Trees (1998) 12: 175–180 

ORIGINAL ARTICLE

**Andreas Hamann**

# Adventitious root formation in cuttings of loblolly pine (*Pinus taeda* L.): developmental sequence and effects of maturation

Received: 3 June 1997 / Accepted: 15 August 1997

**Abstract** Adventitious root formation in cuttings from fascicular shoots in loblolly pine (*Pinus taeda* L.) consists of four more or less discontinuous stages: (1) proliferation of cells at the base of the cutting, (2) differentiation of wound vascular tissue and periderm, (3) dedifferentiation of a zone near the wound cambium and wound phloem to form a root initial, and (4) formation of a root meristem. Anatomical changes during adventitious root initiation are described in cuttings from donors of different types and ages. Cuttings from seedlings and 3- to 7-year-old hedged stock plants rooted better than cuttings from 3-year-old tree form donors. It is concluded that the loss of rooting capacity in loblolly pine can be arrested by shearing loblolly pine stock plants to low hedges. The process of root initiation, however, was similar in cuttings from all sources and is apparently not the cause for the rapid decline of rooting potential with increasing age of the donor plant.

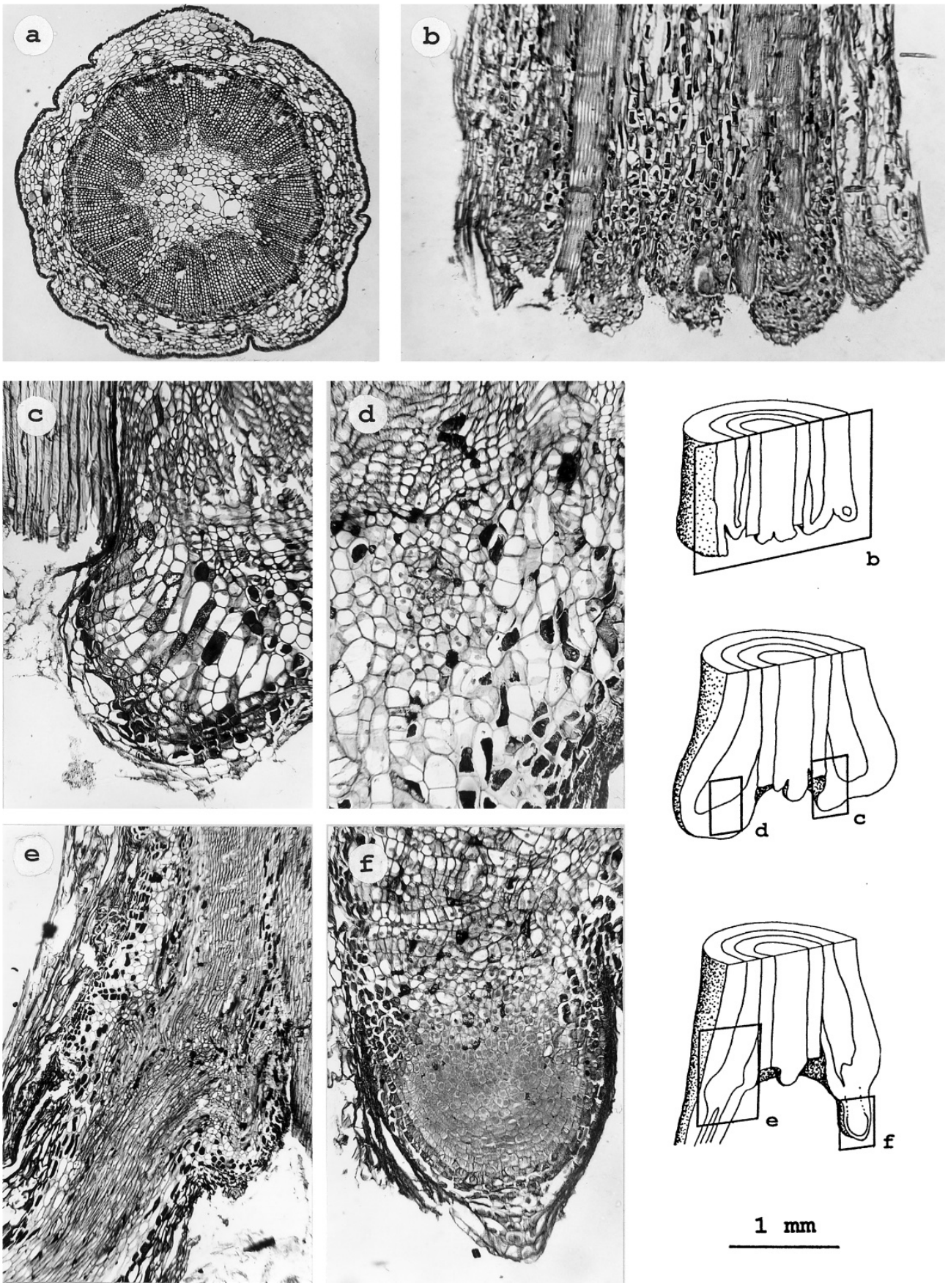
**Key words** Loblolly pine · Rooted cuttings · Adventitious root formation · Maturation

## Introduction

Loblolly pine (*Pinus taeda* L.) is a species of major economic importance and is widely planted for pulpwood and lumber in south-eastern USA. Vegetative propagation of loblolly pine has only been an important technique for the establishment of grafted seed orchards. More recently propagation by rooted cuttings has also been considered for production of commercial planting stock, either for a clonal forestry system or to bulk up small quantities of seeds from elite families for commercial plantations. Unfortunately, loblolly pine is among the species most difficult to root, and consequently much research has been carried out to investigate various factors influencing the rooting process (Hare 1974; van Buijtenen et al. 1975; Greenwood et al. 1980; Frampton and Hodges 1989; Weir and Goldfarb 1993). An accurate understanding of the process of root initiation, however, is lacking although this problem has previously been addressed (Stickney 1987). The aim of this study is to extend the knowledge of the anatomical changes in loblolly pine cuttings undergoing the rooting process.

The ease with which cuttings of loblolly pine can be rooted declines rapidly with the age of the parent stock (McAlpine and Jackson 1958; Greenwood and Nussbaum 1981; Greenwood and Hutchinson 1993). Age related processes underlying such phenomena are generally referred to as maturation. In most economically important conifers, maturation of stock plants is an important problem for vegetative propagation systems and has been subject of research for several species, reviewed by Hackett (1985) and Greenwood and Hutchinson (1993). In most species that are difficult to root, initiation of roots occurs within callus tissue. Examples are *Pinus eliotii* (Reines and McAlpine 1959), *Pseudozuga menziesi* (Haeman and Owens 1972) or *Picea abies* (Dalgas 1975). Conversely, in easy-to-root species adventitious roots arise directly from stem tissues (Haissig 1974; Lovell and White 1986). Sometimes the developmental sequence of root formation changes when the stock plant matures, resulting in a sudden loss of rooting capacity. Such patterns have been described in *Pinus radiata* (Cameron and Thomson 1969; Smith and Thorpe 1975) and *Hedera helix* (Geneve et al. 1988). In these cases direct formation of roots occurs in juvenile cuttings and formation of roots from callus occurs in cuttings from more mature donors. For an operational propagation system it is necessary to arrest maturation in stock plants and maintain juvenile rooting characteristics. In some conifers this has been achieved by shearing of stock plants to low hedges (Libby et al. 1972; Russell and Grossnickel 1989). In this study, cuttings from seedlings, trees and stock plants of a hedged cutting orchard were used to investigate effects of putatively different levels of maturation on the developmental sequence of root formation.

A. Hamann
Department of Forest Science, University of British Columbia, Vancouver B.C., Canada, V6T 1Z4,
Fax: (604) 822-8737; e-mail: hamann@unixg.ubc.ca


a
b
c
d
e
f
b
d
c
e
f
1 mm

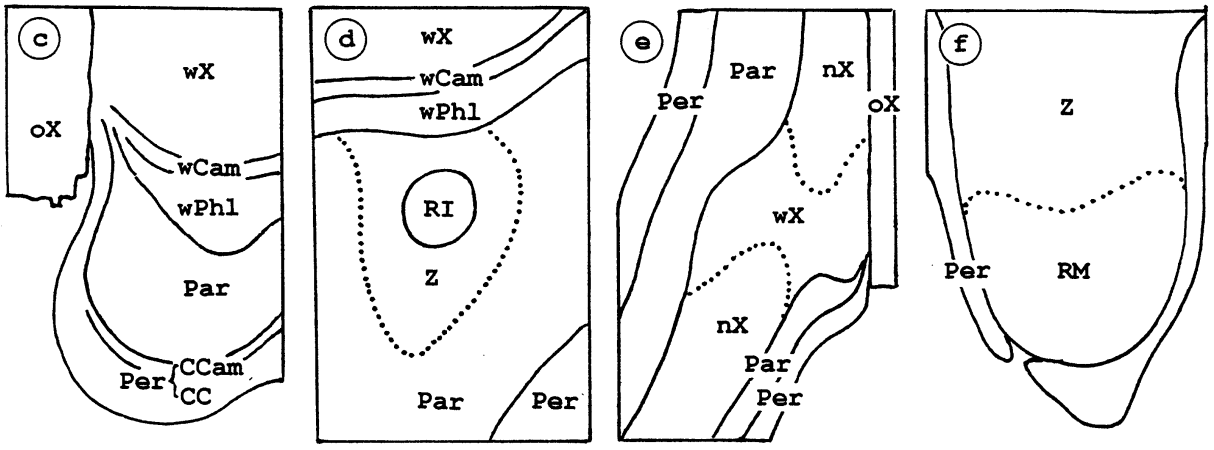



**Fig. 1** Anatomical changes of loblolly pine cuttings undergoing root development. Cross-section at the time of severance (**a**); callus development after 10 days (**b**) and after 20 days (**c**); root initial after 20 days (**d**); formation of vascular tissue (**e**) and root primordium (**f**) after 30 days. The line drawings provide scale and position of the sections (*oX* = old xylem, *nX* = new xylem, *wX* = wound xylem, *wPhl* = wound phloem, *wCam* = wound cambium, *Par* = parenchyma, *Per* = periderm, *Ccam* = cork cambium or phellogen, *CC* = cork cells, *RI* = root initial, *RM* = root meristem, *Z* = zone of cell division)

## Materials and methods

### Plant material

Cuttings for histological investigations were obtained from stock plants that were sheared 2–3 times per year to maintain a height of 20–30 cm. Hedged stock plants were grown outdoors in 15 l polypropylene pots in a commercial potting mixture. Stock plants were irrigated, fertilized, and treated with pesticides as required. The hedges were 3, 5 and 7 years old. In addition 8-month-old seedlings and 3-year-old non-sheared trees served as putatively more juvenile and more mature material. All donor plants were seedling grown and of the same full-sib family of the Westvaco corporation tree breeding program. Shearing of hedged stock plants caused quiescent meristems in needle fascicles to develop into fascicular shoots. Cuttings from such shoots can be rooted relatively easily. Seedlings had been decapitated to induce elongation of fascicular shoots. Likewise the terminal meristems of first order side branches in the upper crowns of 3-year-old trees were removed to force needle fascicles to break; 290 cuttings were sampled for histological investigations at 10-day intervals over a period of 3 months. Cuttings were taken from ten different donor plants for each treatment type when fascicular shoots were about 10 cm long. After 10, 20, 30, 40 and 90 days ten cuttings of all treatments were investigated, and after 50 and 60 days only cuttings from 3-year-old hedges and 3-year-old trees were sampled.

### Rooting procedures

Cuttings were placed in the rooting medium, consisting of equal parts Perlite and coarse Vermiculite, to a depth of 1.5–2 cm. Rooting took place in a shaded fiberglass greenhouse. The air temperature was maintained at approximately 27 °C during the day and 20 °C at night. The temperature of the rooting medium was kept at 25 °C using a root zone heating system (Biotherm Engineering, Petaluma, Calif.). Day length was artificially extended to 14 h with high intensity discharge (HID) lamps (Ruud Lighting) and a uniform intermittent mist was provided by a gantry boom sprayer ITS Grower Jr (J.M. McConkey, Sumner, Wash.). The mist was operated at varying intervals that were automatically adjusted based on light intensity. No rooting hormones were used.

### Histological investigations

The basal 5–6 mm of cuttings were evacuated in FAA for 15 min and then fixed for at least 12 h in a fresh solution of FAA. The material was then dehydrated in a tertiary-butyl-alcohol series. After 25% chloroform was added, the material was gradually embedded with increasing concentrations of paraffin at increasing temperatures (Berlyn and Miksche 1976). For embedding in pure paraffin a vacuum infiltrator (Lipshaw, model 224) was used. Embedded tissue on one side of each block was exposed by manually slicing away the paraffin, followed by soaking for 12 h in a 20% aqueous solution of glycerol at 40–45 °C to soften the tissue (Jewell 1958). The material was then immediately sectioned with a rotary microtome at a thickness of 10–12 µm. Serial

**Table 1** Time course of callus and root formation in cuttings from seedlings (S), hedges 3, 5 and 7 years old (H3, H5, H7) and 3-year-old non-sheared trees (T3). Frequencies of observations in 10 samples are given

| Days after sectioning | Callus | | | | | Root initials | | | | | Root primordia | | | | | Roots | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | H3 | H5 | H7 | T3 | S | H3 | H5 | H7 | T3 | S | H3 | H5 | H7 | T3 | S | H3 | H5 | H7 | T3 |
| 10 | 8 | 9 | 7 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 9 | 10 | 8 | 10 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 10 | 9 | 9 | 10 | 3 | 2 | 1 | 3 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 40 | 9 | 8 | 10 | 10 | 3 | 4 | 5 | 7 | 4 | 0 | 2 | 0 | 1 | 1 | 0 | 2 | 3 | 1 | 5 | 1 |
| 50 | | 10 | | | 4 | | 6 | | | 1 | | 2 | | | 0 | | 8 | | | 0 |
| 60 | | 9 | | | 2 | | 3 | | | 0 | | 0 | | | 0 | | 7 | | | 2 |
| – | | | | | | | | | | | | | | | | | | | | |
| – | | | | | | | | | | | | | | | | | | | | |
| 90 | 10 | 10 | 9 | 10 | 3 | 3 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 6 | 9 | 7 | 0 |

sections were mounted on slides prepared with a chromium-potassium-sulfate adhesive and stained for 2–3 min with toluidine blue (0.05% in aqueous solution). Longitudinal (tangential and radial) sections and transverse sections were examined and photographed on a Leitz diaplan microscope. Line-drawings were made on the basis of complete serial sections.

## Results

### Effects of maturation on timing and anatomy of root development

The observations made in samples from various donors taken during a 90-day period are summarized in Table 1. The anatomical changes during root formation and the origin of adventitious roots were the same in material from different sources. However, cuttings from 3-year-old trees show a general loss in rooting potential. Callus development and root initiation was less frequently observed, and the wound healing process was slightly delayed, with cuttings from 3-year-old donors showing callus formation only after 20 days. The numbers of root primordia observed was low at all sampling dates and in cuttings from all donors, suggesting that once meristems have formed, they develop rapidly into roots. Within a 90 day period around 80% of the cuttings from seedlings and hedged donor plants showed development of roots, while those from 3-year-old trees ranged from 0 to 20% (Table 1). It was further observed that callus development in cuttings from 3-year-old trees was less vigorous and emerging roots were thicker and more brittle compared to those of cuttings from hedges or seedlings.

### Developmental sequence of adventitious root formation in cuttings of loblolly pine

Figure 1a shows the general cross-sectional anatomy of a cutting base at the time of severance from the donor plant. The xylem had already formed a closed ring and the epidermis is not yet replaced by a periderm. Wound healing processes after 10 days in the rooting environment are shown in Fig. 1b. Callus, a mass of rather unorganized, large parenchymatic cells, proliferated from the most proximal cells of the cortex, vascular cambium and pith. It should be noted that the vascular cambium showed normal formation of new xylem and new phloem in the upper part of the stem whereas the lowest 500 µm of the cambium produced abnormal vascular cells.

At about 20 days parenchyma cells in the periphery of the callus mass differentiated and formed a phellogen. At first the outer cells showed a yellowish staining which indicates that suberization had taken place. Subsequently, a continuous periderm developed with several layers of crushed cork cells formed over the surface of the callus (Fig. 1c). This can also be observed macroscopically as the initially white callus mass turns brown. In cuttings of larger diameter ($>2$ mm), callus proliferations from the pith and the cortex remained separated from each other and the old xylem by the formation of the aforementioned periderm, while in cuttings with a diameter of about 2 mm and smaller, callus from pith, cortex and cambial zone had coalesced before the periderm had formed. The whole wound area was consequently covered by a single callus mass (Fig. 2). Complex differentiation had also taken place within the callus after 20 days. In many cuttings wound vascular cambium developed hemispherically around the entire base of each cutting, producing wound tracheids towards the center of the callus mass and wound phloem towards the periphery.

After 30 days root initials could be observed in the vicinity of wound vascular tissue (Fig. 1d). At some sites a fully formed root meristem had ruptured the periderm (Fig. 1f). The formation of a new root continued with the development of a vascular system in the callus, continuous with that of the original cutting. Vascular cells were produced in a zone of highly active cell division and differentiation approximately 1 mm behind the root primordium. Figure 1e shows newly differentiated xylem strands which are connected to wound vascular tissue and stretch into an approximately 1 cm long root.

One interesting observation which did not seem to be related to the rooting process was the formation of somewhat spirally oriented "nests" of wound tracheids (Fig. 2). Root initials could also be observed in the vicinity of tracheid nests, but zones of proliferation arising from isolated tracheid nests did not form meristems or give rise to roots. Instead, differentiation of new periderm and wound parenchyma took place resulting in finger-like protuberances, which macroscopically could easily be mistaken for root primordia. Sometimes new tracheid nests formed in protuberances and new protuberances developed from those tracheid nests, leading to complex patterns. The line drawing in Fig. 2 shows the arrangement of wound tissues within the callus mass leading to both root development and parenchymatic protuberances in one cutting.

## Discussion

The results indicate that loss of rooting capacity can be arrested effectively by shearing stock plants to low hedges. Without such a cultural treatment, rooting success in loblolly pine cuttings from the upper crown of tree form donors declines to a very low percentage within the first 5 years of age of the donor plant (Greenwood and Nussbaum 1981). In this experiment there is no indication of a loss in rooting potential in cuttings from hedged donor plants up to 7 years old when compared to cuttings from seedlings. This can be interpreted as a delay of maturation by a cultural treatment. However, Greenwood (1995) pointed out that rooting is only one of many maturational traits that vary independently of one another, and rooting competence should not be considered as a marker for a juvenile state in general. Also, cultural effects cannot be completely excluded because hedges and seedlings were grown in

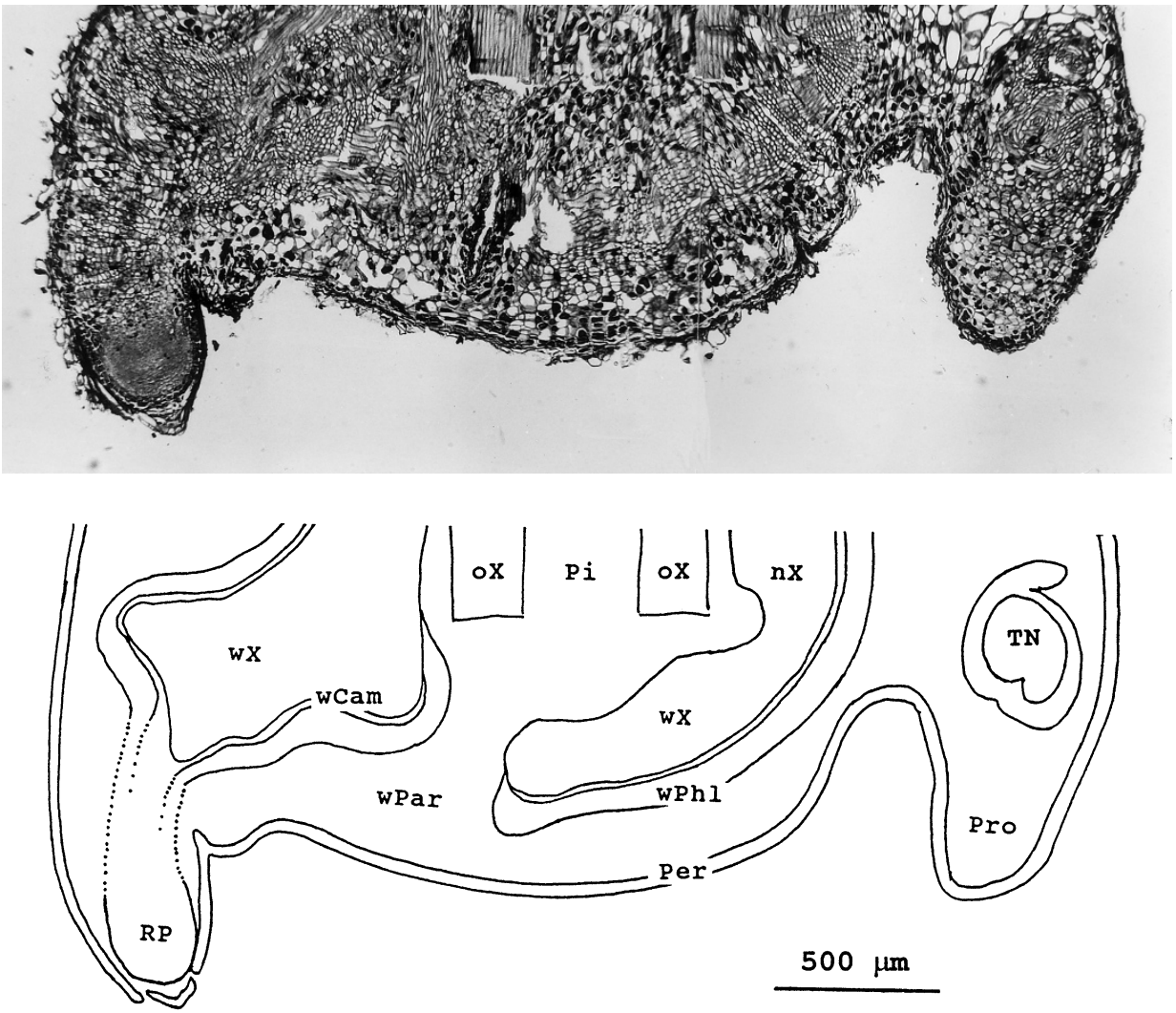



**Fig. 2** Arrangement of wound tissue leading to both, root development and protuberances in one cutting (*oX* = old xylem, *nX* = new xylem, *wX* = wound xylem, *wPhl* = wound phloem, *wCam* = wound cambium, *wPar* = wound parenchyma, *TN* = tracheid nest, *Per* = periderm, *Pro* = protuberance, *RP* = root primordium)

containers, while tree-form donors had to be grown in the field.

The loss of rooting capacity with increasing age of the donor plant over the first 3 years does not seem to be related to changes in root initiation. The developmental sequence observed in cuttings from all sources is indirect via a wound tissue. However, in material more juvenile than 8-month-old seedlings, direct root formation has been observed. In hypocotyl cuttings from seedlings that were only several weeks old, roots usually originated in the vicinity of a resin duct. Direct root formation has been described for *Pinus radiata* (Smith and Thorpe 1975), *P. sylvestris* (Grönroos and von Arnold 1987), *P. contorta* (Grönroos and von Arnold 1988), and *P. taeda* (Diaz-Sala et al. 1996), while indirect root formation in cuttings from more mature material has been found in *P. radiata* (Cameron and Thomson 1969), and *Pinus taeda* (this study). The previous studies suggest a sudden decline in rooting capacity of hypocotyl versus epicotyl cuttings, which is associated with changes in the anatomy of root formation. Direct rooting also appears to be faster than indirect rooting via a wound tissue (15–30 days vs 40–90 days). A more gradual decline in rooting capacity of cuttings with increasing age of the donor plant over several years, however, is apparently not associated with changes in the anatomy of root formation. This suggests the existence of two different maturational processes underlying changes in rooting capacity in pines.

Anatomical changes during rooting in loblolly pine are similar to those in other difficult-to-root conifers (Haissig 1974). Roots do not develop directly from certain stem tissues or from preformed and dormant root primordia, but callus formation and differentiation of wound vascular tissue within the callus apparently precedes root initiation. The anatomical changes that occurred in the basal portion of the cutting and lead to formation of roots were different from those Stickney (1987) observed. In his investigations cuttings from fascicular shoots received an auxin pulse treatment for 9–14 days in vitro before transfer of cuttings to the rooting environment, leading to excessive callus production which made histological observations difficult.

The current study reveals that root development is initiated in the vicinity of wound vascular tissue, as it is in the case of radiata pine (Cameron and Thomson 1969), and Douglas-fir (Bhella and Roberts 1975).

The observation of tracheid nests has previously been made by several authors in other conifers (Reines and McAlpine 1959; Dalgas 1973). In this study it was observed that protuberances are the result of a possibly unsuccessful root initiation originating from isolated wound tracheids. These protuberances can frequently be recognized macroscopically and easily mistaken for root primordia. Similar observations may have led to the conclusion that the development of root meristems and the elongation of roots are discontinuous stages in root development (Dalgas 1973; Hackett 1985), and that improvements of the rooting environment or chemical treatments aimed at breaking the presumed dormancy of root meristems may enhance rooting success. This study suggests that root meristem development requires vascular continuity with the cutting. Once root meristems have formed, they always develop rapidly into roots.

**Acknowledgements** Provision of plant material, greenhouse space and laboratory facilities by Westvaco Forest Research Laboratories is gratefully acknowledged. The author is particularly indebted to Dr. Farrel Wise of Westvaco Corporation, and to Dr. Miklos Graetzer of SUNY-ESF for help and good advice throughout the study.

## References

Berlyn GP, Miksche JP (1976) Botanical microtechnique and cytochemistry. Iowa State University Press, Ames, Iowa

Bhella HS, Roberts AN (1975) Seasonal changes in origin and rate of development of root initials in Douglas-fir stem cuttings. J Am Soc Hortic Sci 100:643–646

Buijtenen JP van, Toliver J, Bowere R, Wendel M (1975) Mass production of loblolly pine and slash pine cuttings. Tree Planter's Notes 26:4, 26

Cameron RJ, Thomson GV (1969) The vegetative propagation of *Pinus radiata*: root initiation in cuttings. Bot Gaz 130:242–251

Dalgas KF (1973) Anatomical studies on cuttings of Norway spruce undergoing the rooting process. For Tree Improv 5:1–20

Diaz-Sala C, Hutchinson KW, Goldfarb B, Greenwood MS (1996) Maturation-related loss in rooting competence by loblolly pine stem cuttings: the role of auxin transport, metabolism and tissue sensitivity. Physiol Plant 97:481–490

Frampton LJ, Hodges JF (1989) Nursery rooting of cuttings from seedlings of slash and loblolly pine. South Journal of Appl For 13:127–132

Geneve RL, Hackett WP, Swanson BT (1988) Adventitious root formation in de-bladed petiols from juvenile and mature phases of English ivy. J Am Soc Hortic Sci 113:630–635

Greenwood MS (1995) Juvenility and maturation in conifers: current concepts. Tree Physiol 15:433–438

Greenwood MS, Hutchinson KW (1993) Maturation as a developmental process. In: Ahuja MR, Libby WJ (eds) Clonal forestry. I. Genetics and biotechnology. Springer, Berlin Heidelberg New York, pp 14–33

Greenwood MS, Nussbaum ES (1981) Rooting ability and vegetative growth performance of stem cuttings from one- and five-year-old ortets of loblolly pine. Proceedings of the 16th Southern Tree Improvement Conference, May 27–28, Blacksburg

Greenwood MS, Marino TM, Meier RD, Shahan KW (1980) The role of mist and chemical treatments in rooting loblolly and shortleaf pine cuttings. For Sci 26:651–655

Grönroos R, Arnold S von (1987) Initiation of roots on hypocotyl cuttings of *Pinus sylvestris* in vitro. Physiol Plant 69:227–236

Grönroos R, Arnold S von (1988) Initiation of roots on hypocotyl cuttings of *Pinus sylvestris*, with emphasis on direct rooting, root elongation, and auxin uptake. Can J For Res 18:1457–1462

Hackett WP (1985) Donor plant maturation and adventitious root formation. In: Davis TD, Haissig BE, Sankhla N (eds) Adventitious root formation in cuttings. Dioscorides, Portland, Oregon

Haeman JC, Owens JN (1972). Callus formation and root initiation in stem cuttings of Douglas-fir. Can J For Res 2:121–134

Haissig BE (1974) Origins of adventitious roots. N Z J For Sci 4:299–310

Hare RC (1974) Chemical and environmental treatments promoting rooting of pine cuttings. Can J For Res 4:101–106

Jewell FF (1958) Softening slash pine tissue for serial sectioning. Stain Technol 33:191–192

Libby WJ, Brown AJ, Fielding JM (1972) Effects of hedging radiata pine on production, rooting and early growth of cuttings. N Z J For Sci 15:281–297

Lovell PH, White J (1986) Anatomical changes during adventitious root formation. In: Jackson MB (ed) New root formation in plants and cuttings. Martinus Nijhoff, Dordrecht, pp 111–140

McAlpine RG, Jackson LWR (1959) Effects of age on rooting of loblolly pine air layers. J For 57:565–566

Reines M, McAlpine RG (1959) The morphology of normal, callused and dwarf shoots of slash pine. Bot Gaz 120:118–124

Russell JH, Grossnickle SC (1989) Rooting of yellow cypress cuttings, part 1: influence of donor plant maturation. FRDA Research Memo No. 083

Smith DR, Thorpe TA (1975) Root initiation in cuttings of *Pinus radiata* L.: developmental sequence. J Exp Bot 26:184–192

Stickney RH (1987) Anatomy of adventitious root formation in hypocotyl and stem cuttings of *Pinus taeda*. M. S. Thesis, North Carolina State University, Raleigh

Weir RG, Goldfarb B (1993) Loblolly and slash pine rooted cutting research at N. C. State University. Proceedings of the 22nd Southern Forest Tree Improvement Conference, June 14–17, Atlanta, Georgia