incompatibility process is inactivated by the removal of either. We call them here P (pollen) and S (stigma) factors.

Developed and used by us, these methods have produced large numbers of hybrid populations which otherwise could not have come into existence. The base for poplar breeding is broadened very greatly.

Improving poplars for disease resistance becomes very important in Australia. In 1972 the poplar rust species, *Melampsora medusae*, appeared and, in the following year — 1973, *Melampsora larici-populina* became widespread. Several biotypes of these two rust races have developed and more will come into existence. Their virulence may change and, as a result, the level of resistance of the poplar clones may change also. Work done by Dr. W. Heather has helped us immensely to understand development and behavior of the *Melampsora* fungus as well as its enemy, the hyperparasites *Cladosporium* and *Eudarluca*. Breeding of rust resistant poplar clones is the basis of poplar silviculture in Australia.

The introduction of semi-evergreenness into all other clones of the remaining sections can now be easily achieved. Species and clones, difficult to strike from cuttings, but otherwise of interest and value, can be used as parents to produce progenies which root from stem cuttings. Soon we shall know if hybrids of those wide crosses can produce fertile seed. If they do, back-crosses can be made and all the hopes of the poplar breeder can come true.

THE NATURE OF CALLUS AND ITS IMPORTANCE TO THE PLANT PROPAGATOR

FREDERICK C. HELLRIEGEL

Burnley Horticultural College
Melbourne, Victoria

Abstract. The ability of wounded plant tissue to regenerate is important for successful plant propagation. It enables a union to occur in budding and grafting, healing to occur at the base of cuttings, and allows rapid multiplication of selected plants in tissue culture techniques.

Regenerating cells from wounded tissues emanate primarily from the cambium region of the plant and they grow over the wound both radially and laterally. The overwalling of these cells is called callus and the nature of callus and its part in the rooting and unification process is explained in this paper.

With some species excess callus forms, inhibiting root primordia from emerging and preventing the formation of a viable root system on cuttings. This problem is examined with reference to the pH and nature of the propagating medium, the time of year of selection of cuttings, auxin application, wounding, trimming of callus, environment, and the type and condition of stock material. Certain recommendations are given for problems encountered with the propagation of some Australian native plants.

1.0 Grafting. Success in grafting depends on various factors including species characteristics and compatibility, correct timing, protection of all cut surfaces, proper aftercare, and close cambial contact between the stock and the scion (13).

1.1 The Graft Union. The graft union may be considered to be the healing of a wound with an extra piece of tissue, the scion, incorporated into the wound. This healing process involves callus proliferation and is dependent on physiological and environmental conditions which are suitable for high cell activity. These normally occur during early spring.

The cambial regions of both the stock and scion are meristematic (the tissues are capable of dividing and forming new cells). Mass proliferation of parenchyma cells interlock and form callus tissue in this region. Once the wound has completely healed some of these cells will differentiate into cambial cells producing xylem and phloem and enable a vascular connection to be made between the stock and the scion.

The expert grafter will aim to align the two cambial regions as closely as possible because it is in this area that callus proliferation is the highest. Poor matching may result in a failed union or a delay in the healing process. In the technique of cleft grafting *Camellia reticulata* hybrids, which are poor callus producers, some nurserymen tend to slightly offset the scion to ensure intimate cambial contact for at least one point.

1.2 Role of Temperature, Oxygen and Moisture. More than 50 years ago Shippy (18) showed that with bench grafting of apples the rate of callus formation increases directly with temperature (range 4° - 32°C). In bench grafting and hardwood cutting operations, callusing can proceed steadily by storing the grafts at low temperatures (7 - 10°C) or rapidly by storing at higher temperatures (25 - 28°C) for a shorter time. The grafts or hardwood cuttings may then be kept at reduced temperatures to prevent further callus and root development dependent on the logistics of the grafting operation.

Shippy (18) also showed that because of the high respiration rate of actively dividing cells, oxygen and moisture are important factors in the production of callus tissue. Waxing or taping the union protects the developing callus tissue from desiccation. For some plants this may be a restrictive factor in callus development, e.g. some *Vitis* spp., and a water saturated environment without waxing is more desirable. In practice, callusing in peat provides this environment.

1.3 Graft Chimeras. Chimeras can occasionally occur from adventitious buds which arise from the callus around the graft union. The Bizzarria orange is one such chimera which has been propagated by vegetative methods.

2.0 Budding. Budding may be considered to be a form of grafting, the main difference being in the technique. Only one bud is used whereas grafting uses a scion with several buds. The importance of good callus production is as essential with budding as it is with grafting.

2.1 The Bud Union. Mosse and Labern (16) have shown that in the technique of T budding the apple, the cambial layer of the stock actually remains on the inside of the bark flaps. The rootstock is responsible for producing most of the callus and it emanates from the exposed cells of the xylem cylinder. Providing environmental conditions are suitable, the callus begins rapid growth 2 days after insertion and continues for several weeks. The second stage involves formation of a continuous cambium between the bud and rootstock and complete lignification of the callus is completed in about 12 weeks.

Callus development differs marginally with other budding techniques such as chip, patch or flute, but in each case callus development is an essential part of the healing and unification process.

CUTTINGS

3.1 Callus Development. The development of callus at the base or along wounded surfaces of cuttings is, as it is in budding and grafting, a response by the plant to wounding. The regenerating cells from the wounded tissues emanate primarily from the cambial region (see Figures 1 and 2) and they grow over the wound both radially and laterally (1). This overwalling can, with some species and given certain conditions, occur to excess and a large thick mass of lignified callus may form at the base of the cutting. Some Australian members of the Proteaceae family commonly exhibit this problem, including species in the genera *Grevillea*, *Buckinghamia*, *Hakea*, and *Stenocarpus*.

Conditions favorable for the normal development of callus are also generally favorable for root initiation. This is why callus development was once considered an important factor in the root initiation of cuttings. It needs to be clearly stated that callus is part of the healing process and therefore should be considered separately to the rooting process. Only very rarely do roots actually originate in the callus tissue. The main advantage of callus development is in sealing the end of the cutting and preventing decay.

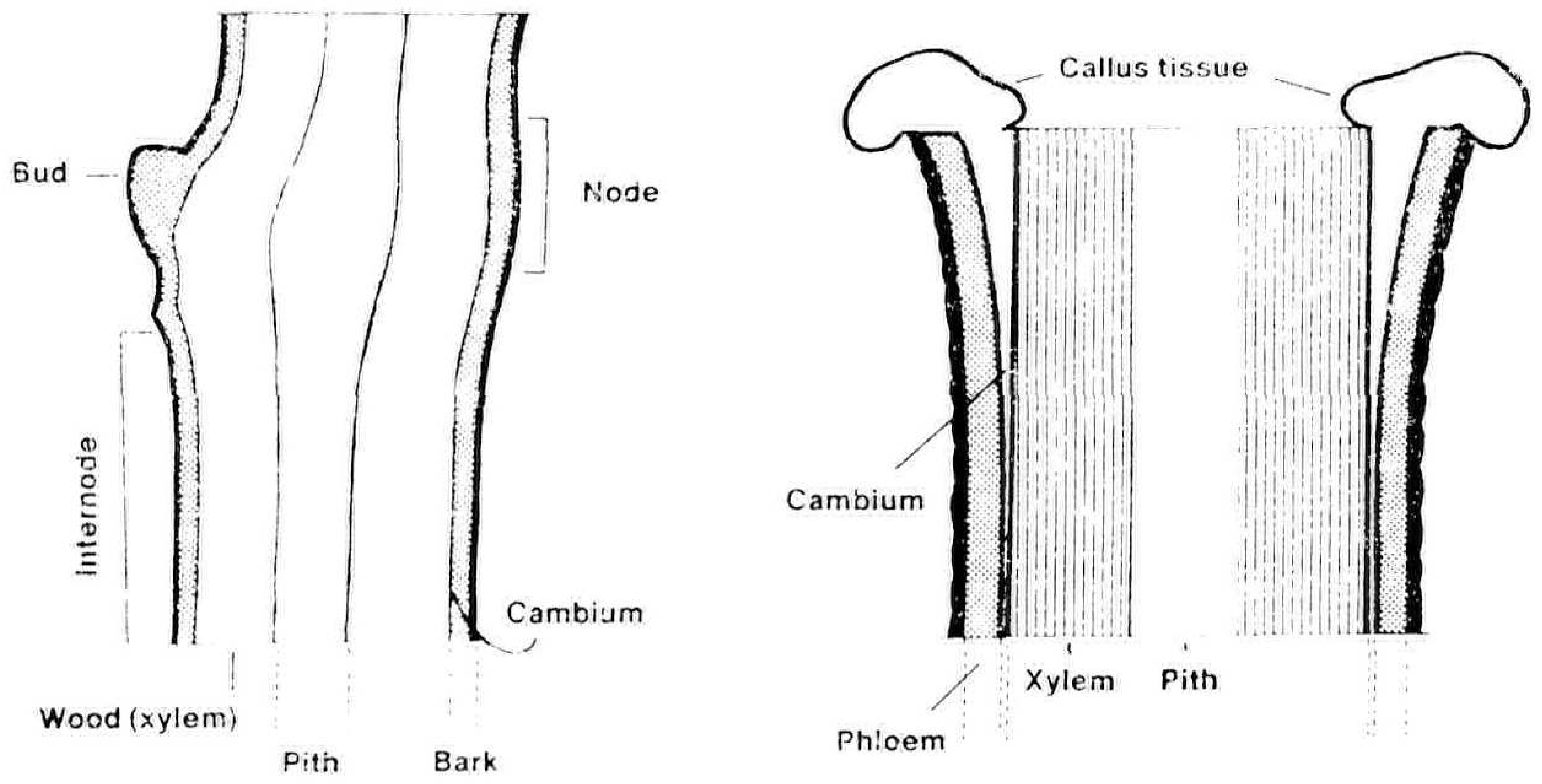

Figure 1. Anatomy of a vine cane and site of origin of callus tissues.

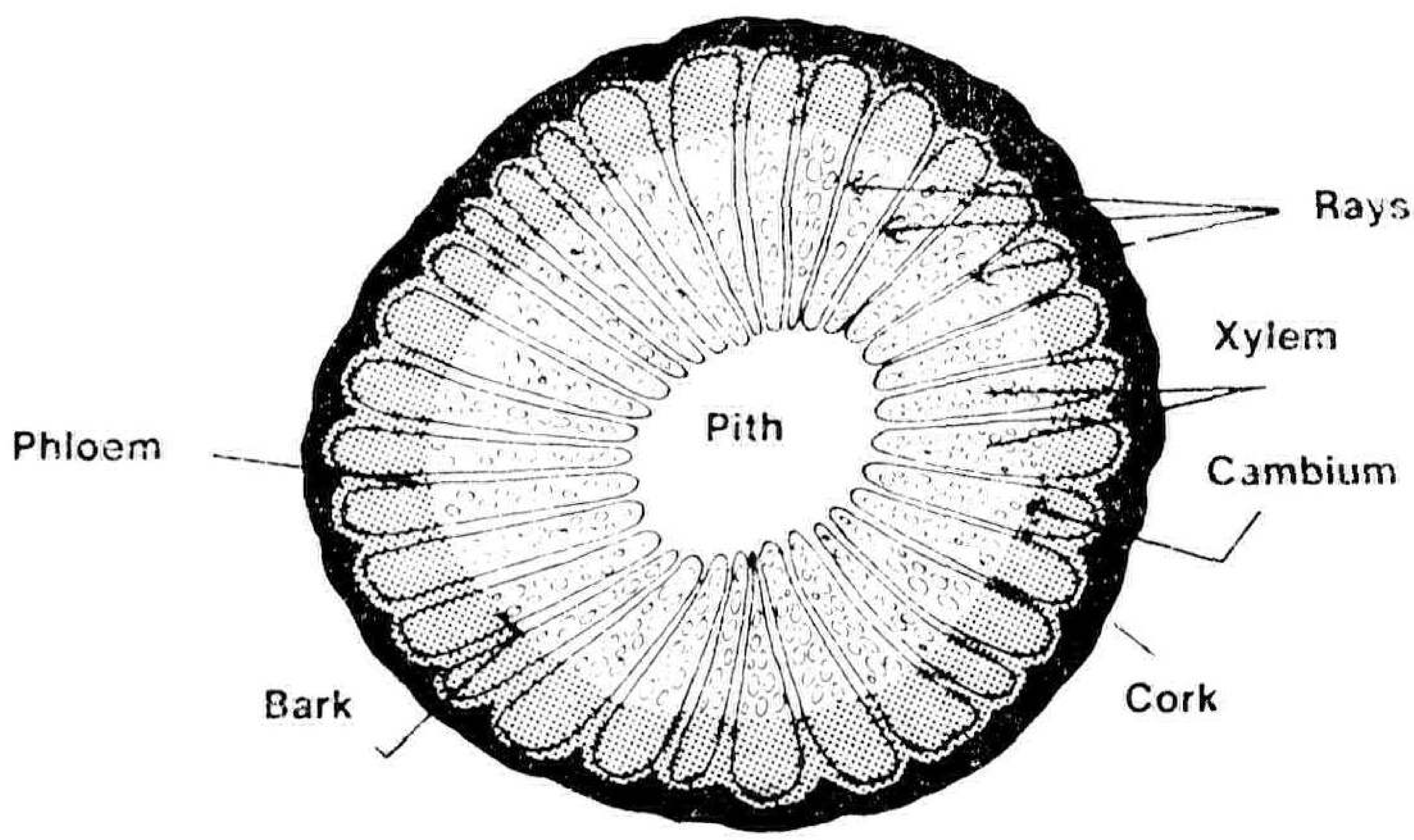

Figure 2. Cross-section of a vine cane illustrating the position of the main tissue components.

3.2 Root Initiation. The regeneration of plants from cuttings involves a careful manipulation of factors such as:

(i) the environment surrounding the cutting.

(ii) the anatomical, morphological, and physiological characteristics of the plant material

(iii) any treatment, either physical or chemical.

Garner and Hatcher (11) point out that a "carefully regulated interplay" of factors is necessary to stimulate rooting, particularly for plants which are considered to be shy-rooting. This interplay needs to be borne in mind when considering callus development and root initiation.

4.0 Factors Affecting Callus Development and Root Initiation:

4.1 Type and Selection of Cutting Material. Three main categories can be recognized in classifying the type of plant material to be selected from cuttings of woody perennial plants. They are softwood, semi-ripe, and hardwood. It is impossible to generalize about any type of material being the most suitable for optimum rooting for a range of plants. However, it is possible to generalize about the relationship between the type of plant material and the degree of callus development. Generally callus proliferation will be greatest on hardwood cuttings and the least development will occur on softwood material.

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Experimental Trial — Effect on Rooting and Callusing of Tip and Basal Cuttings of Grevillea 'Ivanhoe'

Aim: To examine the degree of callus development and root initiation on tip and basal cuttings of *Grevillea* 'Ivanhoe'.

Materials and Methods: Tip and basal cuttings were selected from 1 yr old healthy stock plants in February, 1982. The cuttings were surface sterilized in a 0.5% sodium hypochlorite solution. The rooting medium was 2 pts coarse river sand, 1 pt peat and 1 pt perlite. Treatments were a control, a hormone talc preparation, [Seradix 2 (IBA4 g/kg)] and a liquid dip equivalent (IBA at 3000 ppm for 5 secs). Each treatment consisted of 30 cuttings (two replicates of 15 each). Cuttings were set into 15 cm pots — 15 cuttings per pot and placed under intermittent mist, with bottom heat at 23°C ± 1°C. All cuttings were drenched with a fungicide, (Captan) at a rate of 15 g/10 litres water. All cuttings were harvested after 4 weeks.

Tip Cuttings. Sixty percent of the tip cuttings had formed a strong, well branched root system. Liquid dip preparations performed marginally better than talc preparations. Cuttings which failed to root looked healthy and although the bases had callused, little over-callusing was evident at this stage.

Results: (See Tables 1 and 2)

Table 1. Effect of auxins on rooting and callusing of basal cuttings of *Grevillea* 'Ivanhoe'.

Treatment	Percent Excess Callus	Percent Rooted	Average Root Length	Average no. Roots/Cutting
Control	50	—	—	—
Talc, IBA 3g/kg	60	10	1 cm	8
Liquid, IBA 3000 ppm	50	10	1	8

Table 2. Effect of auxins on rooting and callusing of tip cuttings of Grevillea 'Ivanhoe'.

Treatment	Percent Excess Callus	Percent Rooted	Average Root Length	Average no. Roots/Cutting
Control	5	20	1 cm	6
Talc, IBA 3g/kg	10	55	5 cm	8
Liquid, IBA 3000 ppm	10	60	5 cm	8

Some of the control cuttings (no hormone treatment) had begun to form roots. Little callus development had occurred.

Basal Cuttings. Minimal root development occurred on the basal cuttings. Varying degrees of callus development were evident and some of the cuttings had clearly begun to over-callus.

Discussion: It is fairly well established in commercial horticulture that for plants which are difficult to root, young growth and juvenile growth will often give improved rooting over older growth. The increased rooting ability of shoot tips is likely because of higher concentrations of naturally occurring rooting auxins and co-factors, coupled with the fact that there is less differentiation in the primary (shoot) growth and more cells are potentially meristematic.

Poor rooting with a stronger tendency to over-callus on basal cuttings of *Grevillea* 'Ivanhoe' is likely because of the change in the carbon/nitrogen ratio of the plant material and the lignification of cell walls which occurs during secondary growth.

Other Species. Persoonia pinifolia is considered to be difficult to propagate by conventional methods (10). Elliot (8) has achieved 90% success with this species by selecting young, soft growth in late spring which has slightly firmed.

* * * * * *

4.2 Source of Cuttings. For difficult-to-root species the genotype of the parent plant can be an important factor in ability to root adventitiously (14). This is to be expected when considering the sort of variation which occurs amongst sexually propagated species. It may also explain why very young cuttings of *Grevillea* 'Robyn Gordon' selected from physiologically stable, tissue culture raised plants are so easy to strike.

4.3 Physiological Condition of Plant Material. Factors which influence the physiological condition of the propagation material are climate, production schedules for mother stock, nutrition, and protected environment structures. In Queensland the vigour of *Grevillea* 'Robyn Gordon' is far greater than in Victoria, and suitable cuttings may be larger and taken over a longer period of time.

Generally with species which tend to over-callus during the late autumn/late winter period should be avoided. This is because of the physiological condition of the stock plant. The plant has a higher C/N ratio and increased lignification of cell walls. Flower initiation also occurs for most Proteaceae during this period and vegetative growth is minimal. It has been shown by Dore (7) that cuttings are best taken either before or after, rather than during, the flowering period.

Hard pruning of stock plants during late autumn/early winter, coupled with a light, slow-released fertilizer application will encourage young healthy growth suitable for propagation in late spring/early summer.

4.4 Auxins. The important role of rooting hormones is well established in modern horticulture. IBA, NAA, and to a lesser extent, IAA and 2,4-D, are utilized to hasten root initiation and encourage more uniform rooting.

Empirical trials are necessary to determine the optimum hormone treatment for a particular species. Ellyard (9,10) has recommended concentrated, quick liquid dips as consistently yielding optimum results for a range of Australian native plants.

It has been my experience that liquid formations may be applied more accurately to plant material with marginally better results than talc formulations. Excess callusing for many of the "holly-leaved" grevilleas is more likely to occur with talc preparations, especially if excess talc is allowed to remain on the base of the cutting.

4.5 Rooting Media. Rooting media should have the general qualities of good drainage, aeration, and moisture retention. As a result of trials carried out at Burnley Horticultural Center (B.H.C.), a medium consisting of 2 parts coarse, washed river sand, 1 part sieved German peat moss, and 1 part perlite is recommended for a wide range of plants. Excess sand or poorly drained sand will encourage a hard, lignified callus and excess peat contribute to poor drainage and aeration, increasing the likelihood of basal rot.

4.6 Temperature. Temperature has a definite effect on the rooting of cuttings and callus development. Ooishi *et al* (17) showed that callus formation and rooting of camellia cuttings were greatly stimulated with rising temperature; 16%, 36%, and 87.5% root formation was shown at 17°, 23°, and 30°C, respectively.

Preliminary trials at B.H.C. with a species which is very difficult to root and consistently over-calluses, *Grevillea john-*

sonii, have suggested that increased temperatures encourages callus development but does not alone stimulate rooting.

4.7 pH. The pH of the propagating medium will influence the ability of some plants to regenerate roots from cuttings and may affect the nature of the callus. Studies on *Thuja occidentalis* showed that rooting increased between pH 5.1 and 9.3 but with no evident change in the callus. However studies on *Populus balsamifera* (5) showed that increasing the pH from 6.8 to 11.0 resulted in increasingly calcified and dense callus which inhibited the emergence of root primordia.

Most standard propagating media have a pH of approximately 5. Trials to determine pH responses for a range of plant material under propagation may yield interesting results.

4.8 Wounding. Wounding the base of difficult-to-strike cuttings can hasten root and callus development and improve the quantity and quality of roots (21), especially along the margins of the wound. This is probably due to a concentration of naturally occurring hormones and increased carbohydrate levels along the wounded area. Day (6) suggests that wounded cuttings are able to absorb more water from the propagating medium and, therefore, probably increased absorption of rooting hormones.

4.9 Trimming of Callus. Cuttings which have over-callused may be removed from the propagating bed, the callus trimmed, and the cuttings re-dipped in hormone. After re-setting in the propagating medium, rooting will often follow. Balfour (2) proposed that the reason this occurs is that the paring of the callus reduces its growth and the food supply it draws on is left available for the vascular cambium. This then grows more actively and, as a result, produces root initials. It is more likely that the factors which have combined to cause this excess callus also contribute to discourage root development. When the outer layers of lignified cells are removed, developing primordia are sometimes evident. With the physical barrier removed and an increased application of hormone, rooting often results.

4.10 Nodal Cuttings. Most propagators tend to prepare cuttings by cutting either through, or just below a node. This procedure is based on tradition rather than scientific fact as the vascular cambium which is responsible for most root initiation forms a continual longitudinal cylinder in the steam. Chadwick (4) reported that large callus growth on *Weigela florida* 'Eva Rathke' could be avoided and better roots initiated, by preparing tip cuttings and making the basal cut 1 to 2 cm below the node. This technique may have application for many Australian plants which tend to over-callus. It can also make more economical use of propagating material.

5.0 Tissue Culture. Tissue culture is the development of new plants in an artificial medium under sterile conditions. For rapid multiplication of most woody perennial plants the multiple shoot technique is utilized. Callus tissue will still develop on species such as *Grevillea* 'Robyn Gordon' (12) using this technique.

It is possible to develop this callus tissue in a test tube and grow it indefinitely on nutrient agar. From this undifferentiated callus mass, adventitious roots and shoots can be encouraged by applying the correct amount of auxins and cytokinins for the species. However these plants may show considerable variation as "somaclonal variation" often occurs in these plants due to complex changes at the cellular level (15). Therefore the main horticultural value in these variants is in plant breeding and scientific research. This is an exciting new field with great potential for the horticultural industry.

Most of the research in this area has occurred on food crops, e.g. sugar cane, potato, *Brassica* spp., but Skirvin and Janick (19,20) have observed variation in callus-derived somaclones of five *Pelargonium* cultivars. It is interesting to note that one of these variants has been released as a new cultivar in U.S.A. called 'Velvet Rose'.

Acknowledgements. Figures 1 and 2 reprinted with kind permission from Ag. Bulletin, *Propagating and Grafting of Grapevines*. John A Considine. Victoria Dept. of Agriculture.

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PROPAGATION OF PIN OAK *(QUERCUS PALUSTRIS)* TO PREVENT WINTER LEAF RETENTION

R. BODEN, J.H. FRYER, AND G. KING

Department of the Capital Territory
Canberra, A.C.T.

INTRODUCTION

The pin oak (*Quercus palustris*) is a tree of considerable amenity value in the cooler areas of southeast Australia. It is vigorous and hardy on most soils, it is quite drought tolerant and gives heavy summer shade and brilliant autumn colours. To date it has shown only a low susceptibility to the oak leaf miner.

However pin oak has one obviously unattractive habit. In mature trees the upper crown loses its leaves in late autumn but most of the dead leaves on the lower crown persist and are shed gradually during winter and spring, giving the tree a rather tattered appearance for much of this period. This gradual loss of leaves necessitates a number of winter clean-ups by residents or park authorities, rather than one concerted effort in autumn.