formation, tissue differentiation, and root grafting in the present study.

The movement of isotope through some of the root grafts, although only in small quantities and in inconsistent patterns, is encouraging. The isotope was added only 20 weeks after the grafts were made. It is possible that anatomical continuity, particularly of the vascular elements between roots, was insufficient at this time for free movement through the graft unions. Also, the number of seedling pairs used in each treatment was minimal. However, the results show the feasibility of the technique which could be extremely valuable in studying the function of root grafts.
CONCLUSIONS

This study has shown that root grafting is widespread in radiata pine plantations in the A.C.T. In the relatively low site quality stands examined, root grafting appears to commence at a stand age of about 10 years, and then to increase rapidly with age until at least 25 years when it is likely that most standing trees have at least one functional root graft. Compared with other areas of radiata pine of higher site quality, the age at which root grafting commences in the A.C.T. is probably high and the incidence of grafting throughout the life of stands is probably low (e.g., Will 1966).

There was no evidence, from an examination of the variation in root grafting in different stands, that silvicultural practices such as initial spacing, thinning frequency or intensity, affected the incidence of root grafting. The one factor which did appear to have a marked influence on root grafting was soil depth: grafting was much less prevalent on shallow soils. The range of variation in silvicultural treatment and site quality in the stands studied was not great, and the available evidence, viz.,
infrequency of root grafting in trees of lower crown classes (Grose 1968, Pawsey 1962, Yli Vakkuri 1953), abundance of root grafting in radiata pine on high site qualities (Will 1966), and the paucity of root grafting in the A.C.T. on shallow soils, suggest that any factor which encourages vigorous root growth would encourage root grafting. The extent to which roots permeate the available soil volume must also be important, and it seems certain that initial spacing and thinning practices must have some influence on root grafting. The absence of such effects in the present study is probably due to the lack of sufficiently large differences in these factors in the stands examined.

Various techniques are available, at least in theory, for measuring the incidence of root grafting in forest stands. These include excavation of root systems, estimates based on the number of living stumps in thinned stands, and the use of tracer materials such as isotopes and dyes. For obvious reasons of expense and time, the possibility of excavating root systems on a large scale is limited. Estimates based on the number of living stumps are confined to stands which have been thinned, and there is no guarantee that the results apply to the retained trees. In addition, determination of the time at which
particular root grafts are actually formed can only be made by excavation and ring counts of fused root systems. The most meaningful expression of root grafting based on counts of living stumps may vary according to the state of the stand as discussed in Chapter 2. In theory, the use of radioisotopes, and less ideally, dyes to detect the presence of root grafts in undisturbed forest stands would seem to be the best technique.

In this study, however, serious limitations of the isotope technique were apparent. In some cases, although the isotope moved through a root graft into the base of a tree or stump, its presence could not be detected by the monitoring equipment used because of the depth of the isotope in the wood. In other cases, injected isotopes did not move through functional root grafts. Isotopes (and dyes) moved readily from live stumps to associated live trees, but in no case did the tracers move in the reverse direction nor did they move from one living tree to another. These results show that movement of the tracer depends on undefined factors and, until more is known of the factors affecting movement of substances through root grafts, the field application of tracers for the detection of root grafts has limited value.

The occurrence of root grafting in forest stands has
obvious importance for some silvicultural and management practices. For example, the possibility of carrying out chemical thinning or debarking is severely limited by the risk of damage to crop trees. Also, there is a distinct possibility of diseases such as root rots being transmitted between trees through root grafts.

Other less obvious effects of root grafts may also influence the growth of trees. In the past, many authors have speculated on the physiological implications of root grafts in thinned stands, but quantitative evidence available indicates that the association of living stumps with a live tree has no effect on the growth of that tree (see Graham and Bormann 1966). The same conclusion was reached in this study following analysis of the growth of trees associated with varying numbers (from 0 to 4) of live stumps. The allocation of stumps to trees was based purely on the relative positions of stumps and trees and, as pointed out in Chapter 4, this allocation may have been in error. However, the overall conclusion that the association of living stumps with a live tree had no effect on the growth of that tree, even under conditions of severe environmental stress such as drought, is probably valid.

Since living stumps continue to grow in diameter for
many years (although growth may only be slight) they must receive some organic nutrients from the associated living tree. Conversely, as shown by the movement of tracers from living stumps to living trees through root grafts, the living tree is able to draw on the root system of the stump for at least some water and inorganic nutrients. Possibly the exchange of organic material to, and inorganic material from, the living stump reaches a state of balance and the overall growth of the associated tree is unaffected.

The quantity of material passing through root grafts, however, is unknown and knowledge of the factors affecting such movement is likewise unknown. Until more information is available on questions such as these our knowledge of the precise function and potential importance of root grafting is incomplete. The patterns of movement of isotope through a root (Figs. 3.3 and 3.4) showed fluctuations which were not readily explicable in terms of environmental factors such as solar radiation and water stress. The causes of these fluctuations in translocation patterns should be sought. Also, more detailed examinations should be made of the plant, soil, and climatic conditions which affect the rate and direction of
movement of substances through root grafts.

Methods of successfully producing grafts between seedling roots are described in this study, and there appear to be no real difficulties with the technique. With such material the movement of substances, both organic and inorganic, through polar, antipolar and right-angular root grafts could be studied. The effects of environmental conditions, and of the relative growth rates of donor and receptor seedlings, could be examined under controlled conditions using root-grafted seedling material. It is considered that work of this nature could help elucidate the fundamental nature of root graft function.
## APPENDIX A

Reported occurrence of root grafts additional to those given by Graham and Bormann (1966)

<table>
<thead>
<tr>
<th>Species</th>
<th>Living Stumps</th>
<th>Self Grafts</th>
<th>Intra-specific</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acer saccharum</strong></td>
<td></td>
<td></td>
<td>x</td>
<td>Berchenko (1959)</td>
</tr>
<tr>
<td><strong>Eucalyptus delegatensis</strong></td>
<td></td>
<td></td>
<td>c</td>
<td>Grose (1969)</td>
</tr>
<tr>
<td>&quot; marginata</td>
<td></td>
<td></td>
<td>r</td>
<td>Kimber (1967)</td>
</tr>
<tr>
<td>&quot; regnans</td>
<td></td>
<td></td>
<td>c</td>
<td>Grose (1969)</td>
</tr>
<tr>
<td>&quot; tessellaris</td>
<td></td>
<td></td>
<td>o</td>
<td>Bisset &amp; Shaw (1954)</td>
</tr>
<tr>
<td><strong>Ficus globosa</strong></td>
<td></td>
<td>c</td>
<td></td>
<td>Rao (1966)</td>
</tr>
<tr>
<td><strong>Fraxinus viridis</strong></td>
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<td></td>
<td>x</td>
<td>Savel'eva (1968)</td>
</tr>
<tr>
<td><strong>Gleditschia (?)</strong></td>
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<td></td>
<td>x</td>
<td>Berchenko (1959)</td>
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<tr>
<td><strong>Picea glauca</strong></td>
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<td>c</td>
<td>c</td>
<td>Grose (1968)</td>
</tr>
<tr>
<td><strong>Pinus radiata</strong></td>
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<td>c</td>
<td>c</td>
<td>Adams (1940)</td>
</tr>
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<td>Boomsma (1949)</td>
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<td>c</td>
<td>c</td>
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<td><strong>Populus (?)</strong></td>
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<td>&quot; dumosa</td>
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<td>x</td>
<td>Hellmers et al (1955)</td>
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? = best inference    r = rare  
c = common            m = specifically missing  
o = occasional        x = present but frequency not stated
**APPENDIX B**

Summary of information on F.R.I. experimental plots used in this study. Total stems per acre after each thinning and basal area per acre (in parenthesis) are shown (data obtained from Cremer 1969).

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<td>399 (198)</td>
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APPENDIX C

Plot diagrams showing living trees and living stumps on all plots examined in the study.

Live portions of living stumps are shaded black.

Location of soil pits for examination of soil profiles referred to in Chapter 2 are shown by an X.

Drainage path in Plot 4 is shown by dotted line.
COMPARTMENT 48 STROMLO PLOT 1.

APPENDIX C

○ = living tree. • = living stump. o = partially alive stump

- Diagram showing the distribution of living trees and stumps in a compartment.

- Scale indicating distances along the x and y axes.
COMPARTMENT 48 STROMLO PLOT 2. APPENDIX C

○ = living tree. ● = living stump. ○ = partially alive stump.

0 20 40 feet
COMPARTMENT 48 STROMLO PLOT 3 APPENDIX C

• = living tree. • = living stump. o = partially alive stump.

0 20 40 feet
APPENDIX C

PLOT B

STROMLO.

COMPARTMENT 51

PLOT A

living tree. • = partially alive stump.

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o = living stump.
APPENDIX C

URIARRA

COMPARTMENT 132

O = living tree. • = living stump. ◯ = partially alive stump.
APPENDIX C

COMPARTMENT 140 URIARRA

PLOT 4

PLOT 3

PLOT 1

\( o = \text{living tree.} \quad \bullet = \text{living stump.} \quad \circ = \text{partially alive stump.} \)

\( x = \text{unlabeled stump.} \)
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