part three
Vegetative Propagation

The chapters in Part III deal with vegetative procedures to propagate clones. The importance of clones as a category of cultivar cannot be overestimated. Essentially, all fruit and nut tree cultivars, as well as many rootstocks, are clones. Many major floriculture crops (e.g., roses, chrysanthemum, and carnation) are clones. Some important vegetable crops are clones, including potatoes and sweet potato. Some major plantation crops (i.e., sugar cane, banana, and pineapple) are also clones. Although forest species have traditionally been produced as seedlings, bamboo, poplar, and willow have been propagated vegetatively for centuries. Methods of vegetative propagation discussed include cutting, grafting, budding, layering, separation, and division.

9

Principles of Propagation by Cuttings

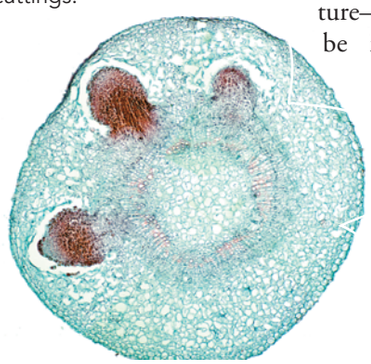
learning objectives

The first section of this chapter explores the biological approaches utilized to understand the regenerative process of adventitious root and bud (and shoot) formation. After reading the first section, you should be able to:

- Describe the observations made of adventitious root and bud (and shoot) formation.
- Explain how hormonal control affects root and bud (and shoot) formation.
- Explain the biochemical basis for adventitious root formation.
- Discuss the biotechnological advances in asexual propagation.

The second section of the chapter deals with the management and manipulation of adventitious root and bud (and shoot) formation. After reading the second section, you should be able to:

- Discuss the management of stock plants to maximize cutting propagation.
- Describe the factors involved in the treatment of cuttings.
- List the environmental conditions necessary in the manipulation of cuttings.



INTRODUCTION

The main focus of this chapter is on **adventitious root formation,** since it is the primary regenerative process required in most cutting propagation. **Adventitious bud and shoot development,** events important in the regeneration of leaf and root cuttings, are also discussed. **Adventitious organs** include new roots and buds that are formed from cells and tissue of previously developed shoots and roots.

Cutting propagation is the most important means for **clonal regeneration** of many horticultural crops: ornamentals, fruits, nuts, and vegetables. Adventitious root formation is a prerequisite to successful cutting propagation. In forestry, cutting propagation has been around for hundreds of years. Vegetative propagation of forest planting stock through adventitious rooting is one of the most exciting emerging technologies in forestry. Yet, many economically important woody plants have a low genetic and physiological capacity for adventitious root formation, which limits their commercial production. Furthermore, rooting and acclimatization of tissue-culture–produced plants will need to be improved if biotechnology (manipulating genes for new flower color, disease resistance, fruit yield, etc.) is to be incorporated into the propagation and production of genetically transformed woody plant species. Labor costs contribute more than

adventitious roots Roots that arise on aerial plant parts, underground stems and old root parts.

adventitious buds (and shoots) Arise from any plant part other than terminal, lateral, or latent buds on stems. Adventitious buds form irregularly on older portions of a plant and not at the stem tips or in the leaf axils. Unlike dormant buds, adventitious buds do not have a bud trace all the way to the pith. An adventitious bud is an embryonic shoot.

adventitious organs Organs that rise from the dedifferentiation of parenchyma cells; when they originate from callus (also composed of parenchyma cells) their **organogenesis** is termed **indirect.**

cutting propagation The clonal multiplication of plants with propagules of stems, leaves, or roots.

clonal regeneration or reproduction The asexual reproduction of genetically uniform copies **(clones)** of plants using propagules such as stem, leaf, and root cuttings.

50 percent of propagation costs, so there is considerable financial incentive to streamline propagation techniques and improve rooting success.

Commercial propagators have developed technologies that successfully manipulate environmental conditions to maximize rooting (i.e., *intermittent mist* and *fog systems,* temperature, and light manipulation). What has lagged behind is the knowledge of the biochemistry, genetic and molecular manipulation of rooting. While we know a lot about the biology and manipulation of cuttings, the fundamental events of what triggers adventitious root formation remain largely unknown. The new tools of biotechnology offer exciting opportunities to understand the molecular keys to rooting and to enable propagators to develop new cultivars that can be commercially rooted.

DESCRIPTIVE OBSERVATIONS OF ADVENTITIOUS ROOT AND BUD (AND SHOOT) FORMATION

Propagation by **stem** and **leaf-bud cuttings (single-eye cuttings)** requires only that a new adventitious root system be formed, because a potential shoot system (a bud) is already present. **Root cuttings** and **leaf cuttings** must initiate both a new shoot system—from an adventitious bud—as well as new adventitious roots.

The formation of adventitious roots and buds is dependent on plant cells to **dedifferentiate** and develop into either a root or shoot system. The process of **dedifferentiation** is the capability of previously developed, differentiated cells to initiate cell divisions and form a new meristematic growing point. Since this characteristic is more pronounced in some cells and plant parts than in others, the propagator must do some manipulation to provide the proper conditions for plant regeneration. A sound understanding of the underlying biology of regeneration is very helpful in this regard.

dedifferentiation
The early stage of adventitious root or bud formation when differentiated cells are triggered to form new meristematic regions.

Adventitious Root Formation

Adventitious roots form naturally on various plants. Corn, screwpine (*Pandanus utilis*), and other monocots develop "brace" roots, which arise from the intercalary regions at the base of internodes. Screwpine produces long, aerial, prop roots from their shoots that grow into the ground and support the tree (Fig. 9–1). Plants that are regenerated from rhizomes, bulbs, and other such structures also develop adventitious roots (see Chapter 15).

Figure 9–1
The ultimate in adventitious root production is shown on this screwpine (Pandanus utilis). Prop roots (arrow) arise from the shoots, grow into the soil, and support the tree.

Adventitious roots are of two types:

- **preformed roots** (Figs. 9–2 and 9–3)
- **wound-induced roots** (Figs. 9–3 and 9–4)

preformed root initials and primordia
Develop naturally on stems while they are still attached to the parent plant and roots may or may not emerge prior to severing the stem piece.

Figure 9–2
Preformed aerial roots at node of Ficus pumila.

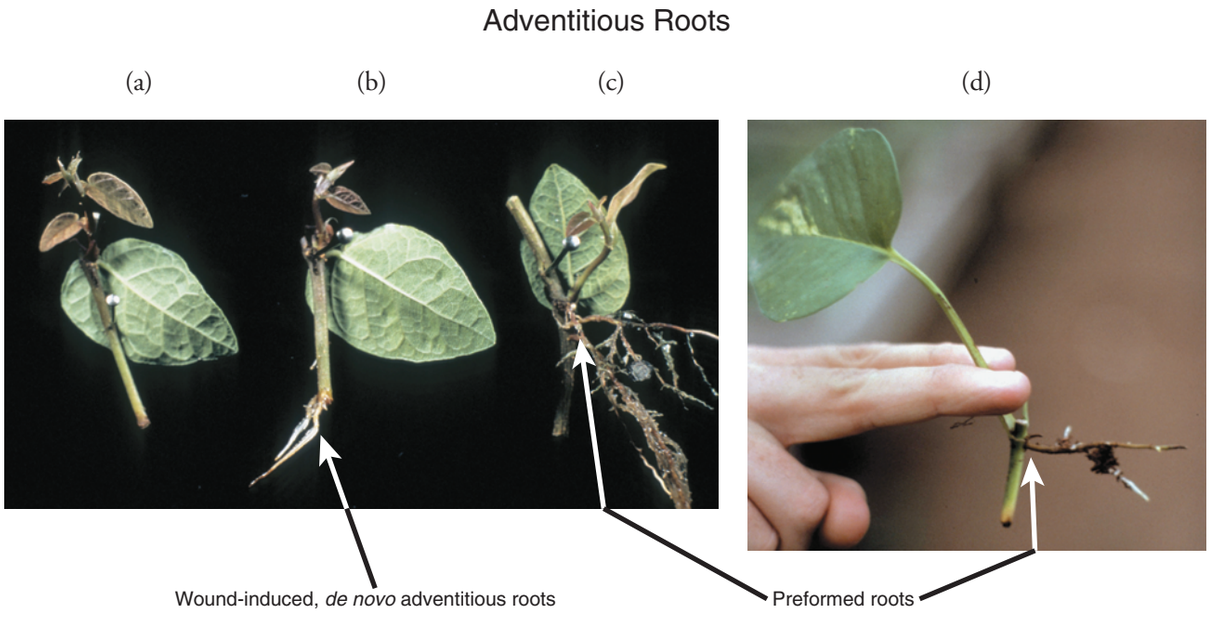


Figure 9–3
Leaf bud cuttings of Ficus pumila with (a) unrooted cutting, (b) wound induced, de novo and (c) preformed adventitious roots. (d) Philodendron cutting with preformed adventitious root from node.

Preformed or Latent Root Initials Preformed or latent root initials generally lie dormant until the stems are made into cuttings and placed under environmental conditions favorable for further development and emergence of the primordia as adventitious roots. In poplar (*Populus xrobusta*), root initials form in stems in midsummer and then emerge from cuttings made the following spring (257).

Figure 9–4
Emergence of adventitious roots in mung bean (Vigna) stem cuttings. Observe the tendency of the roots to form in longitudinal rows.

In some spices, primordia develop into aerial roots on the intact plant and become quite prominent (Figs. 9–1 and 9–2). Such preformed root initials occur in a number of easily rooted genera, such as willow (*Salix*), hydrangea (*Hydrangea*), poplar (*Populus*), coleus, jasmine (*Jasminum*), currant (*Ribes*), citron (*Citrus medica*), and others. The position of origin of these preformed root initials is similar to ***de novo* adventitious root formation** (Table 9–1) (185). In some of the clonal apple rootstocks and in old trees of some apple and quince cultivars, these preformed latent roots cause swellings, called **burr knots.** Species with preformed root initials generally root rapidly and easily, but cuttings of many species without such root initials root just as easily.

In willow, latent root primordia can remain dormant, embedded in the inner bark for years if the stems remain on the tree (2, 43). Their location can be observed by peeling off the bark and noting the protuberances on the wood, with

de novo adventitious roots Roots that are formed "anew" (from scratch) from stem or leaf cells that experience a stimulus, such as wounding, to dedifferentiate into roots.

burr knots Preformed roots that are not desirable and are selected against in modern apple rootstock breeding programs. Though rooting of cuttings is easier, clusters of burr knots can later girdle the stem.

Table 9–1
ORIGIN OF PREFORMED ROOT INITIALS (PRIMORDIA, BURR KNOTS, AND/OR ROOTGERMS) IN STEMS OF WOODY PLANTS

Origin	Genera
Rays	
Wide rays	*Populus*
Medullary rays, associated with buds	*Ribes*
Nodal and connected with wide radial bands of parenchyma	*Salix*
Internodal medullary rays	*Salix*
Medullary ray	*Citrus*
Phloem ray parenchyma	*Hydrangea*
Cambium	
Cambial ring in branch and leaf gap; 1 and 2° medullary rays	*Malus*
Cambial region of an abnormally broad ray	*Acer, Chamaecyparis, Fagus, Fraxinus, Juniperus, Populus, Salix, Taxus, Thuja, Ulmus*
Leaf and bud gaps	
Bud gap	*Cotoneaster*
Median and lateral leaf trace gaps at node	*Lonicera*
Parenchymatous cells in divided bud gap	*Cotoneaster*

Source: M. B. Jackson (154).

corresponding indentations on the inside of the bark that was removed.

Wound-Induced Roots On the other hand, wound-induced roots develop only after the cutting is made, in response to wounding in preparing the cutting. In effect, they are considered to be formed ***de novo*** (anew) (59, 154). Any time living cells at the cut surfaces are injured and exposed, a **response to wounding** begins (48).

Wounding Response. The subsequent wound response and root regeneration process includes three steps:

1. The outer injured cells die, a necrotic plate forms, the wound is sealed with a corky material (suberin), and the xylem may plug with gum. This plate protects the cut surfaces from desiccation and pathogens.
2. Living cells behind this plate begin to divide after a few days and a layer of **parenchyma cells** form *callus* which develops into a **wound periderm.**
3. Certain cells in the vicinity of the vascular cambium and phloem begin to divide and initiate *de novo* adventitious roots.

Parenchyma cells
The basic cells from which all other differentiated cells and tissues are derived, including adventitious organs.

Wound periderm
A mass of callus cells that forms a protective layer behind the wounded surface of a cutting.

Stages of De Novo Adventitious Root Formation. The developmental changes that occur in *de novo* adventitious root formation of wounded roots can generally be divided into four stages:

Stage I: Dedifferentiation of specific differentiated cells.

Stage II: Formation of root initials from certain cells near vascular bundles, or vascular tissue, which have become meristematic by dedifferentiation.

Stage III: Subsequent development of root initials into organized **root primordia.**

Stage IV: Growth and **emergence of the root primordia** outward through other stem tissue plus the formation of vascular (conducting) tissue between the root primordia and the vascular tissues of the cutting.

While most scientists divide the process of adventitious root formation into four stages, rooting of Monterrey pine hypcotyl cuttings are divided (*Pinus radiata*) into three stages: preinitiative, initiative, and postinitiative with continuous division of derivatives to form **meristemoids** (255, 256).

meristemoid A cell or group of cells constituting an active locus of meristematic activity in a tissue composed of somewhat older, differentiated cells; they can develop into root primordia or adventitious buds.

Table 9–2
TIME OF ADVENTITIOUS ROOT FORMATION IN JUVENILE AND MATURE LEAF-BUD CUTTINGS OF *FICUS PUMILA* TREATED WITH IBA

	Juvenile	Mature
Anticlinal cell divisions of ray parenchyma	Day 4	Day 6
Primordia	Day 6	Day 10
First rooting[a]	Day 7	Day 20
Maximum rooting[b]	Day 14	Day 28

[a]Based on 25 percent or more cuttings with roots protruding from stem.
[b]Based on 100 percent rooting and maximum root number.

Source: Davies et al. (59).

Time to Form Adventitious Roots The time for root initials to develop after cuttings are placed in the propagating bed varies widely. In one study (260), they were first observed microscopically after 3 days in chrysanthemum, 5 days in carnation (*Dianthus caryophyllus*), and 7 days in rose (*Rosa*). Visible roots emerged from the cuttings after 10 days for the chrysanthemum, but 3 weeks were required for the carnation and rose.

Phloem ray parenchyma cells in juvenile (easy-to-root) cuttings of creeping fig (*Ficus pumila*) undergo early anticlinal cell division and root primordia formation more quickly than mature (difficult-to-root) plants under optimal auxin treatments (Table 9–2). Once primordia are formed, there is a comparable time period (7 to 8 days) between root primordia elongation (emergence) and maximum rooting in both the easy-to-root and difficult-to-root plants (59). This delay was also reported with *Agathis australis*, where primordia formation was variable in cuttings from different-aged stock plants—but once root primordia formed, root emergence consistently occurred within a three-to-four-week period (185, 294, 295).

The Anatomical Origin of Wound-Induced Adventitious Roots The precise location inside the stem where adventitious roots originate has intrigued plant anatomists for centuries. Probably the first study of this phenomenon was made in 1758 by a French dendrologist, Duhamel du Monceau (72). A great many subsequent studies have covered a wide range of plant species (10, 185).

Adventitious roots usually originate on **herbaceous plants** just outside and between the vascular bundles (224), but the tissues involved at the site of origin can vary widely depending upon plant species and propagation technique (1). In tomato, pumpkin, and mung bean (22), adventitious roots arise in the phloem parenchyma; in *Crassula* they arise in the epidermis, while in coleus they originate from the pericycle (42). Root initials in carnation cuttings arise in a layer of parenchymatous cells inside a fiber sheath; the developing root tips, upon reaching this band of impenetrable fiber cells, do not push through it but turn downward, emerging from the base of the cutting (260).

Adventitious roots in stem cuttings of **woody perennial plants** usually originate from living parenchyma cells, in the young, secondary phloem (Figs. 9–6 and 9–7, page 286), but sometimes in vascular rays, cambium, phloem, callus, or lenticels (Table 9–3, page 286) (101, 126, 185).

Generally, the origin and development of *de novo* adventitious roots takes place next to and just outside the central core of vascular tissue. Many easy-to-root woody plant species develop adventitious roots from phloem ray parenchyma cells. Figure 9–7, page 286, depicts the first **anticlinal division** of a phloem ray cell during dedifferentiation (Stage I). Further cell divisions occur and the meristematic area becomes more organized with the formation of a root initial (Stage II) (Fig. 9–8, page 287). Ultimately a fully developed root primordia forms in the phloem and cortex (Fig. 9–9, page 287). Upon emergence from the stem (Fig. 9–10, page 287), the adventitious roots have already developed a root cap as well as a complete vascular connection with the originating stem.

anticlinal division
Cell division that occurs when the cell wall plate is formed perpendicular to the circumference of the stem.

The Relationship of Stem Structure and Rooting Ability There have been attempts to correlate stem structure with the rooting ability of cuttings. A continuous **sclerenchyma**

sclerenchyma ring
Composed of sclereid cells that are highly lignified and used for structural support of the stem. In some rare occasions these cells may impede the rooting process.

BOX 9.1 **GETTING MORE IN DEPTH ON THE SUBJECT**

DEVELOPMENTAL PHASES IN ADVENTITIOUS ROOT AND SHOOT FORMATION

Figure 9–5 depicts the developmental phases in the organogenesis of adventitious root and shoot formation. Cells in potential sites must become competent to respond to chemical/metabolic signals that trigger induction, which enables subsequent dedifferentiation and adventitious organ development. See page 283 for a discussion of developmental stages of wound-induced roots, page 299 for biochemical and page 303 for molecular implications on cell competency to root.

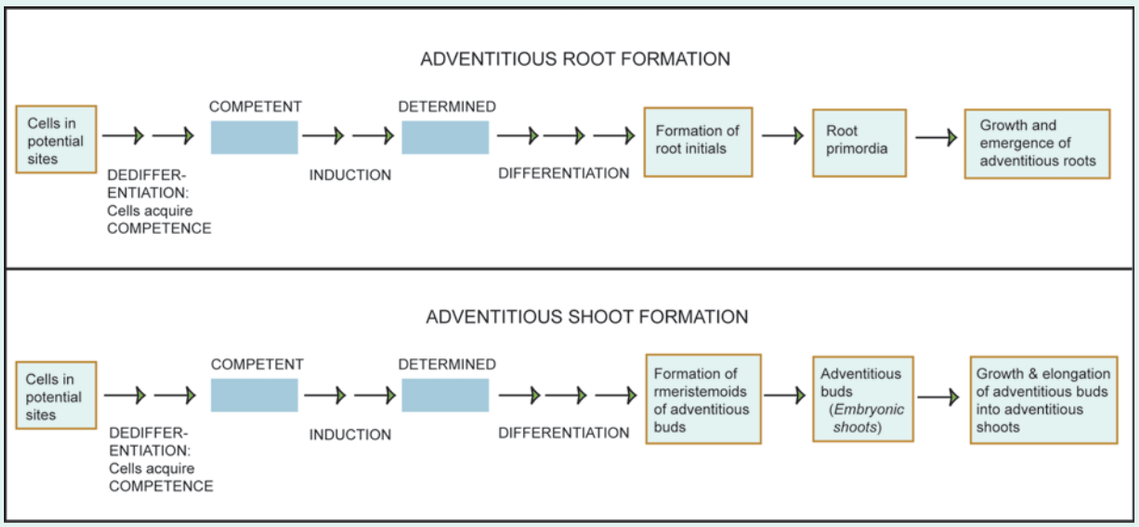


Figure 9–5
Developmental phases in the organogenesis of adventitious root and shoot formation. Modified from Christianson and Warnick (46); Davies et al. (57, 59).

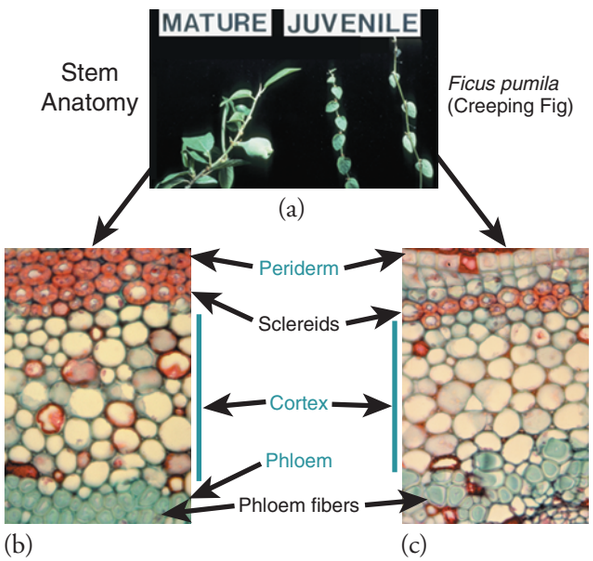


Figure 9–6
Shoot morphology (a) in juvenile and mature Ficus pumila. Cross section from (b) mature and (c) juvenile stems from the outside periderm to phloem fibers. Rarely are sclereids or phloem fibers a barrier that prevents adventitious rooting.

ring (Fig. 9–6) between the phloem and cortex, exterior to the point of origin of adventitious roots, occurs as the stem matures and gets older. Sclereids and fibers are impregnated with **lignin,** which provides structural support and mechanical barriers for pest resistance.

lignin An abundant plant polymer in cell walls that provides structural support and mechanical barriers for pest resistance.

Sclereids occur in difficult-to-root species such as olive stem cuttings, mature English ivy (*Hedera helix*) (102), and creeping fig (*Ficus pumila*) (59), while easy-to-root types are characterized by discontinuity or fewer cell layers of this sclerenchyma ring (Fig. 9–6) (15).

Easily rooted carnation cultivars have a band of sclerenchyma present in the stems, yet the developing root primordia emerge from the cuttings by growing downward and out through the base (260). In other plants, in which an impenetrable ring of sclerenchyma could block root emergence, this same rooting pattern can occur. Rooting is related to the genetic potential

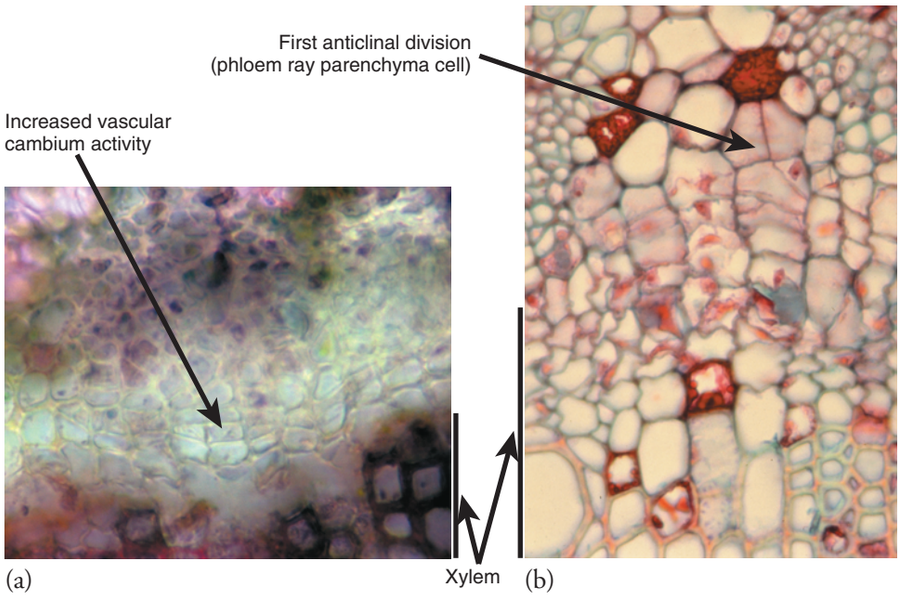


Figure 9–7
Early events of rooting with (a) increased vascular cambium activity and (b) first anticlinal division of phloem ray parenchyma cell during stage I—dedifferentiation in Ficus pumila (59).

Table 9–3
ORIGIN OF WOUND-INDUCED *DE NOVO* ADVENTITIOUS ROOTS IN STEMS OF WOODY PLANTS

Origin	Genera
Cambial and ray	
Cambial and phloem portions of ray tissues	*Acanthopanax, Chamaecyparis, Cryptomeria, Cunninghamia, Cupressus, Metasequoia*
Medullary rays	*Vitis*
Cambium	*Acanthus, Lonicera*
Fascicular cambium	*Clematis*
Phloem ray parenchyma	*Ficus, Hedera*
Secondary phloem in association with a ray	*Malus* (Malling stocks), *Camellia*, 'Brompton' plum
Phloem area close to the cambium	*Pistacia*
Cambium and inner phloem ray also in leaf gap	*Griselinia*
Bud and leaf gaps	
Outside the cambium in small groups	*Rosa, Cotoneaster, Pinus, Cephalotaxus, Larix, Sciadopitys, Malus, Acanthus*
Pericycle Callus, internal	
Irregularly arranged parenchymatous tissues	*Abies, Juniperus, Picea, Sequoia*
Callus, external	
Callus tissues (external)	*Abies, Cedrus, Cryptomeria, Ginkgo, Larix, Pinus, Podocarpus, Sequoia, Sciadopitys, Taxodium, Pinus*
Bark and basal callus	*Citrus*
Within callus at base of cutting	*Pseudotsuga*
Other	
Hyperhydric outgrowth of the lenticels	*Tamarix*
Margin of differentiating resin duct or parenchyma within the inner cortex	*Pinus*

Source: M. B. Jackson (154).

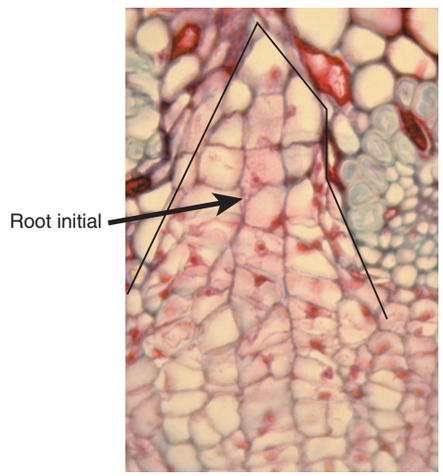


Figure 9–8
Root initial development in Ficus pumila with the meristematic zone in the phloem ray becoming more organized during stage II of adventitious root formation—root initial formation.

and physiological conditions for root initials to form, rather than to the mechanical restriction of a sclerenchyma ring barring root emergence (59, 245, 293).

Thus, two patterns of adventitious root formation emerge: **direct root formation** of cells in close proximity to the vascular system (i.e., generally more easy-to-root species); and **indirect root formation,** where nondirected cell divisions, including callus formation, occur for an interim period before cells divide in an organized pattern to initiate adventitious root primordia (i.e., generally more difficult-to-root species). See the flow diagram of adventitious root formation (Fig. 9–11, page 288) (98, 185).

Callus Formation: Rooting and Bud (and Shoot) Organogenesis

Root Organogenesis
Callus is an irregular mass of parenchyma cells in various stages of

callus An irregular mass of parenchyma cells in various stages of lignification.

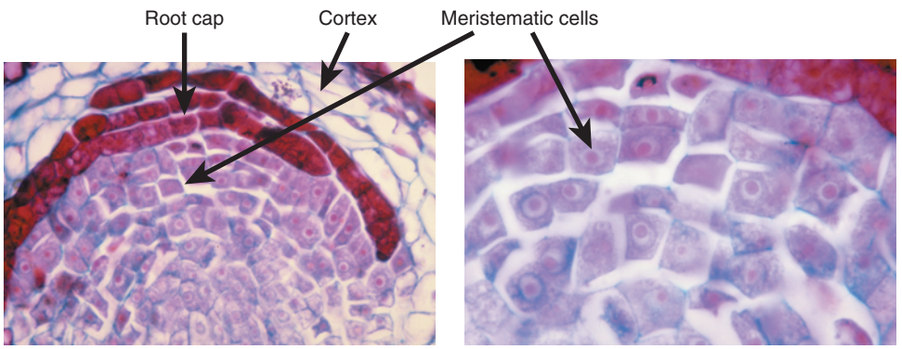


Figure 9–9
Development of a fully organized meristem during stage III of adventitious root formation—root primordia formation. (a) The root cap of the adventitious root has become organized, and (b) meristematic cells are characterized with isodymetric cell walls, deeply staining cytoplasm, and large nuclei in a Ficus pumila cutting.

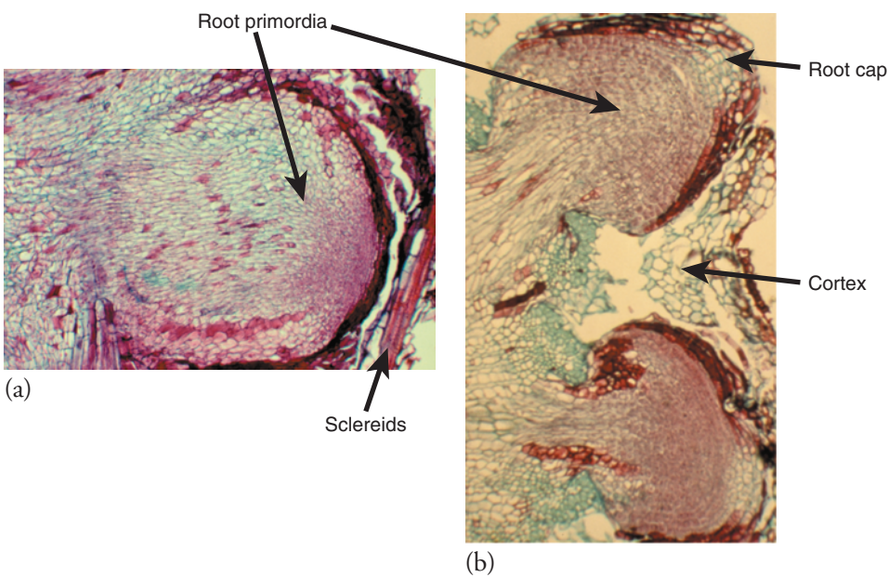


Figure 9–10
Elongation of root primordia during stage IV of adventitious root formation—root elongation. (a) Longitudinal section with root primordia elongating through the cortex, pushing out sclereids in the exterior of the cortex. (b) Cross-section of two adventitious primordia elongating through the cortex and periderm in a Ficus pumila cutting.

BOX 9.2 **GETTING MORE IN DEPTH ON THE SUBJECT**

STEM STRUCTURE AND ROOTING

With most difficult-to-root species, stem structure does not influence rooting potential. While a sheath of lignified tissue in stems may in some cases act as a *mechanical barrier* to root emergence, there are so many exceptions that this **is not** the primary cause of rooting difficulty (Fig. 9–10). Moreover, auxin treatments and rooting under mist (15, 59) cause considerable cell expansion and proliferation in the cortex, phloem, and cambium, resulting in breaks in continuous sclerenchyma rings—yet in some difficult-to-root cultivars, even with wounding, there is still no formation of root initials.

lignification that commonly develops at the basal end of a cutting placed under environmental conditions favorable for rooting. Callus growth proliferates from cells at the base of the cutting, primarily from the vascular cambium, although cells of the cortex and pith may also contribute to its formation (Table 9–3).

Roots frequently emerge through the callus, leading to the belief that callus formation is essential

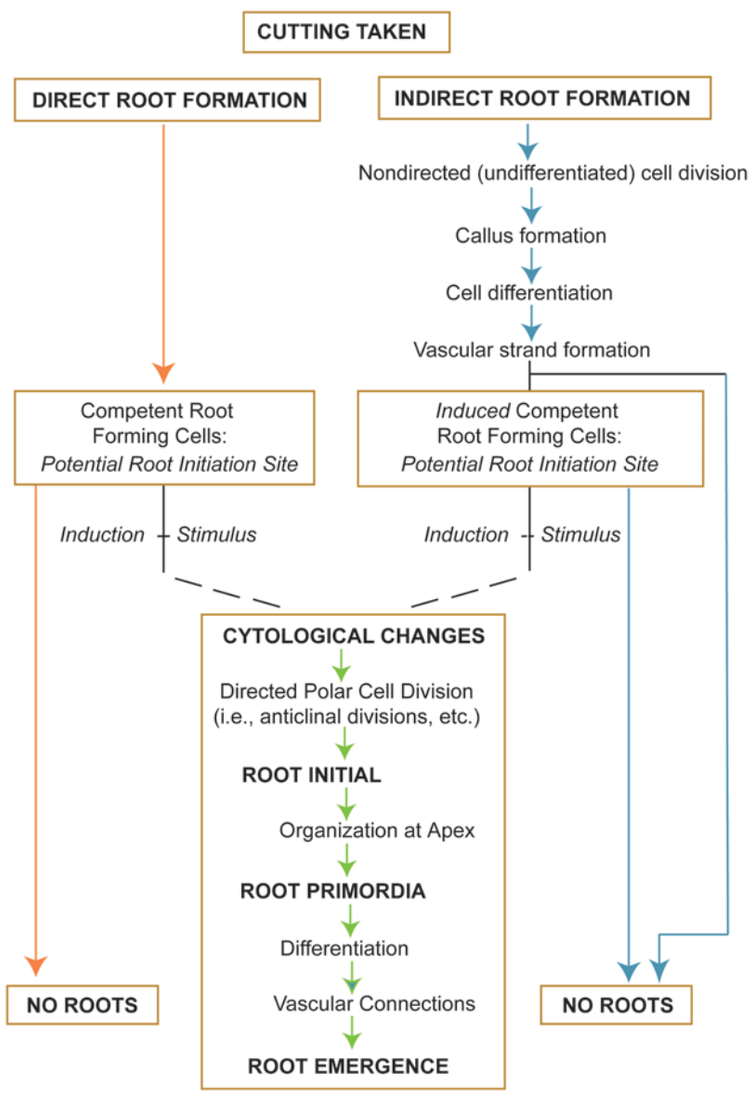


Figure 9–11
Flow diagram of adventitious root formation through direct (cells in close proximity to vascular system—i.e., generally more easy-to-root species) and indirect model (interim period of undifferentiated cell division—i.e., generally more difficult-to-root species). When a potential root initiation site is already present the initial cell divisions lead to root production in situ. When a site is not present, alternative routes leading to the creation of a site are shown. Rooting does not always occur.
Modified from Lovell and White (185) and Geneve (98).

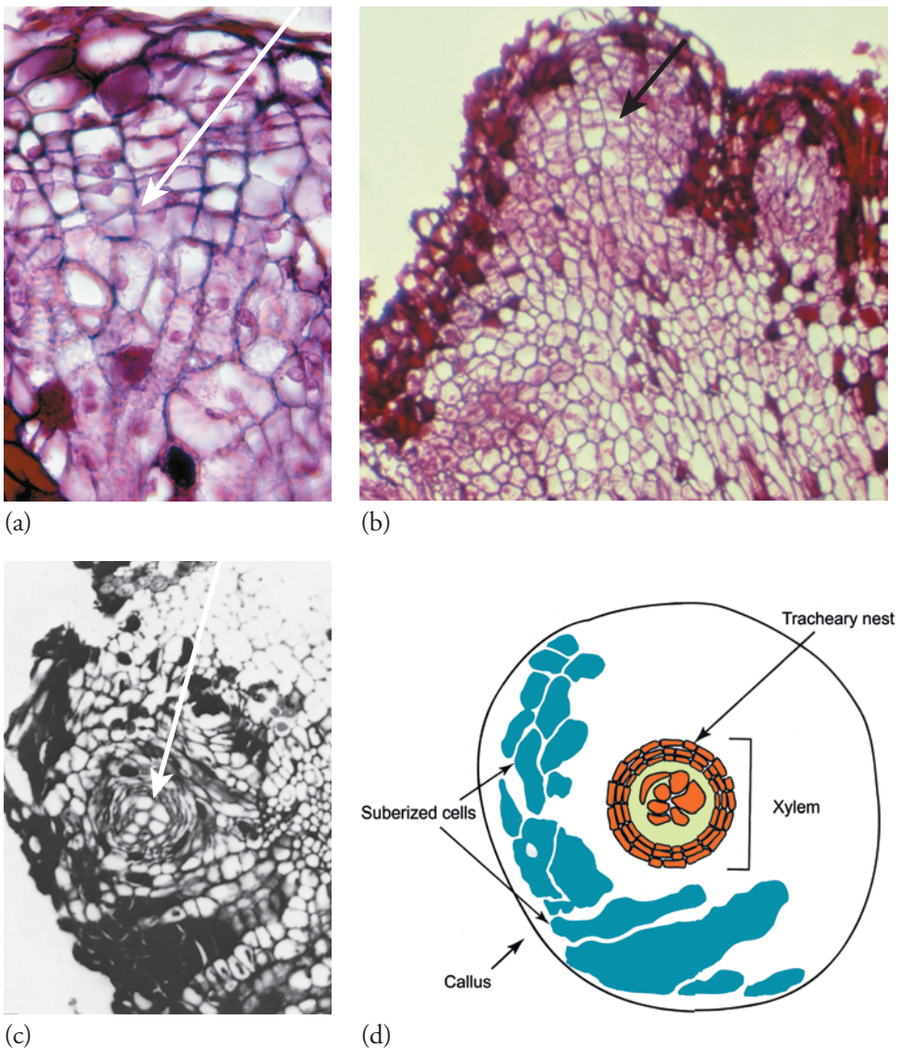


Figure 9–12
(a and b) Cell divisions in early de novo root primordia initiation from callus formation at base of mature, difficult-to-root Ficus pumila cutting. (c and d) Primordia originating in the vicinity of differentiating tracheary elements that have been described as "callus xylem" or "tracheary nests" (59).

for rooting. In easy-to-root species, the formation of callus and the formation of roots are independent of each other, even though both involve cell division. Their simultaneous occurrence is due to their dependence upon similar internal and environmental conditions.

In some species, callus formation is a precursor of adventitious root formation, while in other species excess callusing may hinder rooting. Origin of adventitious roots from callus tissue has been associated with difficult-to-root species (Table 9–3) (59, 142), such as pine (*Pinus radiata*) (41), *Sedum* (310), and the mature phase of English ivy (*Hedera helix*) (98). Adventitious roots originate in the callus tissue formed at the base of the cutting and from "tracheary nests," such as in callus of creeping fig (*Ficus pumila*) (Fig. 9–12). It is possible to have adventitious roots originating from different tissues on the same cutting—epicotyl stem cuttings of pine (*Pinus sylvestris*) can form roots from resin duct wound (callus) tissue, central and basal wound (callus) tissue, and vascular tissue (Fig. 9–13, page 290) (93).

Shoot Organogenesis Adventitious bud differentiation and subsequent adventitious shoot formation may also be obtained by direct organogenesis or via secondary organogenesis from disorganized calli (95). Shoot formation occurs by direct morphogenesis when the apical ends of epicotyl microcuttings of Troyer citrange are inserted vertically in a solid medium (204); conversely at the basal end, shoot formation occurs by indirect organogenesis through callus formation. When epicotyl explants are placed horizontally on the medium, shoot regeneration at both ends occurs by indirect organogenesis through callus formation.

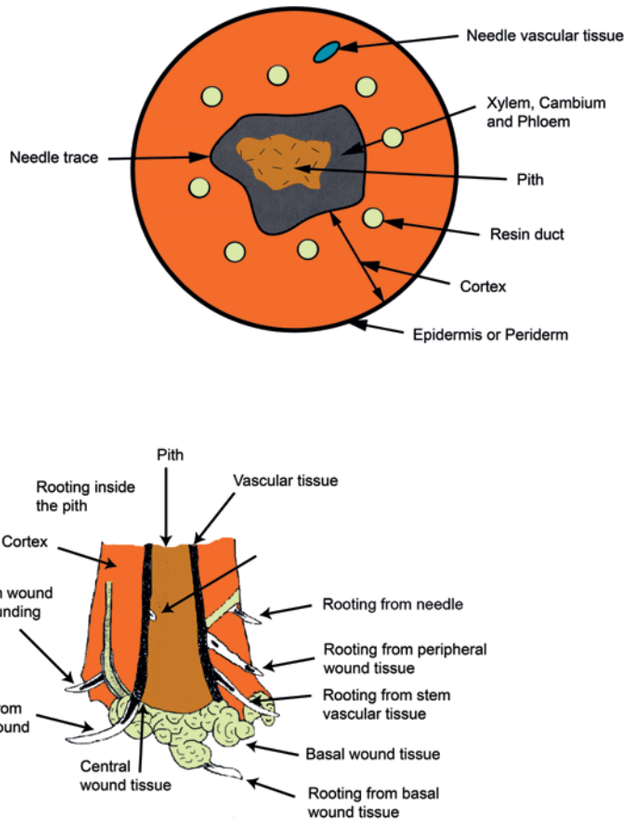


Figure 9–13
It is possible to have adventitious rooting originating from different tissues on the same cutting. Top: Tissue map of transverse section of epicotyl stem of one-year-old Pinus sylvestris. Bottom: Schematic longitudinal section showing examples of rooting occurring from resin duct wound (callus) tissue. No single cutting developed roots from all potential tissues.
Redrawn from Flygh et al. (93).

Leaf Cuttings—Adventitious Bud (and Shoot) and Root Formation

Many plant species, including both monocots and dicots, can be propagated by leaf cuttings (113). The origin of new shoots and new roots in leaf cuttings is quite varied and develops from primary or secondary meristems:

- **Preformed, primary meristems** are groups of cells directly descended from embryonic cells that have never ceased to be involved in meristematic activity.
- **Wound-induced, secondary meristems** are groups of cells that have differentiated and functioned in some previously differentiated tissue system and then dedifferentiate into new meristematic zones (*de novo*), resulting in the regeneration of new plant organs. This is the most common type of **meristem** in leaf cuttings.

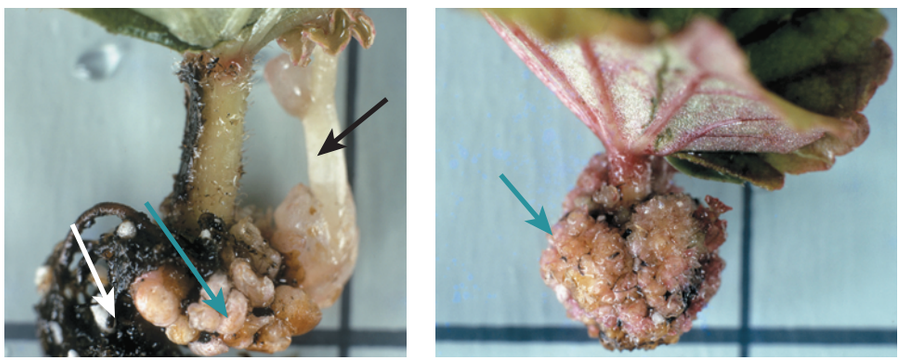
meristem tissue
Tissue composed of undifferentiated cells that can continue to synthesize protoplasm and produce new cells by division.

Leaf Cuttings with Preformed, Primary Meristems Detached leaves of *Bryophyllum* produce small plantlets from notches around the leaf margin (see Fig. 10–19, page 301). These small plants originate from so-called ***foliar "embryos,"*** formed in the early stages of leaf development from small groups of vegetative cells at the edges of the leaf. As the leaf expands, a foliar embryo develops until it consists of two rudimentary leaves with a stem tip between them, two root primordia, and a "foot" that extends toward a vein (134, 309). As the leaf matures, cell division in the foliar embryo ceases, and it remains dormant. If the leaf is detached and placed in close contact with a moist rooting medium, the young plants rapidly break through the leaf epidermis and become visible in a few days. Roots extend downward, and after several weeks many new independent plants form while the original leaf dies. Thus the new plants develop from ***latent primary meristems***—from cells that have not fully differentiated. Production of new plants from leaf cuttings by the renewed activity of primary meristems is found in species such as the piggyback plant (*Tolmiea*) (see Fig. 10–19) and walking fern (*Camptosorus*).

Leaf Cuttings with Wound-Induced, Secondary Meristems In leaf cuttings of *Begonia rex, Sedum,* African violet (*Saintpaulia*), snake plant (*Sansevieria*) (see Fig. 10–18, page 297), *Crassula,* and lily, new plants may develop from secondary meristems arising from differentiated cells at the base of the leaf blade or petiole as a result of wounding.

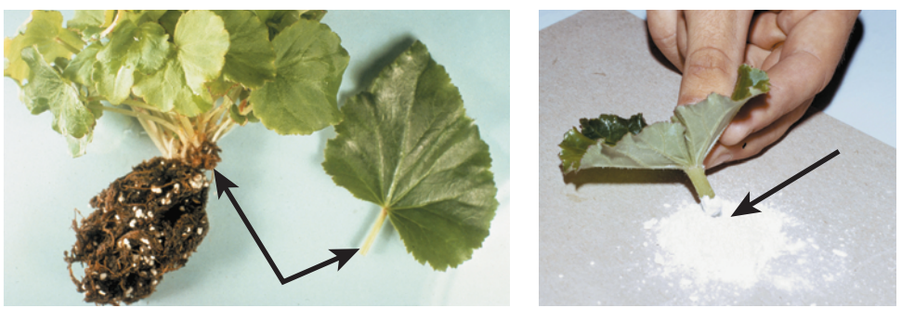
meristematic cells Cells that synthesize protoplasm and produce new cells by division. They vary in form, size, wall thickness, and degree of vacuolation, but have only a primary cell wall.

In African violet, new roots and shoots arise *de novo* by the formation of **meristematic cells** from previously differentiated cells in the leaves. The roots are produced from thin-walled cells lying between the vascular bundles. The new shoots arise from cells of the subepidermis and the cortex immediately below the epidermis. Adventitious roots first emerge, form branch roots, and continue to grow for several weeks before adventitious buds and their subsequent development into adventitious shoots occurs. Root initiation and development are independent of adventitious bud and shoot formation (284). The same process occurs with many begonia species (Figs. 9–14 and 9–15). Although the original leaf supplies metabolites to the young plant, it does not become a part of the new plant.

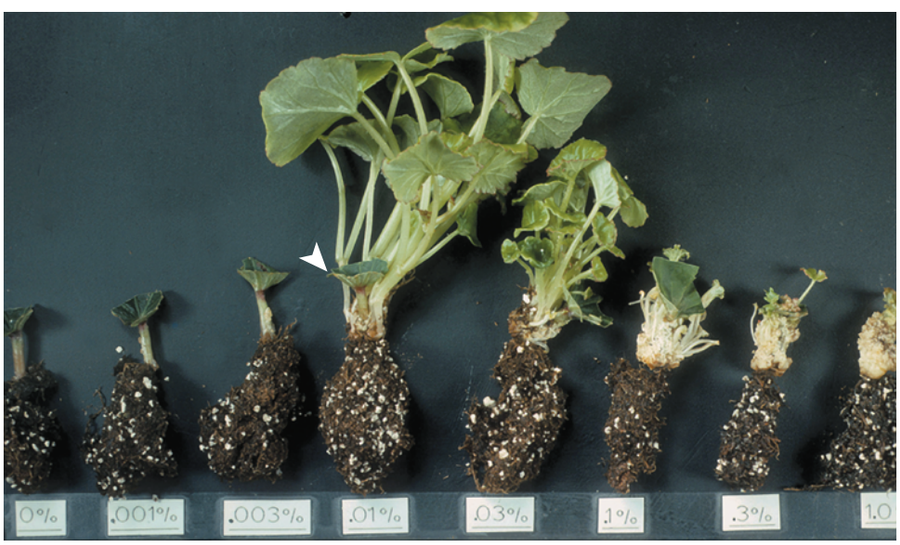


(a) (b)

Figure 9–14
(a) Adventitious shoot (upper black arrow), adventitious buds (blue arrow) and roots (white arrow) from a leaf cutting of Rieger begonia. An adventitious bud is an embryonic shoot. (b) At high cytokinin concentration, only buds and budlike tissue are visible (arrow) with poor shoot development; roots formed but were removed before the photograph was taken (57).



(a) (b)



(c)

Figure 9–15
(a) Adventitious shoots and adventitious roots arise at the base of the petiole (arrow) of a leaf cutting of Rieger begonia. (b) Application of a cytokinin mixed with talc to leaf cutting petiole base. (c) For sufficient, normal-appearing adventitious shoot production from a leaf cutting, without excessive adventitious bud formation, the 0.01 percent (100 ppm) treatment was optimal (arrow) (57). The original leaf blade was removed prior to taking the photo.

In lily (*Lilium longiflorum*) and *L. candidum,* the bud primordium originates in parenchyma cells in the upper side of the bulb scale (see Figs. 15–3 and 15–15), whereas the root primordium arises from parenchyma cells just below the bud primordium. Although the original scale serves as a source of food for the developing plant, the vascular system of the young bulblet is independent of that of the parent scale, which eventually shrivels and disappears (287).

In several species (e.g., sweet potato, *Peperomia,* and *Sedum),* new roots and new shoots on leaf cuttings arise in callus tissue that develops over the cut surface through the activity of secondary meristems. The petiole of *Sedum* leaf cuttings forms a considerable pad of callus within a few days after the cuttings are made. Root primordia are organized within the callus tissue, and shortly thereafter four or five roots develop from the parent leaf. Following this, bud primordia arise on a lateral surface of the callus pad and develop into new shoots (310).

Root Cuttings—Adventitious Bud (and Shoot) and Root Formation

Development of adventitious shoots, and in many cases adventitious roots, must take place if new plants are to be regenerated from root pieces (root cuttings) (251). Regeneration of new plants from root cuttings takes place in different ways, depending upon the species. Commonly, the root cutting first produces an adventitious shoot, and later produces roots, often from the base of the new shoot rather than from the original root piece itself. With root cutting propagation of apples, and the storage roots of sweet potato, these adventitious shoots can be removed and rooted as stem cuttings when treated with auxin (239). In other plants, a well-developed root system has formed by the time the first shoots appear.

In some species, adventitious buds form readily on roots of intact plants, producing **suckers.** When roots of such species are dug, removed, and cut into pieces, buds are even more likely to form. In young roots, such buds may arise in the pericycle near the vascular cambium (248). The developing buds first appear as groups of thin-walled cells having a prominent nucleus and a dense cytoplasm (80). In old roots, buds may arise in a callus-like growth from the phellogen; or they may appear in a callus-like proliferation from vascular ray tissue. Bud primordia may also develop from wound callus tissue that proliferates from the cut ends of injured surfaces of the roots (224), or they may arise at random from cortex parenchyma (239).

Sometimes regeneration of new root meristems on root cuttings is more difficult than the production of adventitious buds (2, 33). New roots may not always be adventitious and can develop from latent lateral root initials contained in the root piece or attached lateral roots.

BOX 9.3 **GETTING MORE IN DEPTH ON THE SUBJECT**

IMPORTANCE OF ADVENTITIOUS BUD FORMATION IN LEAF CUTTINGS

The limiting factor in leaf cutting propagation is generally the formation of adventitious buds, ***not*** *adventitious roots.* Adventitious roots form on leaves much more readily than do adventitious buds. In some plants, such as the India rubber fig (*Ficus elastica*), the cutting must include a portion of the old stem containing an axillary bud (a leaf-bud cutting) because although adventitious roots may develop at the base of the leaf, an adventitious shoot is not likely to form. In fact, rooted leaf cuttings of some species will survive for years without producing an adventitious shoot.

BOX 9.4 **GETTING MORE IN DEPTH ON THE SUBJECT**

PROPAGATION OF CHIMERAL PLANTS FROM LEAF AND ROOT CUTTINGS

One of the chief advantages claimed for asexual propagation is the exact reproduction of all characteristics of the parent plant. With root and leaf cuttings, however, this generalization does not always hold true. In periclinal chimeras, in which the cells of the outer layer are of a different genetic makeup from those of the inner tissues, the production of a new plant by root cuttings (derived from nonmutated, "wild type" inner tissues) results in a plant that is different in appearance from the parent. This is well illustrated in the thornless boysenberry and the 'Thornless Evergreen' trailing blackberry, in which stem or leaf-bud cuttings produce plants that retain the (mutated) thornless condition, but root cuttings develop into (normal, nonmutated) thorny plants. This is because the tissues forming the root cutting originate from normal, nonmutated cells. Likewise, with leaf cuttings, adventitious buds would have to originate from both mutated and normal cells for the chimera to be expressed. See Chapter 16 for more information on chimeras.

Generally, such branch roots arise from differentiated cells of the pericycle adjacent to the central vascular cylinder (21). Adventitious root initials have been observed to arise in the region of the vascular cambium in roots.

A list of plants commonly propagated by root cuttings can be found in Chapter 10, Table 10–2.

Polarity and Organ Formation in Cuttings

The polarity inherent in shoots and roots is shown dramatically in the rooting of cuttings (Fig. 9–16). Polarity is the quality or condition inherent in a cutting that exhibits different properties in opposite parts; that is, stem cuttings form shoots at the distal end (nearest to the shoot tip), and roots form at the proximal end (nearest to the crown, which is the junction of the shoot and root system). Root cuttings of many species form roots at the **distal** end and shoots at the **proximal** end. Changing the position of a stem cutting with respect to gravity does not alter this tendency (Fig. 9–16) (28) . Polarity is also observed in leaf cuttings even though roots and shoots arise at the same position, usually the base of the cutting (see Fig. 9–14).

In 1878, Vöchting (286) advanced the theory that polarity could be attributed to individual cellular components, since no matter how small the piece, regeneration was consistently polar. A general explanation of polarity is that when tissue segments are cut, the physiological unity is disturbed. This must cause a redistribution of some substance, probably auxin, thus accounting for the different growth responses. The correlation of polarity of root initiation with auxin movement has been noted in several instances (115, 188, 240, 251, 289). It is also known that the polarity in auxin transport varies in intensity among different tissues. The polar movement of auxins is an active transport process, mediated by a membrane transport carrier, which occurs in phloem parenchyma cells (154, 176, 307). See page 39 in Chapter 2 for discussion on auxin transport.

Figure 9–16
Polarity of root regeneration in grape hardwood cuttings. Cuttings at left were placed for rooting in an inverted position, but roots still developed from the morphologically basal (proximal) end. Cuttings at right were placed for rooting in the normal, upright orientation with roots forming at the basal end.

CORRELATIVE EFFECTS: HOW HORMONAL CONTROL AFFECTS ADVENTITIOUS ROOT AND BUD (AND SHOOT) FORMATION

The Effects of Buds and Leaves

correlative effect The control of one organ over the development of another, which is mediated by phytohormones. Auxin produced from axillary buds is transported basipetally down the shoot and is important in subsequent root formation at the base of a cutting.

In 1758, Duhamel du Monceau (72) explained the formation of adventitious roots in stems on the basis of the downward movement of sap. Sachs, the noted German plant physiologist (1882), postulated the existence of a specific root-forming substance manufactured in leaves, which moves downward to the stem base where it promotes adventitious root formation (244). It was shown by van der Lek (1925) that sprouting buds promoted root initiation just below the buds in cuttings of such plants as willow, poplar, currant, and grape (175). It was assumed that hormone-like substances formed in the developing buds and these were transported through the phloem to the cutting base where they stimulated root initiation.

The existence of a specific root-forming factor was first determined by Went in 1929 when he discovered that leaf extracts from chenille (*Acalypha*) plants applied back to chenille or papaya (*Carica*) tissue induce root formation (292). Bouillenne and Went found substances in cotyledons, leaves, and buds that stimulated the rooting of cuttings; they called this material **"rhizocaline"** (35, 292).

rhizocaline A hypothetical chemical complex, that was considered important in the biochemical events leading to root initiation.

Bud Effects on Rooting In Went's 1934 pea test for root-forming activity of various substances, it is significant that the presence of at least one bud on the pea cutting was essential for root production (292). After auxins were discovered, it was shown that a budless cutting would not form roots even when treated with an auxin-rich preparation. This finding indicated again that a factor other than auxin, presumably one produced by the bud, was needed for root formation. In 1938, Went postulated that specific factors other than auxin were manufactured in the leaves and were necessary for root formation. Thus, rhizocaline was more than just auxin. Later studies (83, 198) with pea cuttings confirmed this theory.

For root initiation, the presence of a metabolically active shoot tip (or a lateral bud) is necessary during the first three or four days after the cuttings are made (115). But after the fourth day the shoot terminal and axillary buds can be removed without interfering with subsequent root formation.

"rest period"
A physiological condition of the buds of many woody perennial species beginning shortly after the buds are formed. While in this condition, they will not expand into flowers or leafy shoots even under suitable growing conditions. After exposure to sufficient chilling hours (1 to 6°C (33 to 43°F), however, the "rest" influence is broken, and the buds will develop normally with the advent of favorable growing temperatures.

Bud removal from cuttings in certain species will stop root formation, especially in species without preformed root initials (175). In some plants, if the tissues exterior to the xylem are removed, just below a bud, root formation is reduced, indicating that some root-promoting compound(s) travels through the phloem from the bud to the base of the cutting. If hardwood, deciduous cuttings are taken in midwinter when the buds are in the **rest period,** they have either no effect or can inhibit rooting (88, 145). But if the cuttings are made in early fall or in the spring when the buds are active and not at rest, they show a strong root-promoting effect.

Conversely, with cuttings of apple and plum rootstocks, the capacity of shoots to regenerate roots increases during the winter, reaching a high point just before budbreak in the spring; this root regeneration is believed to be associated with a decreasing level of bud dormancy following winter chilling (144). Studies with Douglas-fir cuttings showed a pronounced relationship between bud activity and the rooting of cuttings—cuttings taken in early fall (September to October in the United States) root the poorest (238).

Leaf Effects on Rooting It has long been known that the presence of leaves on cuttings exerts a strong stimulating influence on rooting (Fig. 9–17). The stimulatory effect of leaves on rooting in stem cuttings is nicely shown by studies (234) with avocado. Cuttings of difficult-to-root cultivars under mist soon shed their leaves and die, whereas leaves on the cuttings of cultivars that have rooted are retained as long as nine months. While the presence of leaves can be important in rooting, leaf retention is more a consequence of rooting than a direct cause of rooting. After five weeks in the rooting bed, there was five times more starch in the base of the easy-to-root avocado cuttings than there was at the beginning of the tests. In hibiscus, rooting is also enhanced when leaves are retained on the cuttings (279).

Carbohydrates translocated from the leaves are important for root development. However, the strong root-promoting effects of leaves and buds are probably due to other, more direct factors (38). Leaves and buds produce auxin, and the effects of the polar apex-to-basal

Figure 9–17
Effect of leaves, buds, and applied auxin on adventitious root formation in leafy 'Old Home' pear cuttings. *Top:* Cuttings treated with auxin (indolebutyric acid at 4,000 ppm for five seconds). *Bottom:* Untreated cuttings. *Left to right:* with leaves; leaves removed; buds removed; one-fourth natural leaf area. Courtesy W. Chantarotwong.

(basipetal) transport of auxins enhances rooting at the base of the cutting.

Plant Growth Substances

All classes of growth regulators—auxins, cytokinins, gibberellins, ethylene, and abscisic acid, as well as ancillary compounds such as growth retardants/inhibitors, polyamines, and phenolics—influence root initiation either directly or indirectly (64). However, auxins have the greatest effect on root formation in stem cuttings, while cytokinins are used to stimulate adventitious bud formation in leaf cuttings. The other plant growth regulators and ancillary compounds can influence organogenesis, but not consistently enough to merit their commercial use in propagation. See Table 9–4 for a synopsis on plant growth regulator effects on adventitious bud and shoot formation. Chapter 2 has a more complete description of plant hormones, plant growth regulators, and how they function.

Auxins In the mid-1930s and later, studies of the physiology of auxin action showed that auxin was involved in such varied plant activities as stem growth, adventitious root formation (115, 275, 276, 292), lateral bud inhibition, abscission of leaves and fruits, and activation of cambial cells. Auxins can induce gene activity and are also signaling molecules in developmental events of adventitious root formation (39, 307).

Indole-3-acetic acid (IAA) was identified as a naturally occurring compound having considerable auxin activity (115). Indole-3-acetic acid (see Fig. 2–25) was subsequently tested for its activity in promoting roots on stem segments, and in 1935 investigators demonstrated the practical use of this material in stimulating root formation on cuttings (276). About the same time it was shown that two synthetic materials, **indole-3-butyric acid (IBA)** and a-**naphthalene acetic acid (NAA)** (see Fig. 2–25), were even more effective than the naturally occurring or synthetic IAA for rooting (29). Today, IBA and NAA are still the most widely used auxins for rooting stem cuttings and for rooting tissue-culture–produced microcuttings. It has been repeatedly confirmed that auxin is required for initiation of adventitious roots on stems, and indeed, it has been shown that divisions of the first root initial cells are dependent upon either applied or endogenous auxin (96, 116, 188, 266).

Indole-3-butyric acid, although less abundant than IAA, is also a naturally occurring substance in plants (11, 82, 186). In *Arabidopsis*, endogenously

Table 9–4
PLANT GROWTH REGULATOR EFFECTS ON ADVENTITIOUS ROOT AND BUD (AND SHOOT) FORMATION

Plant growth regulator	Adventitious root formation	Adventitious bud and shoot formation
Auxins	Promote	Inhibit; low auxin: high cytokinin ratio promote
Cytokinins	Inhibit; high auxin: low cytokinin ratio promote	Promote
Gibberellins	Inhibit	Inhibit; can enhance shoot elongation *after* organ formation
Ethylene	Can promote with auxin-induced rooting of some herbaceous plants; with woody plants generally not directly involved in rooting—but in small concentrations and for short durations may enhance competency to root (68)	Not promotive
ABA	Inhibit; however, used in combination with auxin can promote rooting in some species	Inhibits; however was reported to stimulate adventitious bud formation of a herbaceous species
Other potential hormones and ancillary compounds Retardants/inhibitors, polyamines, jasmonate, brassionsteroids, phenolics polyamines, salicylate, flavoinds, peroxidases	Used in combination with auxin can promote or inhibit rooting in some species	Not promotive; may depress shoot development

conjugation of plant hormones Plant hormones that are important in the regulation of physiologically active phytohormone levels, and are deactivated ("bound") hormones attached to other molecules via ester, glycoside, or amide bonds. The conjugated hormones may later be liberated via enzymatic hydrolysis and regain their activity, for example, IAA-aspartate is an auxin conjugate.

formed IAA is more readily transported than endogenously formed IBA (11). IAA also **conjugates** via amide bonds, while IBA conjugates from ester bonds.

In apple (*Malus*), when IBA is applied to stem cuttings or microcuttings to stimulate rooting, it is, in part, converted to IAA (282, 307). IBA may also enhance rooting via increased internal-free IBA or may synergistically modify the action of IAA or endogenous synthesis of IAA; IBA can enhance tissue sensitivity for IAA and increase rooting (282). In avocado microcuttings, IBA increased endogenous IAA and indole-3-acetyl-aspartic acid (IAA-asp) before root differentiation occurred, and as root formation proceeded (94). The same IBA response occurred in juvenile and mature phase microcuttings of chestnut (9); however, more endogenous IAA was detected in mature (recalcitrant) than juvenile (easy-to-root) tissue, indicating that endogenous IAA was not limiting rooting capacity.

In mung bean cuttings, IBA applied to the cutting base was transported to the upper part of the cuttings to a greater extent than IAA, and rapidly metabolized into IBA conjugates. These IBA conjugates were reported to be superior to free IBA in serving as an auxin source during later stages of rooting (297).

Cytokinins Cytokinins have the greatest effect on initiating buds and shoots from leaf cuttings and in tissue culture systems (31, 57, 79, 241, 281). Natural and synthetic cytokinins include **zeatin, zeatin riboside, kinetin, isopentenyladenine (2iP), thidiazuron (TDZ),** and **benzyladenine (BA** or **BAP)** (See Chapter 2). Generally, a high auxin/low cytokinin ratio favors adventitious root formation and a low auxin/high cytokinin ratio favors adventitious bud formation (36, 133) (Figs. 9–15 and 9–18). Cuttings of species with high natural cytokinin levels have been more difficult to root than those with low cytokinin levels (212). Applied synthetic cytokinins normally inhibit root initiation in stem cuttings (217). However, cytokinins at very low concentrations, when applied to decapitated pea cuttings at an early developmental stage (84), or to begonia leaf cuttings (133), promote root initiation, while higher concentrations inhibit root initiation. Application to pea cuttings at a later stage in root initiation does not show such inhibition; the influence of cytokinins in root initiation may thus depend on the particular stage of initiation and the concentration (32, 58, 256). To date, the quantitative determination of endogenous cytokinins at various stages of rooting has yet to be determined (281).

It has been suggested that the few cases of rooting success using exogenous applications indicate that cytokinins have an indirect rather than a direct role on rooting (281). Cytokinins may also be indirectly involved in rooting through effects on rejuvenation and

BOX 9.5 **GETTING MORE IN DEPTH ON THE SUBJECT**

CHANGES IN AUXIN REQUIREMENTS DURING ADVENTITIOUS ROOT FORMATION

With pea cuttings, the role of auxins in the intricate developmental processes of rooting occurred in two basic stages (83, 85, 197):

- A **root initiation stage** in which root meristems were formed (including dedifferentiation, root-initial, and root-primordia formation). This stage could be further divided into:
  a. An **auxin-active stage**, lasting about 4 days, during which auxin had to be supplied continuously for roots to form, coming either from terminal or lateral buds, or from applied auxin (if the cutting has been decapitated) (85, 197).
  b. An **auxin-inactive stage** occurred next. Withholding auxin during this stage (which lasts about 4 days) did not adversely affect root formation.
- **Elongation of root primordia stage,** during which the root tip grows outward through the cortex, finally emerging from the epidermis of the stem (see Fig. 9–10). A vascular system develops in the new root and becomes connected to adjacent vascular bundles of the stem. At this stage there was no further response to applied auxin.

BOX 9.6 **GETTING MORE IN DEPTH ON THE SUBJECT**

DIFFERENCES IN ROOTING RESPONSES OF IBA AND IAA

Variability in forming adventitious roots has been attributed to differences in auxin metabolism (27). However, the endogenous auxin concentration or type of auxin applied, (i.e., IBA compared to IAA), do not always explain rooting differences. Response to type of auxin is also species dependent (67, 225). While the more difficult-to-root *Grevillea* (Proteaceae) species had a reduced rooting response to IBA application when compared to the easy-to-root species, there were no differences in endogenous levels of IAA (170). Both IAA and IBA transport is mediated by different transport protein complexes (228). Difficult-to-root *Prunus avium* conjugated IBA more rapidly than the easy-to-root cultivar (82). Only free IBA was observed in the easy-to-root cultivar, suggesting that the difficult-to-root cultivar could not hydrolyze (de-conjugate) IBA during the appropriate developmental points of ARF. In young (easy-to-root) *Sequoia sempervirens* explant cuttings, higher levels of IAA were found after IBA treatment, whereas the mature (more difficult-to-root clone) had higher free IBA and conjugated IBA (27). Rooting was attributed to differences in auxin metabolism, and not to cell competency or sensitivity to form adventitious roots. In summary, the enhanced rooting of IBA compared to IAA has been attributed to differences in receptor binding, compartmentalization, greater stability and differences in tissue sensitivity between the two auxins (67, 82, 307).

BOX 9.7 **GETTING MORE IN DEPTH ON THE SUBJECT**

AUXIN: ADVENTITIOUS ROOTING AND MOLECULAR STUDIES

One explanation for auxin activity of IBA is that it is a "slow-release" form of IAA (82, 282). IBA may supply plants with a continuous IAA source when it is required for root initiation. Biochemical studies in numerous plants and genetic studies of *Arabidopsis* with IBA-responsive mutants indicate that IBA acts primarily via its conversion to IAA through peroxisomal fatty beta-oxidation (11). Mutants and genes of *Arabidopsis* involved in auxin biosynthesis, conjugation (inactivation of auxin), conjugate hydrolysis (activation of auxin), and degradation are being used to determine the complex mechanisms by which auxins are controlled (307). While we know the gross effects of auxin on rooting, we don't fully know the molecular basis, that is, the function of auxins as signaling molecules during root induction, initiation, and development (11, 39, 258). Molecular biology can help determine upstream and downstream regulators of IAA. Identifying genes involved in converting IBA to IAA is important to understanding auxin regulation and the contribution of IBA to active auxin pools (including *de novo* synthesis and conjugate hydrolysis of IAA.

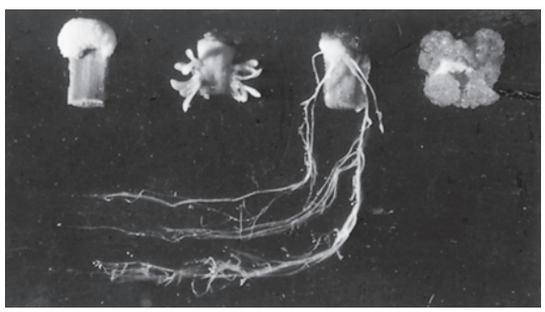


Figure 9–18
Effects of adenine sulfate (a cytokinin precursor) and indoleacetic acid (auxin) on growth and organ formation in tobacco stem segments. *Far left:* Control. *Central left:* Adenine sulfate, 40 mg per liter. Bud formation with decrease in root formation. *Central right:* Indoleacetic acid, 0.02 mg per liter. Root formation with prevention of bud formation. *Far right:* Adenine sulfate, 40 mg per liter plus indoleacetic acid, 0.02 mg per liter. Growth stimulation but without organ formation. Courtesy Folke Skoog.

accumulation of carbohydrates at the cutting base (i.e., carbohydrate loading) (281).

Leaf cuttings provide good test material for studying auxin-cytokinin relationships since such cuttings must initiate both roots and shoots. Cytokinin application at relatively high concentrations promoted bud formation and inhibited root formation of *Begonia* and *Bryophyllum* (134) leaf cuttings, while auxins, at high concentrations, stimulated roots and inhibited buds. Too high a cytokinin concentration applied to leaf cuttings maximizes adventitious bud formation but reduces the quality of new shoots (Figs. 9–14 and 9–15); from a horticultural standpoint, adventitious shoot quality, not just adventitious bud formation, is an important criterion in regenerating new plants from leaf cuttings (57). The considerable seasonal changes in the regenerative ability of *Begonia* leaf cuttings are due to a complex interaction of ***environmental cues***: temperature, photoperiod, and irradiance, which affect the

BOX 9.8 **GETTING MORE IN DEPTH ON THE SUBJECT**

CHANGES IN CYTOKININ REQUIREMENT DURING SHOOT ORGANOGENESIS

As with auxin and rooting, there are also changes in cytokinin requirement during shoot organogenesis (46). Three phases of shoot organogenesis can be distinguished: (a) formation of cell competence, (b) shoot induction, and (c) shoot development (Fig. 9–5). During induction, the leaf cutting or explant perceives exogenous cytokinin and auxin compounds and becomes committed to the development of shoots. With a highly shoot organogenic *Petunia hybrida* line, there was an 1.7-fold increase in endogenous cytokinins during shoot induction and 2.6-fold cytokinin increase during the shift from the induction to shoot development phase; conversely, isoprenoid cytokinins did not accumulate with mutant explants, incapable of shoot induction (6). Hence, the early stages of shoot development are influenced by cytokinin uptake and metabolism, which subsequently affects accumulation of isoprenoid cytokinins and the activity of cytokinin oxidase (6).

levels of endogenous cytokinins, auxins, and other growth regulators (137).

Gibberellins (GA) The gibberellins (see Fig. 2–28) are a group of closely related, naturally occurring compounds first isolated in Japan in 1939 and known principally for their effects in promoting stem elongation. At relatively high concentrations (i.e., 10^{-3} M), they have consistently inhibited adventitious root formation (250). This inhibition is a direct local effect that prevents the early cell divisions involved in transformation of differentiated stem tissues to a meristematic condition. Gibberellins have a function in regulating nucleic acid and protein synthesis and may suppress root initiation by interfering with these processes, particularly transcription (125). At lower concentrations (10^{-11} to 10^{-7} M), however, gibberellin has promoted root initiation in pea cuttings, especially when the stock plants were grown at low light levels (125).

In *Begonia* leaf cuttings, gibberellic acid (138) inhibited both adventitious bud and root formation, probably by blocking the organized cell divisions that initiate formation of bud and root primordia. Inhibition of root formation by gibberellin depends on the developmental stage of rooting. With herbaceous materials, inhibition is usually greatest when GA is applied 3 to 4 days after cutting excision (125). However, woody plant species such as willow (*Salix*) (116) and fig (*Ficus*) (59) were not adversely affected by GA during root initiation but were inhibited if GA was applied after root primordia were initiated. GA caused the reduction in cell numbers in older established primordia, which was deleterious to root formation. *The biochemical and physiological mechanisms by which applied gibberellins inhibit adventitious rooting remains unknown* (115).

Ethylene (C_2H_4) Ethylene can enhance, reduce, or have no effect on adventitious root formation (64). In 1933, Zimmerman and Hitchcock (311) showed that applied ethylene at about 10 mg/liter (ppm) causes root formation on stem and leaf tissue as well as the development of preexisting latent roots on stems. They and other scientists (312) also showed that auxin applications can regulate ethylene production and suggested that auxin-induced ethylene may account for the ability of auxin to cause root initiation. Centrifuging *Salix* cuttings in water, or just soaking them in hot or cold water, stimulates ethylene production in the tissues as well as root development, suggesting a possible causal relationship between ethylene production and subsequent root development (161, 162, 206). High auxin concentrations will also trigger ethylene evolution.

Ethylene promotion of rooting occurs more frequently in intact plants than cuttings, herbaceous rather than woody plants, and plants having preformed root initials. Rooting cuttings of ethylene-insensitive tomato mutants has shown that the promotive effect of auxin on adventitious rooting is enhanced in plants that are responsive to and sensitive to ethylene (47). The commercial ethylene receptor blockers, STS and 1-MCP, also inhibit rooting. However, the effects of ethylene on rooting are not as predictable or consistent as those of auxin (115). While a large body of evidence suggests that endogenous ethylene is not directly involved in auxin-induced rooting of cuttings (206), ethylene may be necessary in minute quantities for initiating cell division as a prerequisite for root initiation in cuttings (34). Ethylene effects are of very short duration, whereas higher concentrations and longer time exposure to ethylene inhibits rooting. It is possible that ethylene changes the competency of cells for receiving auxin signals (68).

Abscisic Acid (ABA) Reports on the effect of abscisic acid (ABA) on adventitious root formation are contradictory (14, 136, 230)—apparently depending upon the concentration, environmental, and nutritional status of

the stock plants from which the cuttings are taken. ABA is important to rooting, since it (a) antagonizes the effects of gibberellins and cytokinins, both of which can inhibit rooting, and (b) influences the ability of cuttings to withstand water stress during propagation. If the role of ABA in rooting is to be understood, then endogenous ABA levels will need to be determined at the site of root initiation, during the developmental stages of rooting (64).

Other Potential Hormones and Ancillary Compounds

There are ancillary compounds that modify main hormone effects on rooting, and adventitious bud and shoot formation. These compounds include growth retardants/inhibitors, flavonoids, peroxidases, and phenolics. Other potential phytohormones include jasmonic acid (jasmonate), polyamines, brassionosteroids and salicylic acid (salicylate). Salicylate has been reported to enhance rooting in combination with auxin (64, 229).

Growth Retardants/Inhibitors. Growth retardants, generally applied to reduce shoot growth, have been used to enhance rooting based on the rationale that they (a) antagonize GA biosynthesis or activity (GA is normally inhibitory to rooting) or (b) reduce shoot growth, resulting in less competition and consequently more assimilates are available for rooting at cutting bases (66). Synthetic anti-gibberellins and inhibitors of GA biosynthesis include chlormequat chloride (CCC), paclobutrazol (PP333, Bonzi), uniconazole (a triazole growth retardant related to PP333), morphactins, ancymidol (Arest), gonadotropins, and daminozide (SADH, Alar) (64, 231). Growth retardants frequently promote rooting (generally in combination with exogenous auxin) (66, 128). However, the mode of action of how these compounds enhance rooting is not well understood. Hence, rooting enhancement by GA biosynthesis inhibitors has been inconsistent, and none are commercially used for rooting (64).

The Polyamines. The effect of polyamines on rooting of woody plant species is quite variable. Putrescine, spermidine, and spermine in combination with IBA improved rooting of hazel microshoots (235). Conversely, higher levels of endogenous putrescine, spermidine, and spermine were found in mature phase (recalcitrant) than juvenile (easy-to-root) microshoots of chestnut (9). The rooting of olive microshoots increased by using polyamines along with NAA, but rooting of almond, pistachio, chestnut, jojoba, apricot, and walnut did not increase (243). In NAA-treated English ivy (*Hedera helix*) cuttings, there were increases in endogenous polyamines, particularly putrescine (99). *Polyamines may serve as secondary messengers for rooting*. To date, polyamine enhancement of rooting occurs only in the presence of auxin.

Classification of Plant Rooting Response to Growth Regulators

Plants can be divided into three classes with regard to growth regulator effects on rooting:

- **Easy-to-Root**—plants that have all the essential endogenous substances **(root morphogens)** plus auxin. When cuttings are made and placed under proper environmental conditions, rapid root formation occurs. Auxin may further enhance rooting, but is generally not required.

root morphogen
An endogenous substance(s) that stimulates rooting. It may be auxin or a combination of substance(s) with auxin that promote rooting.

- **Moderately Easy-to-Root**— plants in which the naturally occurring root morphogen(s) are present in ample amounts, but auxin is limited. Auxin is needed to enhance rooting.
- **Difficult-to-Root (Recalcitrant)**—plants that lack a rooting morphogen(s) and/or lack the cell sensitivity to respond to the morphogen(s), even though natural auxin may or may not be present in abundance. External application of auxin gives little or no rooting enhancement.

recalcitrant plants
Plants that are difficult to root from cuttings. They lack a rooting morphogen(s) and/or lack the cell sensitivity to respond to the morphogen(s), even though natural auxin may or may not be present in abundance. External application of auxin gives little or no rooting response.

THE BIOCHEMICAL BASIS FOR ADVENTITIOUS ROOT FORMATION

The biochemical basis for root formation implies that there are root-promoting and root-inhibiting substances produced in plants and their interaction is thought to be involved in rooting. Therefore, this theory considers that difficult-to-root cuttings either lack the appropriate root-promoting substances or are high in root-inhibiting substances.

While we know much about the biology and manipulation of cuttings, the **primary chemical stimulus** for dedifferentiation and root initial formation (the critical steps of adventitious root formation) and

the subsequent organization of root primordia **remains unknown** (65, 115). The following is a brief history of post–World War II research on the biochemistry of rooting.

Endogenous Rooting Inhibitors

In the early 1950s, endogenous chemical inhibitors were reported to retard rooting in selected plant species, as indicated in the following section. This was found to be the case with selected grape cultivars; leaching cuttings with water enhanced the quantity and quality of roots. Difficult-to-root hardwood cuttings of wax flower (*Chamaelaucium uncinatum*) have a cinnamic acid derivative that inhibits rooting, while no detectable levels of this phenolic compound were found in easy-to-root softwood cuttings (50). Cuttings of difficult-to-root mature eucalyptus (49, 215), chestnut (285), and dahlia cultivars (18, 19) also had higher rooting inhibitors than easy-to-root forms.

Rooting Co-Factors (Auxin Synergists)

rooting bioassay The use of a plant organ or tissue to respond morphologically to chemical stimulation, such as the rooting response of mung bean hypocotyl cuttings to various chemicals.

Various **model rooting bioassay systems** have been used to test adventitious root formation. The easy-to-root mung bean (*Vigna*) was used by Hess (140, 141) as a rooting bioassay to screen biochemical effects on rooting (Fig. 9–4). Hess was not able to demonstrate any difference in rooting inhibitors between the juvenile easy-to-root, and mature difficult-to-root forms of English ivy (*Hedera helix*). Instead, he determined that the juvenile, easy-to-root forms of English ivy, and easy-to-root cultivars of chrysanthemum and *Hibiscus rosa-sinensis* contained greater nonauxin rooting stimuli than their difficult-to-root forms (140, 141). He termed these nonauxin rooting stimuli ***rooting co-factors,*** which was a *modification of the rhizocaline theory* that biochemical factors, other than just auxin, were controlling rooting. These rooting co-factors were naturally occurring substances that appeared to act synergistically with indoleacetic acid in promoting rooting.

Rooting co-factors have since been found in maple (*Acer*) species (168). Fadl and Hartmann (87, 88) isolated an endogenous root-promoting factor from basal sections of hardwood cuttings of an easily rooted pear cultivar ('Old Home'). Extracts from basal segments of similar cuttings of a difficult-to-root cultivar ('Bartlett'), treated with IBA, did not show this root-promoting factor. The action of these phenolic compounds in root promotion was theorized to be in protecting the root-inducing, naturally occurring auxin—indoleacetic acid—from destruction by the enzyme indoleacetic acid oxidase (109).

Jarvis (157) attempted to ***integrate the biochemical with developmental anatomy of adventitious root formation by examining the four developmental stages of rooting*** (Fig. 9–19). His premise was that (a) the initial high concentrations of auxin needed in early rooting events are later inhibitory to organization of the primordium and its subsequent growth—hence the importance of regulating endogenous auxin concentration with the IAA oxidase/peroxidase enzyme complex playing a central role (i.e., IAA oxidase metabolizes or breaks down auxin); and (b) IAA oxidase activity is controlled by phenolics (*o*-diphenols are inhibitory to IAA oxidase), while borate complexes with *o*-diphenols result in greater IAA oxidase activity—and hence a reduction of IAA to levels that are optimal for the later organizational stages of rooting.

With *in vitro* rooting of poplar (*Populus*) shoots, endogenous free IAA activity is highest during root induction, followed by a peak of soluble peroxidase activity and a subsequent decrease in free IAA preceding root emergence (132). These events correspond to the initiative phase of rooting suggested by Jarvis (157).

Biochemical Changes During the Development of Adventitious Roots

Once adventitious roots have been initiated in cuttings, considerable metabolic activity occurs as new root tissues are developed and the roots grow through and out of the surrounding stem tissue. Protein synthesis and RNA production were both shown to be indirectly involved in adventitious root development in etiolated stem segments of willow (*Salix tetrasperma*) (155) and in seasonal rooting of *Ficus* (see Fig. 9–31, page 317) (51). To date, it is not clear to what extent RNA metabolism is altered within that small pool of cells actually involved in root initiation (156). More definitive studies need to include microautoradiographic and histochemical approaches.

During the rooting of hydrangea cuttings, enzymatic changes were identified during the development

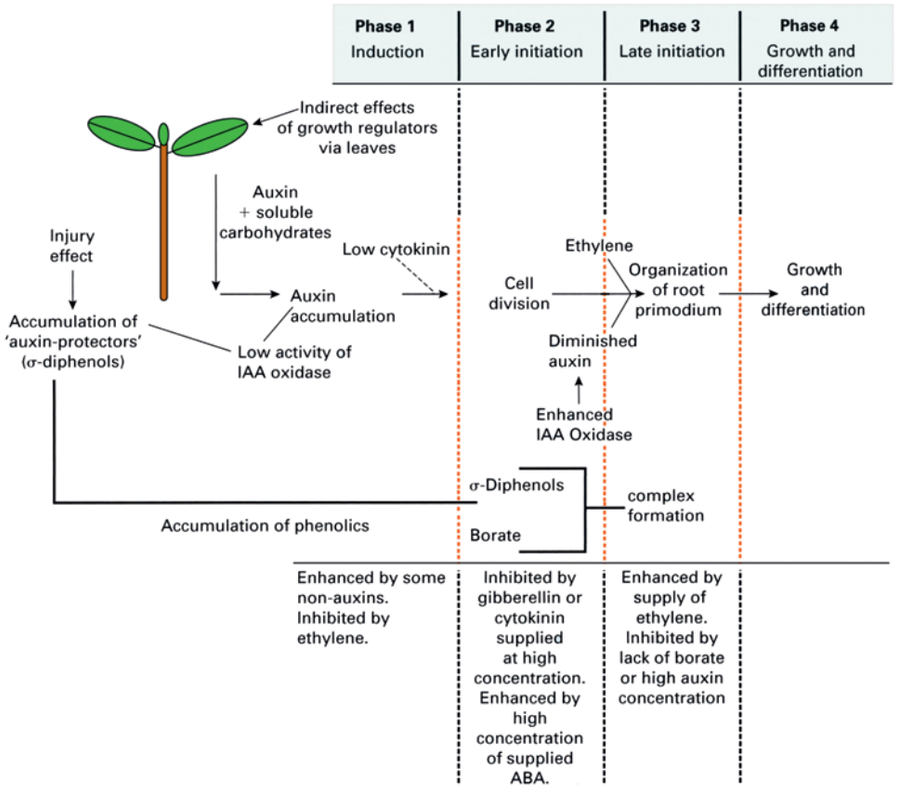


Figure 9–19
Hypothesized scheme of Jarvis (157) which proposes the role of phenolics, IAA oxidase/peroxidase, borate, and phytohormones in the four developmental stages of adventitious root production.

BOX 9.9 **GETTING MORE IN DEPTH ON THE SUBJECT**
A SYNOPSIS OF RHIZOCALINE, INHIBITORS, AND ROOTING CO-FACTORS IN ROOTING

In recent years, the role of phenolic compounds in the control of rooting has been seriously challenged. Wilson and Van Staden (300) argue that the concept of rhizocaline, inhibitors, and promoters (including rooting co-factors) represents a traditional approach to understanding rooting. The concept is founded on a bioassay principle, in which plant extracts or known compounds promote or inhibit rooting when supplied to cuttings. It is sometimes assumed that activity in a rooting bioassay reflects *in vivo* activity, and hence has physiological significance. They argue that this assumption is not warranted because even though a large diversity of known and unknown compounds has been found to promote or inhibit rooting in rooting bioassays, no well-substantiated mode of action has been established. Furthermore, promoter-inhibitor systems have not been universally observed in plants.

There is no good existing evidence that hypothesized rhizocaline consists of an auxin-phenolic conjugate, and other explanations for the actions of phenolics are not well substantiated. *Possibly the action of rooting promoters and inhibitors is mediated by chemical injury (see the later discussion on wounding in this chapter). Irrespective of their chemical identity, low concentrations promote rooting, while higher concentrations are inhibitory* (300).

Wilson (301) further proposed that a **rooting morphogen** can be assumed to induce roots in woody stem cuttings. Whereas auxins promote rooting of most herbaceous cuttings, they may have little effect on more difficult-to-root woody cuttings. The interaction between a rooting morphogen(s) of vascular origin and potential sites for root initiation are likely to be dynamic and variable. Potential rooting sites are not equally sensitive to the rooting morphogen, since each cell has a unique lineage, ontogeny, and position (i.e., the competency of cells varies, which affects their ability to respond to the morphogen and root). Hence, he concluded that no simply defined morphogen can be said to limit rooting.

BOX 9.10 **GETTING MORE IN DEPTH ON THE SUBJECT**

TYING IT ALL TOGETHER–INTEGRATING THE MORPHOLOGY: HORMONAL, PHYSIOLOGICAL, AND BIOCHEMICAL RELATIONSHIPS OF ADVENTITIOUS ROOTING

Much of the research dealing with hormones and rooting has been based on exogenous treatments (115). In contrast, little work has critically tested the roles of endogenous hormones (9) and their interactions with applied hormones. Particularly lacking is research aimed at determining how hormones might regulate gene expression and thereby influence rooting, directly and indirectly. Hence, it is difficult to distinguish between possible controlling roles of hormones on rooting and indirect hormonal effects on other physiological processes of cuttings (115).

Likewise, physiological and biochemical studies have largely addressed the influences of plant growth regulators on the biochemistry of rooting without focusing on changes in gene expression (Fig. 9–20) (121). Essentially, these studies are post-translational and are geared on finding the missing chemical component(s) of rooting.

Figure 9–21 attempts to synthesize the early morphological, physiological, and biochemical events of adventitious root formation—commencing with the severing of the stem cutting from the stock plant, wounding, perceived dehydration, decline in photosynthesis, the signaling cascade of chemicals and phytohormones, and gene expression.

Using the tools of molecular biology with auxin and ethylene mutants, microarray analysis and proteomics, more is being learned about gene expression and the primary control of rooting (11, 39, 258, 307). See Figures 9–22 and 9–23 on microarray analysis of gene expression **during the synchronized development of different stages of adventitious root formation** of *Pinus contorta* hypocotyl cuttings (39).

INVESTIGATION OF ROOTING BY PROCESS

EXPERIMENTAL TREATMENTS	MEDIATING PROCESSES	OBSERVATION OF EFFECTS
Light Temperature Oxygen Carbon Dioxide Water Auxins Gibberellins Cytokinins Ethylene Minerals Organic Nitrogen Phenolics Carbohydrates Amino acids Polyamines Histones Acid / Bases Nucleotides Nucelosides Nucleic Acids Root Symbionts Various Inhibitors Many Others	TRANSCRIPTIONAL	LIMITED ASSESSMENTS AVAILABLE
	TRANSLATIONAL	LIMITED ASSESSMENTS AVAILABLE
	POST-TRANSLATIONAL	**MOST PAST ASSESSMENTS HERE**

INVESTIGATION OF ROOTING BY DISCIPLINE

Morphology	Cytology Anatomy	Physiolgogical Biochemistry (Most past assessments here)	Molecular Genetics

Figure 9–20
Some environmental and chemical factors (in the left column) that have been implicated in rooting. Investigation of rooting research is by process (upper section) and investigation by discipline (lower section). In past research, effects of experimental treatments may have been at any or all process levels, but were usually assessed only post-translationally, in physiological and/or biochemical studies. From Haissig et al. (121).

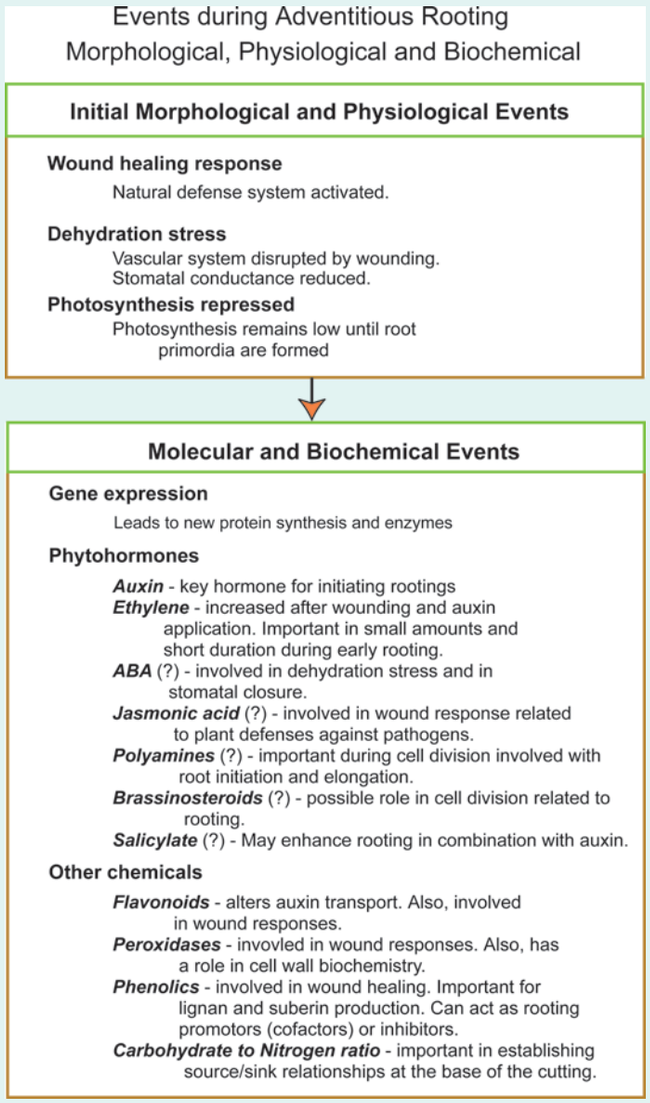


Figure 9–21
Early morphological, physiological and biochemical events in rooting a cutting. See Fig. 9–23 for detailed description of gene expression during discrete rooting stages.

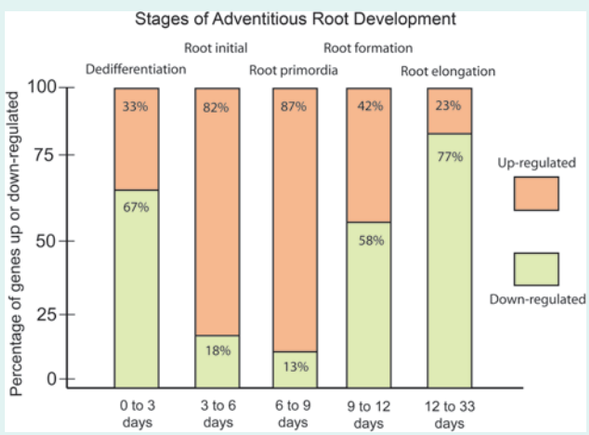


Figure 9–22
Some 220 genes are differentially expressed during the five phases (time period–days) of adventitious root development in Pinus contorta. The histogram shows the percentage of genes up-regulated (increased gene expression) or down-regulated (decreased) during rooting (39).

(*Continued*)

Days	Phase of Development	Up ↑ or Down ↓ Gene Regulation
0 to 3	Dedifferentiation	↑ Cell replication ↑ Cell wall weakening ↑ Water stress ↓ Cell wall synthesis ↓ Auxin transport ↓ Photosynthesis
3 to 6	Root Intitial	↑ Flavonoid pathway enzymes
6 to 9	Root Primordia	↑ Auxin transport ↑ Auxin responsive ↑ Cell wall synthesis ↑ Hypersensitive response proteins ↑ Pathogenesis proteins ↓ Cell wall weakening ↓ Cell wall modification ↓ Water stress
9 to 12	Root Formation	↑ Auxin transport
12 to 33	Root Elongation	↓ Water stress ↓ Cell replication

Figure 9–23
Microarray analysis of gene expression during the synchronized development of different stages of adventitious root formation of Pinus contorta hypocotyl cuttings. Transcript levels of 220 genes and their encoding proteins were up-regulated (↑increased expression) or down-regulated (↓decreased expression) (39).

of preformed root initials into emerging roots (201, 202). Initially, the enzymes peroxidase, cytochrome oxidase, succinic dehydrogenase, and starch-hydrolyzing enzymes increased in the phloem and xylem ray cells of the vascular bundles. During subsequent root development, enzyme activity shifted from the vascular tissues to the periphery of the vascular bundles. These increases in enzyme activity occurred 2 to 3 days after the cuttings were made. Peroxidase activity has been used as a predictive marker of the inductive phase of rooting (97).

During rooting, starch is converted to soluble carbohydrate. In hydrangea, starch disappeared from the endodermis, phloem and xylem rays, and pith—in tissues adjacent to the developing root primordia—and was converted to soluble carbohydrate. Similarly, in the development of adventitious roots on IBA-treated plum cuttings, as soon as callus and roots started forming, pronounced carbohydrate increases of sucrose, glucose, fructose, and sorbitol—and starch losses—occurred at the base of the cuttings where rooting occurs (37). While soluble carbohydrates are not the cause of rooting, the developing callus and roots at the cutting base act as a "sink" for the movement of soluble carbohydrates from the top of the cutting.

MOLECULAR/BIOTECHNOLOGICAL ADVANCES IN ASEXUAL PROPAGATION

Biotechnological Advances In Asexual Propagation

While the physiology of adventitious root formation is better known than the genetic and molecular events of rooting, researchers are identifying specific genes affecting rooting in model systems (i.e., using plants such as *Arabidopsis,* tobacco, loblolly pine, lodgepole pine, and English ivy). They are trying to discover the regulatory sequencing of genes in the rooting process. Artificially inducing roots by nonpathogenic *Agrobacterium,* and the potential transformation of cells using a disarmed **plasmid** from a root-inducing bacterium or from an auxin-inducing fragment of the **T-DNA** may play important roles in the vegetative propagation of plants (see Chapter 2). Applying biotechnology studies at the earlier transcriptional and translational

plasmids Small molecules of extrachromosomal DNA that carry only a few genes and occur in the cytoplasm of a bacterium.

T-DNA The portion of the root-inducing (Ri) plasmid (e.g., from *Agrobacterium rhizogenes*) that is inserted into the plant genome (e.g., of a difficult-to-root species) and stabilized; hence this normally difficult-to-root species is potentially "transformed" to an easy-to-root clone. See Chapter 2 for molecular biology terminology.

periods to determine gene expression can reveal the controls of rooting, adventitious bud formation, tuberization, and other developmental processes important to vegetative propagation. Once the regulatory sequences between genes and the rooting process of a species are known, plants may be genetically transformed with a higher rooting potential. As an example, if an enzyme negatively affected rooting, then antisense DNA or RNA could be used to turn off the gene that produced the enzyme. Initially, the genetically transformed plant would be micropropagated, and then once established *ex vitro* (outside the test tube), conventional cutting propagation techniques would be used to mass-produce the genetically transformed plant (54).

It has not yet been fully determined which genes or gene groups affect rooting. Changes in gene expression were observed during the formation of adventitious root primordia of sunflower (*Helianthus annus*) hypocotyl cuttings (213), rooting of *Arabidopsis* (67), and rooting of juvenile and mature English ivy (246).

Today, difficulties in rooting *in vitro* and *ex vitro*, developing successful tissue culture multiplication systems, and transformation systems for rooting limit the production of transgenic woody plants, (e.g., commercially important plants for the production of fruits, nuts, wood, paper, and landscape ornamentals (60, 236, 249). Some difficult-to-root woody species have been genetically "transformed" to easy-rooters. Rooting of kiwi (*Actinidia deliciosa*) cuttings was improved by introducing genes from the root-inducing bacterium *Agrobacterium rhizogenes* (242, 243) (see Chapter 2).

Progress is being made by using this root-inducing bacterium to enhance root regeneration of bare-root almond stock (265) and *in vitro* rooting of difficult-to-root apple (214). *Agrobacterium rhizogenes* have been used as an effective rooting agent in hazelnut (*Corylus avellana*) cuttings (12), and with *in vitro* and *ex vitro* rooting of pine (*Pinus*) and larch (*Larix*). How the bacterium enhances rooting is not well understood. It may be modifying the root environment by secreting hormones or other compounds, or by transforming plant cells (194).

MANAGEMENT AND MANIPULATION OF ADVENTITIOUS ROOT AND SHOOT FORMATION

Great differences in the rooting ability of cuttings exist among species and cultivars. Stem cuttings of some cultivars root so readily that the simplest facilities and care give high rooting percentages. On the other hand, cuttings of many cultivars or species have yet to be rooted. Cuttings of some "difficult" cultivars can be

BOX 9.11 GETTING MORE IN DEPTH ON THE SUBJECT
ADVANCES IN THE BIOTECHNOLOGY OF ROOTING

Because rooting potential is complex and likely controlled by many genes with unknown modes of action and inheritance, molecular studies are essential to revealing the basic mechanism of rooting. While few results have been obtained to date, there are rootless mutants (89, 112) and some differences in genes and gene products that have been identified in physiologically mature and juvenile materials (67, 107, 254, 306).

In studies of tobacco plants transformed with root-inducing (Ri T-DNA) of *Agrobacterium rhizogenes*, rooting of the transformed tobacco explants was due to *genes* that *increased auxin sensitivity* of the tissue. Rooting of transformed plants was not due to genes that regulated auxin production, or to a substantially altered balance of auxin to cytokinin ratio (259). In other studies with nonrooting tobacco mutants, sensitivity to auxin was due to general alteration of the cellular response to auxin and was not due to the increased rate of conjugation of auxins by these tissues, or by disruption of auxin transport (40). *Thus, there are implications that the lack of cell competency in difficult-to-root species may be due to a lack of cell sensitivity to auxin rather than to a suboptimal level of endogenous auxin.*

Just as in biochemical studies, understanding the molecular events of rooting is difficult because only a very few cells in an explant or cutting are directly involved in regeneration—the specific features of these cells are swamped by those of the other cells. Therefore, validation by microscopic studies is needed to determine characteristics specifically in the cells involved in the regeneration event.

BOX 9.12 **GETTING MORE IN DEPTH ON THE SUBJECT**

GENE REGULATION IN ROOTING OF *PINUS CONTORTA* HYPOCOTYL CUTTINGS

In a very challenging study, the histological events of adventitious root formation of *Pinus contorta* hypocotyl cuttings were correlated to gene expression during five rooting stages using microarray analysis (39). Essentially RNA was harvested at discrete stages of rooting and hybridized to microarrays. The transcript levels of 220 genes and their encoding proteins were either up-regulated (↑increased expression) or down-regulated (↓ decreased expression) (39). Not surprisingly, the highest number of genes were differentially expressed (either up- or down-regulated) during days 0 to 3 (response to: severing the cutting, wounding, exogenous auxin treatment, perceived water stress, decreased photosynthesis, and decreased auxin transport) (Figs. 9–22 and 9–23). The highest up-regulation occurred between days 3 (root initial) to 9 (more defined root meristem—root primordia), which included increased auxin transport, auxin-responsive transcription, cell wall synthesis, and pathogenesis- and hypersensitive-induced response proteins—the latter suggesting further development of a defense barrier—as part of the "wound-healing response." Highest down-regulation occurred during days 0 to 3, and days 12 to 33 (fully developed roots were elongating) roots were fully functional in water uptake, so genes affiliated with water stress and cell replication had reduced expression.

BOX 9.13 **GETTING MORE IN DEPTH ON THE SUBJECT**

PROTEOMICS AND ROOTING

Since adventitious rooting is known to be a quantitative genetic trait, research is being done with **proteomic analysis** Using different mutant genotypes of *Arabidopsis* has led to the identification of eleven proteins whose abundance was either positively or negatively correlated with endogenous auxin, number of adventitious root primordia, and/or number of mature adventitious roots (258). The identification of regulatory pathways associated with adventitious rooting could lead to valuable markers for future identification of genotypes with better rooting ability.

Proteomics The large-scale study of proteins, particularly their structures and functions. The complement of proteins and modifications made to a particular set of proteins will vary with time and distinct requirements during the various stages of adventitious root formation.

BOX 9.14 **GETTING MORE IN DEPTH ON THE SUBJECT**

CELL COMPETENCY-TO-ROOT

The formation of new centers of cell divisions—called *de novo* meristems, that differentiate into adventitious roots—requires that a cell or group of cells (e.g., phloem ray parenchyma cells or callus cells) embark upon a new developmental program (199). What is the molecular mechanism that controls adventitious organ formation? What is the molecular basis for the plasticity that allows differentiated cells (phloem ray parenchyma) to start new developmental programs? How many different signals are needed for root induction? Why is there a decline or loss of competence for the formation of adventitious roots in physiologically mature-phase shoot tissue, compared with physiologically juvenile-phase tissue? Competence-to-root can be assessed by determining whether tissue is capable of responding in a specific way to inductive treatments (208). A model of the events in the organogenic process of rooting is given in Figure 9–5. Our understanding of cell competency-to-root will be enhanced via the molecular tools, such as microarray analysis of gene regulation during the five discrete stages of rooting in *Pinus contorta* hypoctyl cuttings (Figs. 9–22 and 9–23) (39), and proteomics (258).

rooted only if specific influencing factors are taken into consideration and if the cultivars are maintained at the optimum condition. With most species, the careful selection of cutting material from stock plants or containerized plants, management of cuttings, and control of environmental conditions during rooting are the difference between commercial success or failure. The remainder of the chapter discusses these influencing factors that include:

1. Management of stock plants to maximize cutting propagation
2. Treatment of cuttings
3. Environmental manipulation of cuttings

BOX 9.15 **GETTING MORE IN DEPTH ON THE SUBJECT**

CURRENT STATUS OF ADVENTITIOUS ROOT BIOLOGY

Significant new biotechnology has not emerged in commercial rooting operations (60, 221). Cuttings are still rooted by a brief exposure (quick-dipped) in a solution containing a moderate to high auxin concentration or via a rooting powder formulation—techniques developed 60 years ago. Where improvements have been made is in the selection and manipulation of stock plants, maximization of environmental controls, and media manipulation during the propagation and transplanting of rooted liner plants.

Much research has focused on finding the **Primary Causes of Rooting: *genetic potential, metabolic factors, and physiological condition***. Generally, cuttings that do not root are considered deficient in rooting promoters, including hormones. The search for the primary chemical stimulus to root initiation (60, 115, 121) is merely one way of looking at the mechanism of adventitious rooting (e.g., the concept of rooting promoters and inhibitors may have led to undue emphasis on the "*ultimate mechanism of adventitious rooting*").

Hopes for genetic engineering techniques reside in their potential power, which is easily manifest in traits under simple genetic control. However, many genes with unknown modes of action and inheritance control rooting potential. Some 220 genes are either up- or down-regulated during the five discrete development stages of *Pinus contorta* hypocotyl cuttings (39).

Often overlooked are the **Secondary Causes of Poor Rooting:** many leafy woody (and herbaceous!) cuttings have major limitations affecting their *survival* (i.e., they are quite susceptible to stress prior to developing roots) and require good management to avoid mortality (302). Among the secondary causes of poor rooting are low photosynthetic and transpirational capacity of cuttings, loss of plant inertia (abscission of leaves; failure of recently rooted cuttings to put on an initial growth flush prior to fall dormancy, thus incurring high winter losses), environmental stress—inadequate water regimes, desiccation, anaerobic conditions—adverse effects of high auxins on cutting buds and shoots, and so on (305). These problems are discussed in greater detail in the remainder of this chapter and in Chapter 10 where the technology to enhance cutting rootability and survival is addressed.

MANAGEMENT OF STOCK PLANTS TO MAXIMIZE CUTTING PROPAGATION

Selection and Maintenance of Stock Plants for Cutting Propagation

Management of stock plants (or containerized plants) to maximize rooting begins with the *selection* of source material that is easy-to-root (juvenile), *maintenance* of stock plants in the juvenile/transition phase to maximize rooting, and *rejuvenation* of stock plant material (reversal from the mature to a juvenile/transition phase) to reestablish high rooting potential (Table 9–5).

The remainder of this chapter addresses these factors in detail.

Since many containerized ornamental nurseries no longer use stock plants, it is essential to maintain quality control of all production container plants from which propagules are taken. Propagules should be collected from stock plants free of viruses, bacteria,

Table 9–5
STOCK PLANT MANAGEMENT. SELECTION AND MAINTENANCE OF STOCK PLANTS FOR CUTTING PROPAGATION REJUVENATION OF STOCK PLANTS: HEDGING, PRUNING, GRAFTAGE, MICROPROPAGATION MANIPULATION OF ENVIRONMENTAL CONDITIONS AND PHYSIOLOGICAL STATUS

- Water status
- Temperature
- Light: duration (photoperiod), irradiance, spectral quality (wavelength)
- Stock plant etiolation: banding, blanching, shading
- Girdling
- Carbon dioxide enrichment
- Carbohydrates
- Managing carbohydrate/nitrogen levels of stock plants

Selection of Cuttings from Stock Plants

- Type of wood selected
- Seasonal timing
- Predictive indices of rooting

fungi, and other pathogenic organisms. See Chapters 10 and 16 for the discussions on stock plant maintenance including nutritional status, disease control, and the importance of assuring trueness-to-type.

For new cultivars to be commercially successful, they must be relatively easy to propagate and suitable for existing propagation and production systems. New cultivars are, in part, selected for their ease of rooting. Despite how desirable the form, flower color, ornamental characteristics, or yield (fruit crops), it is not economically feasible to use cutting propagation with a new cultivar that has less than 50 percent rooting. Nurseries continually select for plants that are easy to root through the annual harvesting and rooting of cuttings from previously rooted containerized plants in production blocks or stock plants. This **serial propagation** of new generations of rooted cuttings helps *maintain* easy-to-root characteristics of a cultivar.

serial propagation
The annual harvesting and rooting of cuttings from previously rooted, containerized plants to help maintain a high rooting potential from generation to new cutting generation.

There are other horticultural and forestry practices that can maintain stock plants in a physiologically juvenile or transition phase and improve rooting success (53, 146, 151, 167). The development of systems for obtaining whole populations of juvenile and partially juvenile/transition cuttings has revolutionized clonal forestry. For example, seedling and clonal populations of elite germplasm of Monterey pine, loblolly pine, and Douglas-fir are grown as stock plants. They are then subjected to hedging and pruning systems and serial-cutting practices to maintain a high rooting potential. This has exciting opportunities for clonally multiplying elite germplasm and increasing timber yield. The hedging or shearing treatments given Monterey pine (*Pinus radiata*) trees (see Fig. 14–16), stooling of apples (see Fig. 14–1), and pecans (see Fig. 14–12) are quite effective in maintaining juvenility and increasing the rooting potential of cuttings taken from them, compared with nonhedged trees (177, 195).

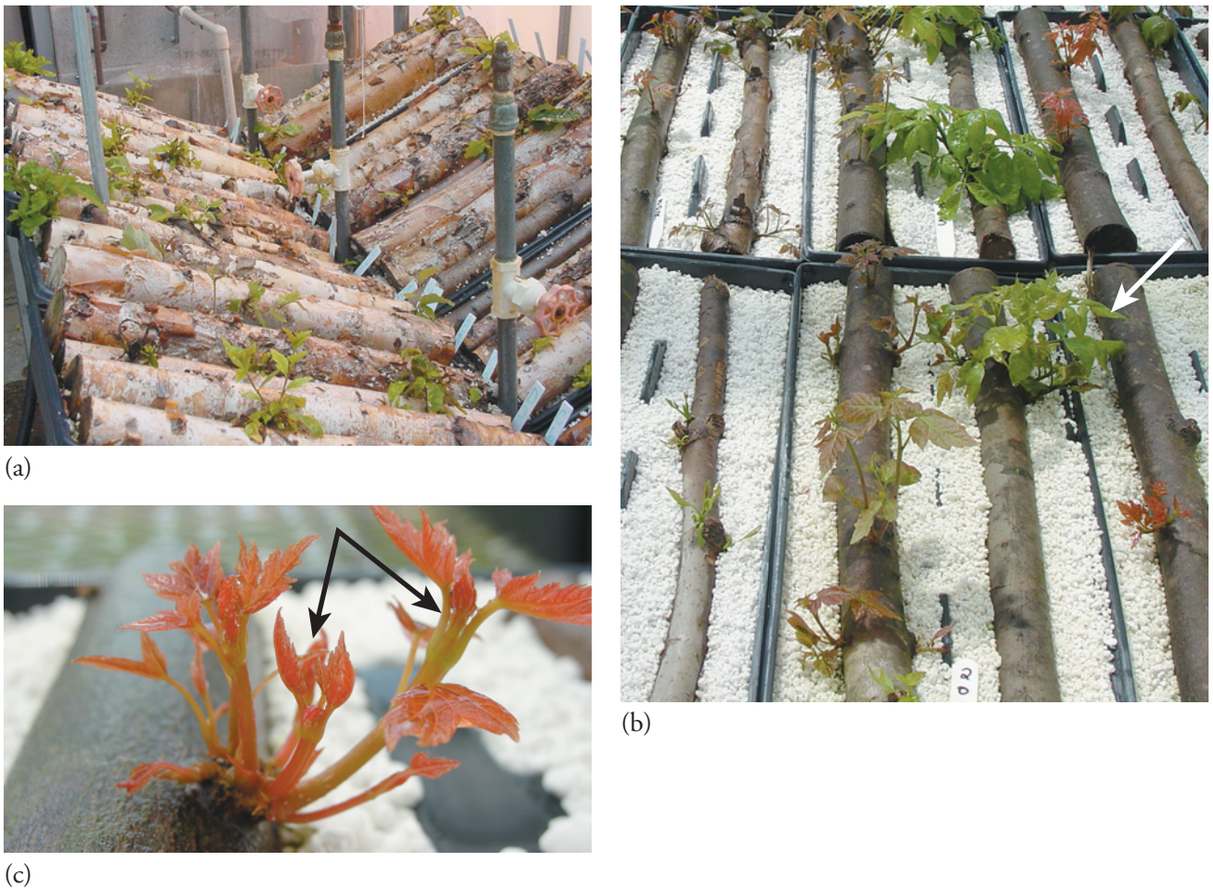
Rejuvenation of Stock Plants

In difficult-to-root woody plant species, the ease of adventitious root formation declines with the age of parent stock, resulting in a propagation enigma, since desirable characteristics are frequently not expressed until after a plant has reached maturity. The transition from the *juvenile* to the *mature* phase has been referred to as *phase change, ontogenetic aging,* or *meristem aging.* There are progressive changes in such morphological and developmental characteristics as leaf shape, branching pattern, shoot growth, vigor, and the ability to form adventitious buds and roots (106, 110, 111, 205) (see Chapter 16). Experiments with apple, pear, eucalyptus, live oak, and Douglas-fir have shown that the ability of cuttings to form adventitious roots decreased with increasing age of the plants from seed; in other words, when the stock plant changed from the juvenile to the mature phase. With many woody species, it is the **physiological** or **ontogenetic age,** not **chronological age,** of the cutting that is most important in rooting success (see Chapter 16).

In some species, such as apple, English ivy, olive, eucalyptus, and Koa tree (*Acacia koa*), differences in certain morphological characteristics, such as leaf size and shape, make it easy to distinguish between the mature and the lower, juvenile portions of the plant. In some kinds of deciduous trees, such as oak and beech, leaf retention late into the fall occurs on the basal parts of the tree and indicates the part **(cone of juvenility)** still in the physiologically juvenile stage (see Fig. 16–22). Ideally, cuttings should be taken from juvenile wood.

Inducing Rejuvenation In rooting cuttings of difficult species it would be useful to be able to **induce rejuvenation** to the easily rooted juvenile or transition stage from plants in the mature form. This has been done in several instances by the following methods:

- **Rejuvenation of apple** can be done with mature trees by causing **adventitious buds/shoots to develop from root pieces,** which are then made into softwood stem cuttings, and rooted.
- **Forcing epicormic sprouts** of 2- to 10-cm (1- to 4-in) wide × 24 cm (9.5 in) long branch segments of adult hardwoods is done to produce softwood cuttings with higher rooting success in red and white oaks, white ash, maple, honeylocust, and other species (Fig. 9–24) (91, 223, 280).
- By **removing terminal and lateral buds and spraying stock plants** of *Pinus sylvestris* with a mixture of cytokinin, tri-iodobenzoic acid, and Alar (daminozide), many fascicular buds can be forced out. With proper subsequent treatment, high percentages of these shoots can be rooted (296).
- **Chemical manipulation with gibberellin sprays** on English ivy stock plants can stimulate growth and reversion of some of the branches to the juvenile stage, and improve rooting of cuttings (264).
- In some plants juvenile wood can be obtained from mature plants by **forcing juvenile growth from sphaeroblasts,** wartlike protuberances containing meristematic and conductive tissues sometimes



(a)

(b)

(c)

Figure 9–24
Forcing softwood cuttings from woody stem segments to propagate hardwood species. (a) River birch shoot forcing under intermittent mist, (b) shoot forcing of white ash and silver maple, and (c) epicormic shoots from forced silver maple—will later be harvested as softwood cutting and rooted under mist (223). Courtesy J. E. Preece.

found on trunks or branches. These are induced to develop by disbudding and heavily cutting back stock plants. Using the mound-layering (stooling) method on these rooted sphaeroblast cuttings produces rooted shoots that continue to possess juvenile characteristics (see Fig. 16–23).

- **Grafting mature forms onto juvenile forms** has induced a change of the mature to the juvenile stage, provided that the plants are held at fairly high temperatures (264); such transmission of the juvenile rooting ability from seedlings to mature forms by grafting has also been accomplished in rubber trees (*Hevea brasilensis*) (209), and with serial graftage of mature difficult-to-root scions onto seedling rootstock of eucalyptus (*Eucalyptus xtrabutii*) (Fig. 9–25, page 310).
- Ready-rooting cuttings can be produced from **stock plants that are produced via micropropagation.** Epigenetic (non-permanent) changes that occur with rejuvenation of tissue *in vitro* has tremendous potential to enhance rooting ability. Stock plants derived from micropropagation exhibit certain juvenile/transition characteristics and produce an increased number of higher-rooting, thin-stemmed cuttings than conventionally produced stock plants (4, 108, 147, 167, 218, 222, 269). The tissue culture effect can be long-lasting depending on the plant species and proper maintenance via severe hedge pruning of stock plants (147, 148). However, without proper stock plant maintenance, the rejuvenation effect may last only one to two generations of cuttings (219). To

epigenetic change
The heritable changes in gene expression, resulting in changes in phenotype (appearance) or physiology (adventitious rooting potential). There is modification of the activation of certain genes, but no changes in basic DNA structure. These changes may remain through cell division and may last for multiple generations.

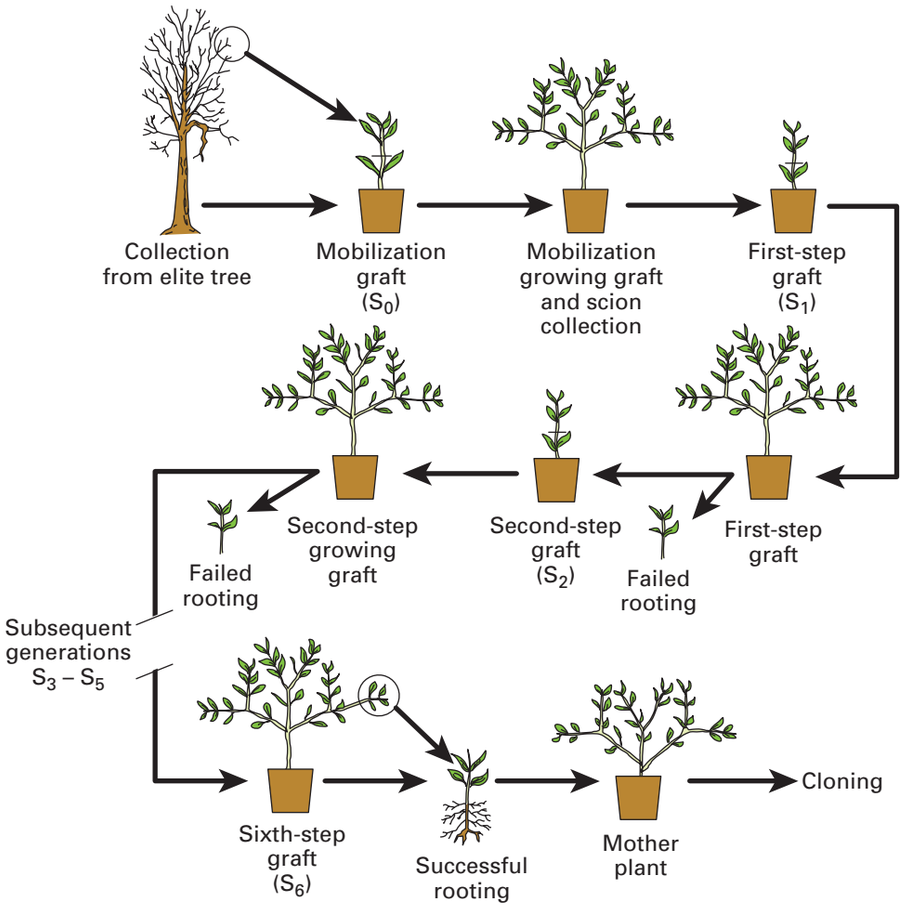


Figure 9–25
Scheme for rejuvenation techniques used in serial graftage of ten-year-old Eucalyptus xtrabutii onto juvenile seedling understock. Six serial grafts (S_1 to S_6) were needed before mature grafted scions could be used as cuttings and rooted.

maintain high rooting potential and avoid clonal variation (i.e., habitation and irregular growth), there are advantages of periodically replenishing tissue culture systems with new explant sources and producing new tissue-culture–derived stock plants from which cuttings are selected (see Chapter 16). Stock plants derived from transgenic plants with higher rooting potential or from somatic embryogenesis (synthetic seed technology) may also be used to restore high rooting potential (74, 210), see Figure 17–45.

Manipulating the Environmental Conditions and Physiological Status of the Stock Plant

The physiological condition of stock plants is a function of genotype (species, cultivar) and environmental conditions (water, temperature, light, CO_2, and nutrition).

Water Status There may be *advantages of periodic, controlled drought stress to stock plants.* Controlled water stress of eucalyptus (*Eucalyptus globus*) stock plants enhanced the survivability and rooting of cuttings (303). However, there is experimental evidence to support the view that extreme drought stress of stock plants is not desirable. Studies with cacao and pea (226) cuttings showed reduced rooting when the cuttings were taken from stock plants having a water deficit. Plant propagators often emphasize the desirability of taking cuttings early in the morning when the plant material is in a turgid condition. Unrooted cuttings are particularly vulnerable to water stress, since rehydration of the tissue is very difficult without a root system. Furthermore, droughted cuttings are more prone to disease and pest problems.

Temperature Information on temperature interactions with stock plant water relations, irradiance, and CO_2 is limited. Research has shown that there is a complex interaction of temperature and stock plant photoperiod on the level of endogenous auxins and other hormones (137). With deciduous woody species (apple, plum), higher air temperatures can produce more rapid growth of stock plants and the production of higher-rooting, thinner-stemmed cuttings (148). *In general*, the air temperature of stock plants (12 to 27°C, 54 to 81°F) appears to play only a minor role in the ease of rooting of cuttings (196).

BOX 9.16 **GETTING MORE IN DEPTH ON THE SUBJECT**

UTILIZING STOCK PLANTS FOR CUTTINGS

Stock plants for cuttings of selected fruit tree rootstocks and woody ornamentals are maintained as hedges rather than allowed to grow to a tree form. Proper hedge management (pruning) of permanent stock plants can maintain large numbers of cuttings in an apparent juvenile stage of development. See Chapter 10 for stock plant pruning and girdling systems.

Severe or hard pruning gives rise to many shoots suitable for cuttings, but their higher rooting potential is not necessarily due to greater vigor (as has long been supposed), but rather to the less vigorous (thinner-diameter), subordinate shoots that root better than the more vigorous ones (e.g., *Prunus, Rhododendron, Syringa,* etc.) (149, 151, 152).

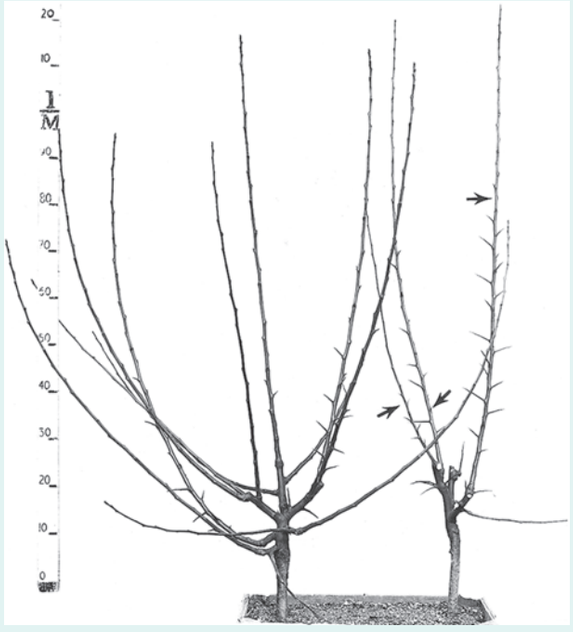


Figure 9–26
Optimum rooting of hardwood cuttings for 'M-26' apple rootstock occurs from the subordinate, thinner shoots that develop in the shoot hierarchy (framework) of severely pruned stock plant hedges. *Left:* An unpruned stock plant with subordinate and dominant (spiny) shoots. *Right:* The thinner, subdominant cuttings have been collected, while only the dominant, spiny shoots (arrows) remain to provide the framework for next year's generation of shoots (cuttings). Courtesy B. H. Howard, Horticulture Research International, East Malling, England.

Enhanced rooting potential, with relatively thin shoots of both hardwood and softwood cuttings, is only achieved with an improved propagation environment. Thin cuttings are more susceptible to basal rotting, so good drainage of media and mist management are critical; when planting hardwood cuttings directly in the field, there must be a compromise between the thinner shoots that root more quickly and larger-stem-diameter cuttings that survive longer in the poor conditions often present in field soil during winter (149).

Competence-to-root appears to be controlled independently in individual shoots and is indirectly related to shoot thickness, which favors the subordinate (subdominant) shoots that develop in the shoot hierarchy of the severely pruned hedges (Fig. 9–26). Rooting potential among shoots in a hedge is then more dependent on their relative position, rather than their proximity to the ground (Fig. 9–27). The most vigorous shoots are the poorest rooters but make better hardwood cuttings (151). Thin-stemmed shoots are better propagated as softwood cuttings. They have a higher leaf-to-stem ratio, and greater accumulation of dry matter at the cutting base before the first roots emerge (150).

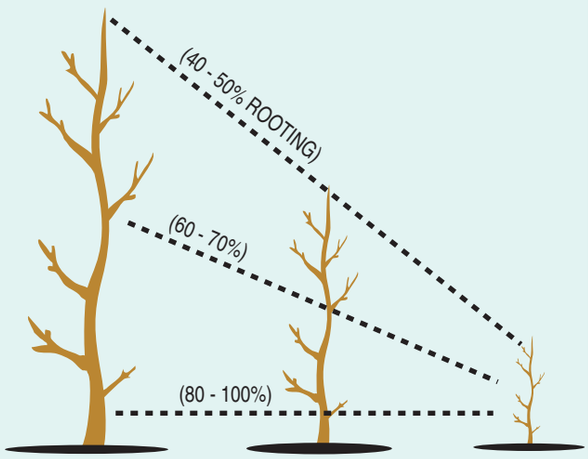


Figure 9–27
Rooting potential (typical values in brackets) of hardwood cuttings in a hardpruned (severely cut back) hedge is more influenced by the relative position of the shoots than by their absolute position in terms of distance between themselves or from the root system (149).

Light *Light duration* (photoperiod), *irradiance* [($W \times m^{-2}$) or photon flux ($\mu mol \cdot m^{-2} \cdot s^{-1}$)], and *spectral quality* (wavelength) influence the stock plant condition and subsequent rooting of cuttings (196). (See Chapter 3 for discussion.) For instance, sufficient irradiance to stock plants is needed to maintain minimal endogenous auxin for rooting chrysanthemum cuttings. Conversely, too high an irradiance can cause photo destruction of auxin or adversely affect stock plant water relations.

There is some evidence that the **photoperiod** under which the stock plants are grown exerts an influence on the rooting of cuttings taken from them (45, 159, 196). This could be a photosynthetic or morphogenic effect. If the photoperiod influences photosynthesis, it may be related to carbohydrate accumulation, with best rooting obtained under photoperiods promoting increased carbohydrates. If manipulation of photoperiod favors vegetative growth (rooting) and suppresses reproductive growth (flowering), then the effect is photomorphogenic (124, 262). Long-day conditions (sufficient hours of light to satisfy the critical photoperiod) have been used with some short-day flowering cultivars of chrysanthemum; where flowering is antagonistic to rooting, the long-day conditions promote vegetative growth and enhance rooting (90). Likewise, with some woody perennials where the onset of dormancy shuts down vegetative growth and/or reduces rooting, propagators can manipulate stock plants by extending the photoperiod with low irradiance from an artificial light source (see Fig. 3–14).

Controlling photoperiod and the daily light integral is not always sufficient to maintain vegetative growth. For many crops, ethephon (Florel) is applied once every two to three weeks at rates ranging from 200 to 750 parts per million or higher. Ethephon releases the gas ethylene, which can abort open flowers and flower beds. Ethephon can also increase the cutting yield of annual stock plants by increasing the laters' branching.

Conflicting reports on the influence of **light quality** on stock plants and subsequent rooting of cuttings is attributed to the effect of red and far-red light on rooting (196). *In vitro* rooting of pear cultivars was enhanced under red light and inhibited under far-red light and darkness, which indicates involvement of the phytochrome system in rooting (17). Using light emitting diodes (LED), rooting of *in vitro Tripterospermum* was inhibited by blue and promoted by red light (203). Red shade cloth, which increases the red, while reducing the blue and green spectra, is used in commercial propagation to enhance root initiation and development of cuttings (see Fig. 3–11).

etiolation The development of plants or plant parts in the absence of light, resulting in such characteristics as small unexpanded leaves, elongated shoots, and lack of chlorophyll, which yields a yellowish or whitish color.

Stock Plant Etiolation Reducing irradiance levels of stock plants can sometimes enhance rooting of difficult-to-root species. By definition, **etiolation** is the total exclusion of light; however, plant propagators also use this term when forcing new stock plant shoot growth under conditions of heavy shade. Softwood cuttings are then taken from new growth and often root more readily. **Banding** is a localized light exclusion pretreatment which excludes light from that portion of a stem that will be used as the cutting base (13, 191). Banding can be applied to etiolated shoots or applied to light-grown shoots which are still in the softwood stage. In the latter case, a band of Velcro or black adhesive tape is said to blanch the underlying tissues, since the stock plant shoot accomplished its initial growth in light prior to banding. Shading refers to any stock-plant growth under reduced light conditions (159). For techniques of banding, blanching, etiolation, and shading, see Chapter 10, Table 10–3 and see Figures 10–25 and 10–26.

Anatomical and physiological changes can occur in etiolated stem tissue that enhance rooting. Etiolation of chestnut (*Castanea*) (i.e., covering the stool bed with soil) caused a greater accumulation of starch grains, but no significant change in stem anatomy; however, girdling and then etiolating the shoots increased parenchyma and storage cells above the girdle, reduced sclerenchyma formation, and was the only treatment that rooted (20). Exclusion of light by etiolation, stem banding, or shading greatly enhances a stem's sensitivity to auxin (192, 193). Translocatable factors produced distal to (above) an etiolated segment also enhanced the etiolation effect (13). Etiolation may also reduce the production of lignin [for structural support cells (sclereids, fibers)]; thus, instead of forming lignin, phenolic metabolites may be channeled to enhance root initiation (53, 81).

Rooting in cuttings of *Syringa vulgaris* 'Madame Lemoine' was enhanced when stock plants were grown in the dark for a short period after bud break. Cuttings grown initially in the dark were found to have relatively thin stems, resulting in a higher leaf-to-stem ratio than normal light-grown ones. This was associated with a net accumulation of dry matter at the cutting base before the first roots emerged (Fig. 9–28) (150). A cutting must produce and/or rely on stored carbohydrates in excess of its maintenance requirement for successful rooting to occur, which is why stock plant manipulation (etiolation, hedging) and the rooting environment of the cutting are so critical for successful rooting.

Girdling Girdling, or otherwise constricting the stem, blocks the downward translocation of carbohydrates, hormones, and other possible root-promoting factors and can result in an increase in root initiation. Girdling

(a) (b)

Figure 9–28
(a) Etiolation frames (arrow) in place over stock plant hedges of Syringa vulgaris. (b) Improved rooting following etiolation of S. vulgaris 'Madame Lemoine' (far left) and S. vulgaris 'Charles Joly' (second from right). Cuttings from nonetiolated stock plants have poor rooting [second from left and far right (arrows)]. Courtesy B. H. Howard, Horticulture Research International, East Malling, England (149).

shoots prior to their removal for use as cuttings can improve rooting. This practice has been remarkably successful in some instances. For example, rooting of citrus and hibiscus cuttings was stimulated by girdling or binding the base of the shoots with wire several weeks before taking the cuttings (263).

In cuttings from mature trees of the water oak (*Quercus nigra*), a threefold improvement in rooting was obtained when cuttings were taken from shoots that had been girdled 6 weeks previously, especially if a talc powder combined with a mixture of auxin, growth retardant, carbohydrate, and a fungicide was rubbed into the girdling cuts (127). Enhanced rooting of cuttings taken from girdled stock plants has also been obtained with sweet gum, slash pine, and sycamore. Girdling just below a previously etiolated stem section was particularly effective in promoting rooting in apple cuttings (69).

Carbon Dioxide Enrichment With many species, carbon dioxide enrichment of the stock plant environment has increased the number of cuttings that can be harvested from a given stock plant, but there is considerable variation of rooting response among species. Principal reasons for increased cutting yields are increased photosynthesis, higher relative growth rate, and greater lateral branching of stock plants (196). Any benefits of CO_2 enrichment have been limited to greenhouse-grown stock plants and cuttings during conditions when propagation house vents are closed and ambient CO_2 becomes a limiting factor to photosynthesis (i.e., October–March in central Europe). Without adequate light (supplementary greenhouse lighting during low-light-irradiance months), CO_2 enrichment is of minimal benefit (200) (see Chapter 3).

Carbohydrates The relationship between carbohydrates and adventitious root formation remains controversial. Since Krause and Kraybill (169) hypothesized the importance of the carbohydrate-to-nitrogen (C/N) ratio in plant growth and development, rooting ability of cuttings has been discussed in relation to carbohydrate content. The carbohydrate pools of sugars (soluble carbohydrates) and storage carbohydrates (starches or insoluble carbohydrates) are important to rooting as building blocks of complex macromolecules, structural elements, and energy sources (105, 119, 120, 267).

Although stock plant carbohydrate content and rooting may sometimes be positively correlated (122, 139), *carbohydrates do not have a regulatory role in rooting.* A positive correlation between carbohydrate content and rooting may reveal that the supply of current photosynthate is insufficient for supporting optimal rooting (283). High C/N ratios in tissue of cuttings promote rooting but do not accurately predict the degree of rooting response (267). Cuttings use stored carbohydrates in root regeneration, but only in small amounts. Differences in C/N ratios are due mainly to nitrogen rather than carbohydrate content. Nitrogen has been negatively correlated to rooting (122), which suggests that the correlation between high C/N ratios and rooting may be due to low N levels.

Managing Carbohydrate/Nitrogen Levels of Stock Plants Rooting can be enhanced by controlling nitrogen fertility of stock plants such that cutting shoot development is not stimulated by high N levels (233, 291). This avoids the disadvantage of adventitious rooting competing with rapidly developing shoots for carbohydrates, mineral nutrients, and hormones (119).

Generally, maintaining stock plants under a high carbohydrate/high nitrogen level is optimal for rooting

cuttings under mist, and a high carbohydrate/low-to-moderate nitrogen ratio is optimal for rooting dormant hardwood cuttings. Cuttings of *Hypericium, Ilex, Rosa,* and *Rhododendron* rooted best when stock plants were suboptimally fertilized, resulting in less-than-maximal shoot growth (233). Very low nitrogen leads to reduced vigor, whereas high nitrogen caused excess vigor; either extreme is unfavorable for rooting. Adequate nitrogen is necessary for nucleic acid and protein synthesis.

It is important to distinguish between the role of carbohydrates in enabling a cutting to survive (*until it roots*) and the role of carbohydrates in rooting itself. In species where unrooted hardwood cuttings were propagated directly in the field without mist, survival is necessary before rooting occurs, hence the need to compromise between thin rooting cuttings (which root better, but have poorer field survival) and larger diameter, carbohydrate-rich cuttings that survive better in the field, but have lower rooting capacity.

To maintain high carbohydrate/low-to-moderate nitrogen ratios of stock plants for optimal rooting of hardwood cuttings, producers can manipulate stock plants as follows:

- Reducing nitrogen fertilization, thus reducing shoot growth and allowing for carbohydrate accumulation.
- Selecting cutting material from lateral shoots, which have slower growth rates and higher carbohydrate storage than fast-growing terminal shoots. [But for plants showing a plagiotropic growth pattern (see Fig. 9–29), use of lateral shoots should be avoided.]
- For maintenance of adequate carbohydrate levels, photosynthate production of greenhouse-grown stock plants can be controlled by increasing light irradiance of supplementary high-pressure sodium-vapor lights (see Fig. 3–14).

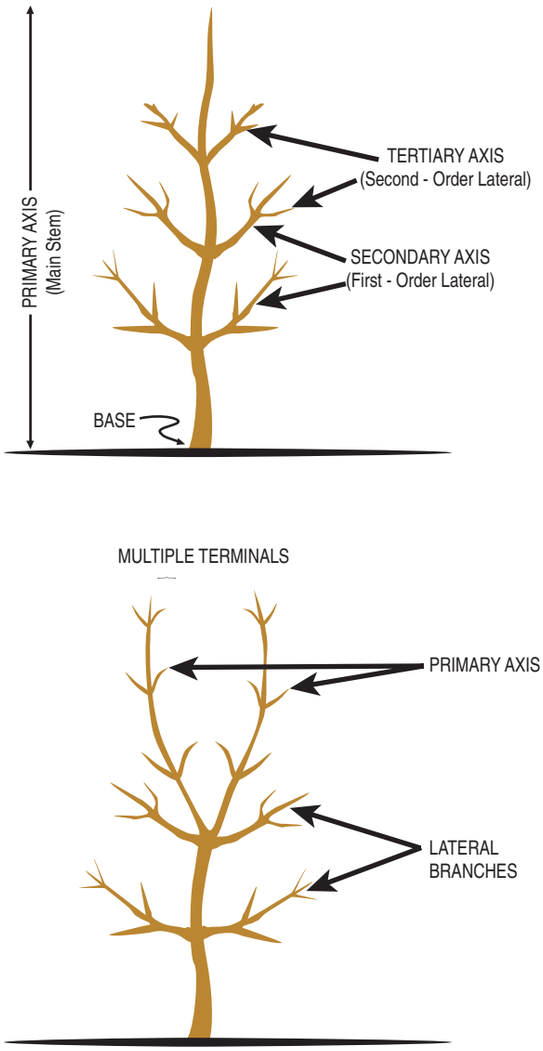


Figure 9–29
The line drawing shows the location where cuttings were taken on stock plants of Frasier fir (Abies fraseri). *Top:* A schematic of the branch order. *Bottom:* Demonstrates multiple terminals used as cuttings. Cuttings from lateral branches root readily, but have an undesirable horizontal growth habit (plagiotropic) after rooting. Cuttings taken from the tips of primary axes (main stem) produce symmetrical, upright (orthotropic) trees. Redrawn from Blazich and Hinesley (26).

Selection of Cuttings from Stock Plants

Type of Wood Selected from Stock Plants In woody perennials, types of materials to use range from softwood terminal shoots of current growth to dormant hardwood cuttings. No one type of cutting material is best for all plants. What may be ideal for one species would be a failure for another. See Table 10–1 for a synopsis of propagation systems with different cutting types. Procedures for certain species or cultivars, however, often may be extended to related species or cultivars (see Chapters 19, 20, and 21).

Differences Between Lateral and Terminal Shoots. In general, with exceptions, softwood cuttings root better from terminal shoots, and the more lignified, semi-hardwood cuttings root better from lateral shoots. In rooting different types of softwood plum cuttings taken in the spring, there was a marked superiority in rooting of lateral shoots, compared with terminal shoots. Similarly, lateral branches of Fraser fir (*Abies*) (Fig. 9–29), white pine, and Norway and Sitka spruce gave consistently higher percentages of rooted cuttings than did terminal shoots (26, 261). In rhododendrons, too, thin cuttings made from lateral shoots consistently

BOX 9.17 **GETTING MORE IN DEPTH ON THE SUBJECT**

SELECTION OF DIFFERENT TYPES OF CUTTINGS

Leafy, softwood cuttings may be the best way to propagate certain difficult-to-root species [e.g., maple (*Acer*), crabapple (*Malus*), redbud (*Cercis*)]. Softwood cuttings tend to have higher auxin and lower endogenous carbohydrate. They have a moderate light requirement, since some photosynthesis enhances their rooting. Their propagation requires more intensive (critical) water management, using mist or fog. Whereas **dormant, hardwood cuttings** have low auxin and high carbohydrate storage, photosynthesis is initially not needed for rooting, and they can be propagated under lower light, without mist or under less critical mist regimes.

give higher rooting percentages than those taken from vigorous, strong terminal shoots. In certain species, however, plants propagated from cuttings taken from lateral branches may have an undesirable growth habit: They tend to become **plagiotropic** and have a horizontal branchlike growth after rooting, whereas cuttings taken from primary axes grow upright **(orthotropically)** and produce symmetrical trees, for example, yew (*Taxus cuspidata*), coffee, Norfolk Island pine, and *Podocarpus* (see Figs. 9–29 and 16–27). This effect on growth is referred to as *topophysis.*

plagiotropic
A horizontal branchlike growth habit that is generally not horticulturally desirable.

orthotropic
A desirable, upright growth allowing production of symmetrical plants.

Differences Between Various Parts of the Shoot. With some woody plants, hardwood cuttings are made by sectioning shoots a meter long and obtaining 4 to 8 cuttings from a single shoot. Marked differences are known to exist in the chemical composition of such shoots from base to tip (277). Variations in root production on cuttings taken from different portions of the shoot are often observed, with the highest rooting, in many cases, found in cuttings taken from the basal portions of the shoot. Cuttings prepared from shoots of three cultivars of the highbush blueberry (*Vaccinium corymbosum*) have greater rooting if taken from the basal portions of the shoot rather than from terminal portions (211). Exceptions are found in rose (122) and other species. The number of preformed root initials in woody stems (in some species at least) distinctly decreases from the base to the tip of the shoot (116). Consequently, the rooting capacity of basal portions of such shoots would be considerably higher than that of the apical parts. This factor is of little importance, however, in cuttings of easily rooted species, which root readily regardless of the position of the cutting on the shoot.

Flowering or Vegetative Wood. With most plants, cuttings can be made from shoots that are in either a flowering or a vegetative condition. Again, with easily rooted species it makes little difference which is used, but in difficult-to-root species the state of the plant can be an important factor. For example, in blueberry (*Vaccinium atrococcum*), hardwood cuttings from shoots bearing flower buds do not root as well as cuttings with only vegetative buds. Herbaceous dahlia cuttings bearing flower buds are more difficult to root than cuttings having only vegetative buds (19).

Flowering is a complex phenomenon and can serve as a **competing sink** to the detriment of rooting. Removal of flower buds increased rooting in rhododendron by eliminating the strong competing sink of flower buds for metabolites necessary for rooting (158). With many ornamental species (e.g., *Abelia, Ligustrum, Ilex,* etc.) it is commercially desirable to remove flower buds from cuttings for more rapid root development, earlier vegetative growth, and more efficient liner production (164).

BOX 9.18 **GETTING MORE IN DEPTH ON THE SUBJECT**

SEASONAL TIMING AND TYPE OF CUTTING WOOD

In propagating **deciduous** species, *hardwood cuttings* can be taken during the dormant season (from leaf fall, when buds are dormant, and before buds start to force out in the spring). Leafy *softwood* or *semihardwood cuttings* could be prepared during the growing season, using succulent and partially matured wood, respectively. The narrow- and broad-leaved evergreen species have one or more flushes of growth during the year, and cuttings can be obtained year-round in relation to these flushes of growth.

Seasonal Timing Seasonal timing, or the period of the year in which cuttings are taken, can play an important role in rooting (51). With many species there is an optimal period of the year for rooting (3). Propagators strive to *maintain the plants momentum* by rooting during these optimal periods to maximize the rooting process and speed up the production of liners. Climate permitting, it is possible to make cuttings of easy-to-root species throughout the year.

Certain species, such as privet, can be rooted readily if cuttings are taken almost any time during the year; on the other hand, excellent rooting of leafy olive cuttings under mist can be obtained during late spring and summer, whereas rooting drops almost to zero with similar cuttings taken in midwinter. Seasonal changes influenced rooting of both juvenile and mature (difficult-to-root) creeping fig (*Ficus pumila*) cuttings; however, treating juvenile (easy-to-root) cuttings with IBA overcame the seasonal fluctuation in rooting (Fig. 9–30). Shoot RNA was found to be an index of bud activity and subsequent seasonal rooting differences (Fig. 9–31). Highest shoot RNA levels and increased vascular cambial activity occurred during peak rooting periods in both the easy-to-root and difficult-to-root forms (51). As previously discussed, micorray analysis of gene expression is being used to better understand rooting events (39). RNA is harvested at distinct developmental periods of rooting and then hybridized to microarrays (Fig. 9–23).

Softwood cuttings of many deciduous woody species [e.g., cherries, lilac (*Syringa*)] taken during spring or summer usually root more readily than hardwood cuttings procured in the winter. The Chinese fringe tree (*Chionanthus retusus*) is notoriously difficult to root, but by taking cuttings during a short period in midspring, high rooting percentages can be obtained.

The effect of timing is also strikingly shown by difficult-to-root deciduous azalea cuttings. These root readily if the cuttings are taken from succulent growth in early spring; by late spring, however, the rooting percentages decline rapidly. For any given species, small experiments are required to determine the optimum time to take cuttings, which is more related to the physiological condition of the plant than to any given calendar date.

Often the effects of timing are merely a reflection of the response of the cuttings to environmental conditions at different times of the year. When hardwood cuttings of deciduous species are taken and planted in the nursery in early spring, after the rest period of the buds has been broken by winter chilling, the results are quite often a complete failure, since the buds quickly

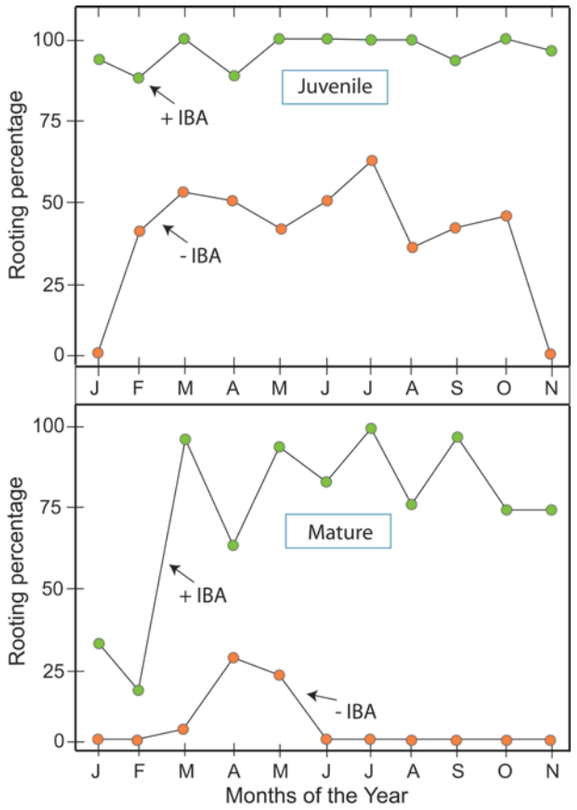


Figure 9–30
Seasonal fluctuation in percent rooting of juvenile (easy-to-root) and mature (difficult-to-root) Ficus pumila cuttings. Mature control (–IBA) plants root only from March to May, while juvenile control roots poorly from November to January. When treated with IBA (+IBA), the juvenile cuttings overcome the seasonal fluctuation in rooting, whereas mature cuttings still show poor rooting in January and February (51).

open with the onset of warm days. The newly developing leaves will start transpiring and remove the moisture from the cuttings before they have the opportunity to form roots, and they soon die. Newly expanding buds and shoots are also competing sinks for metabolites and phytohormones, to the detriment of rooting. This competition has been shown with rose (*Rosa multiflora*) under an intermittent mist system where water stress was not a factor (122). If cuttings can be taken and planted in the fall while the buds are still in the rest period, roots may form and be well established by the time the buds open in the spring.

Broad-leaved evergreens usually root most readily if the cuttings are taken after a flush of growth has been completed and the wood is partially hardened-off or lignifed. This occurs, depending upon the species, from spring to late fall. In rooting cuttings of *narrow-leaved evergreens,* best results may be expected if the cuttings

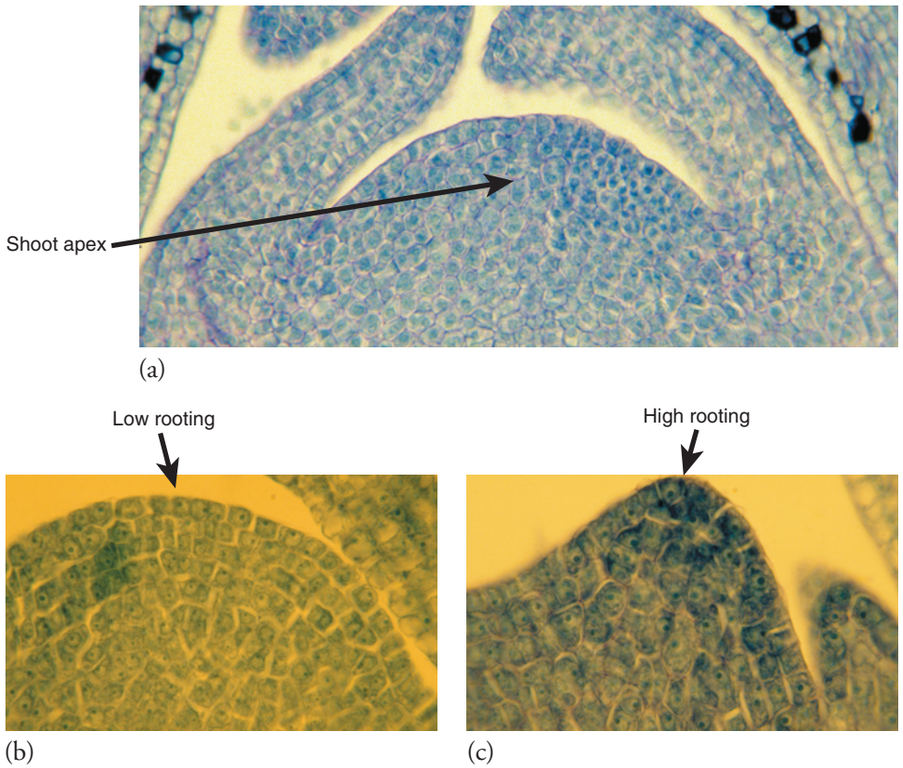


Figure 9–31
Shoot RNA and seasonal effects. (a) Shoot apex of Ficus pumila during (b) low seasonal rooting and (c) high rooting, with greater shoot RNA staining during the latter (51).

are taken during the period from late fall to late winter (171). With junipers and yew (*Taxus*), rooting was lowest during the season of active vegetative growth and highest during the dormant period. Furthermore, the low temperatures occurring at the time when such coniferous evergreens root best apparently is not a requirement, since juniper stock plants held in a warm greenhouse from early fall to midwinter produced cuttings that rooted better than outdoor-grown stock plants exposed to seasonal conditions (172).

In many containerized ornamental nurseries, cuttings from difficult-to-root species are taken early in the propagation season, whereas cuttings of easy-to-root species are taken later in the summer. This seasonal scheduling of propagation also more efficiently utilizes propagation facilities and personnel.

Predictive Indices of Rooting Predictive indices of rooting could facilitate clonal selection for rooting traits and reduce rooting variability within a clone. Rooting can be enhanced through well-directed research for predicting and improving propagation potential, and developing more efficient propagation systems, that is, stock plant selection and manipulation, environmental manipulation of cuttings, etc. (304).

Methods to Document the Most Advantageous Time to Collect Cuttings Various predictors of optimum rooting have been used, including calendar days, days from bud-break, use of plants as phenological indicators (plant growth characteristics), number of hours of sunlight, degree-day chilling units of *Juniper* stock plants (189), and the morphological condition of the

BOX 9.19 **GETTING MORE IN DEPTH ON THE SUBJECT**

ELECTRICAL IMPEDANCE AND SEASONAL ROOTING

Seasonal changes in electrical impedance of shoots and leaves of olive (*Olea europaea*) cuttings is related to rooting ability (190). Impedance measurements reveal information about extra- and intracellular fluids and the condition of cell membranes. They have also been used to estimate general plant health, nutrient status, and tissue stress damage. Seasonal rooting ability has been correlated to intracellar and extracellular resistances of shoots and leaves of olive cuttings.

BOX 9.20 **GETTING MORE IN DEPTH ON THE SUBJECT**

PREDICTIVE INDICES OF ROOTING

Even with relatively easy-to-root clones, rooting ability can vary unpredictably—after 51 harvests of cuttings from 1 clone of eucalyptus (*Eucalyptus globulus*) rooting varied from 14 percent to 100 percent, largely due to environmental variation (304). There are excellent opportunities for developing indices based on *stock plant morphology,* which are more practical if they are sufficiently accurate and easy to measure. For instance, with tropical pine cuttings, shoot attributes of stock plants, including primary needle length, are highly correlated to rooting of cuttings (114). Selection of *Eucalyptus* clones and families has been used to minimize variation in propagation potential, including correlating rooting ability with growth rates of stock plants, leaf thickness, and speculatively, with the frequency of stem sclerenchyma or rays. With *Eucalyptus globulus,* rooting was positively correlated to preharvest extension rates (cm growth per shoot per week) and cutting productivity of stock plants (weekly harvest number per plant) (304).

stock plant. In a novel approach, a degree-day (heat-unit) system was utilized to predict successful rooting in difficult-to-root adult Chinese pistache (*Pistacia*) (73). Maximum rooting occurred when cuttings were collected from stock plants with green softwood stems, which had 380-degree days (using a threshold temperature of 7.2°C/45°F) after bud-break.

TREATMENT OF CUTTINGS

Only high-quality cuttings should be collected for propagation. As the wise instructions to employees in a commercial propagation department go—***"A cutting that is barely good enough is never good enough, so don't put it in the bunch!"*** Quality control of cuttings begins with stock plant quality control. Propagation is the foundation on which production horticulture hinges. Marginal quality propagules delay product turnover and create cultural and quality problems throughout the production cycle (7).

Storage of Cutting Material

Propagators prefer to collect propagules from stock plants early in the day when cuttings are still turgid. If the cuttings cannot be stuck immediately, they are misted to reduce transpiration and held overnight in refrigeration facilities (see Fig. 10–55) at 4 to 8°C (40 to 48°F) and generally stuck the next day.

Cuttings of some temperate-zone woody species have been stored at low temperatures for extended periods without any deleterious effects on subsequent root formation and leaf retention. Storage of rhododendron (*Rhododendron catawbiense*) cuttings in moist burlap bags at either 21 or 2°C (70 or 36°F) for 21 days did not reduce rooting (62), although carbohydrate concentrations in the bases of cuttings changed with time and storage temperature. However, cuttings of Foster's holly root poorly even after the shortest cold storage.

Cuttings of many tropical foliage, greenhouse, and nursery crops are imported from Central and South America, the Middle East and other international locations to be rooted and finished in the United States or Europe. It can take 3 to 10 days to deliver cuttings from Central America to U.S. nurseries. Duration in transit can affect cutting quality due to excess respiration, light exclusion, moisture loss, pathogen invasion, and/or ethylene buildup. Croton (*Codiaeum variegatum*) cuttings had excellent quality when stored 5 to 10 days at 15 to 30°C (59 to 86°F) or 15 days at 15 to 20°C (59 to 68°F) (288).

Unrooted cuttings (URCs) of chrysanthemum, poinsettia, and carnation are routinely shipped by air transportation. See Figure 10–11, page 357 which illustrates URCs being precolled to lower temperatures prior to shipping from a vegetative cutting facility in Central America to the United States. Storage life of URCs of geranium (*Pelargonium xhortorum* Bailey) was improved by high-humidity storage in polyethylene bags at 4°C (39°F) and low-irradiance illumination. Prestorage application of antitranspirants was detrimental, but soaking cutting bases in 2 to 5 percent sucrose for 24 hours prior to storage improved rooting (216). The ethylene inhibitor, silver nitrate, was more effective in maintaining storage life than silver thiosulfate, which reduced rooting (216). Abscisic acid will reduce transpiration in geranium cuttings, which may be of practical value in the shipment and storage of geranium cuttings (5).

In general, successful storage of unrooted cuttings depends on storage conditions, state of the cuttings, and species. It is important that dry matter losses and pathogens be minimized. Within the storage unit, it is best to maintain nearly 100 percent humidity, and the temperature should be as low as the hardiness of the

given species can tolerate (16). Reduced oxygen and ethylene levels and high CO_2 [controlled atmospheric storage (CA)] help to maintain rooting capacity (16). Storage duration can vary from days to several months, depending on cutting carbohydrate reserves, cold hardiness, and degree of lignification (woodiness of the material) (see the discussion in Chapter 10).

Auxins

Before the use of root-promoting growth regulators (auxins) in rooting stem cuttings, many chemicals were tried with limited success (165). The discovery that auxins, such as ***indoleacetic acid (IAA), indole-3-butyric acid (IBA)***, and ***α-naphthalene acetic acid (NAA),*** stimulated the production of adventitious roots in cuttings was a milestone in propagation history (29, 30, 312). The response, however, is not universal. As discussed earlier, cuttings of some difficult-to-root species still root poorly after treatment with auxin, so auxin is not always the limiting chemical component in rooting, as discussed earlier in this chapter.

An ancient practice of some Middle Eastern and European gardeners in early days was to embed grain seeds into the split ends of cuttings to promote rooting. This seemingly odd procedure had a sound physiological basis, for it is now known that germinating seeds are good sources of auxin, which aids root formation in cuttings.

Mixtures of IBA and NAA Mixtures of root-promoting substances are sometimes more effective than either component alone. For example, equal parts of indole-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA), when used on a number of widely diverse species, were found to induce a higher percentage of cuttings to root and more roots per cutting than either auxin alone (64). Species are also known to react differently when treated with equal amounts of NAA or IBA; NAA was more effective than IBA in stimulating rooting of Douglas-fir (225).

Adding a small percentage of certain phenoxy compounds to either IBA or NAA increased rooting and produced root systems better than those obtained with phenoxy compounds alone (64, 143). Amino acid conjugates of IAA sometimes stimulate better rooting than IAA alone. It has been suggested that the activity of IAA in rooting may depend on its covalent bonding to low molecular weight phenolic compounds (i.e., chemical linkage with sugars, sugar alcohols, etc.) (64, 117).

The acid form of auxin is relatively insoluble in water but can be dissolved in a few drops of alcohol or ammonium hydroxide before adding to water. Salts of some auxins may be more desirable than the acid form in some instances because of their comparable activity and greater solubility in water (313). Also, solvents used to dissolve the acid formulations at higher concentrations—alcohol, NaOH, and others—may be toxic to cuttings.

The aryl esters of both IAA and IBA, and amides of IBA [Phenyl-IAA **(P-IAA)**, Phenyl-IBA **(P-IBA),** phenyl thioester **(P-ITB),** and phenyl amide **(NP-IBA)** (see Fig. 2–25)], have been reported to be more effective than the acid forms in promoting root initiation (64, 118, 268). The physiologically active phenyl-modified auxins are probably enzymatically hydrolyzed after cellular uptake, yielding the free parent acid (i.e., IAA or IBA) and phenolic moiety or portion (64). Again, this is species-dependent. It may also be that these formulations are less toxic to plant material than the acid form.

Auxins are commercially applied as a 1- to 5-second basal, quick-dip, or talc application (30). However foliar sprays of auxin on cuttings are gaining in popularity to reduce worker exposure and the amount of auxin used in the propagation industry. The auxin IBA has an LD_{50} (lethal dosage) of 200 and is considered a pesticide, so there are concerns about worker safety and future restrictions. While foliar sprays of auxin may inhibit shoot growth, for most species there has been good rooting success (29, 30). There are advantages of using water soluble IBA salt formulations as foliar sprays, for example, Hortus IBA http://www.rooting-hormones.com/IBAsalts.htm (71).

For general use in rooting stem cuttings of the majority of plant species, IBA and/or NAA are recommended (64). To determine the best auxin and optimum concentration for rooting any particular species under a given set of conditions, small trials are necessary and should be repeated over several occasions, since repeated experiments can give conflicting results. Also, see Chapters 19, 20, and 21 for specific species recommendations.

Auxin Suppression of Bud-Break of Cuttings Application of auxins to stem cuttings at high concentrations can inhibit bud development, sometimes to the point at which no shoot growth will take place even though root formation has been adequate. Application of auxins to root cuttings may also inhibit the initiation and development of shoots from such root pieces. Basally applied IBA increased rooting but inhibited bud-break of single-node rose stem cuttings. IBA was translocated to the upper part of the cutting, where it inhibited bud-break and increased ethylene synthesis of the cuttings (272).

Early bud-break and shoot growth of newly rooted cuttings are important in the overwinter survival

of *Acer, Cornus, Hamamelis, Magnolia, Prunus,* and *Rhododendron* (305). These species need to put on a growth flush (after rooting but prior to winter dormancy) so that sufficient levels of carbohydrates are stored in the root system to ensure winter survival. Hence, there is concern about auxin-suppressing budbreak and growth of rooted cuttings—and reduced winter survival. See Chapter 10 for discussion on postpropagation care of rooted cuttings.

Shelf-Life of Auxins There is often a question of how long the various root-promoting preparations will keep without losing their activity. Bacterial destruction of IAA occurs readily in unsterilized solutions. A widely distributed species of *Acetobacter* destroys IAA, but the same organism has no effect on IBA. Uncontaminated solutions of NAA and 2,4-D maintained their strength for as long as a year. Of course, alcohol solutions of auxin will depress microbial activity.

IAA is sensitive to light and is readily inactivated. Concentrated IBA solutions in 50 percent isopropyl alcohol are quite stable and can be stored up to 6 months at room temperature in clear glass bottles under low-light conditions without loss in activity (237). Both NAA and 2,4-D seem to be light-stable. Indoleacetic acid oxidase in plant tissue will break down IAA but has no apparent effect on IBA or NAA.

Movement of Auxins in Cuttings In stem tissue, auxin generally moves in a basipetal direction (apex to base). The naturally occurring auxins, IAA and IBA, and the synthetic auxin, NAA, are translocated via polar transport, while the synthetic auxin, 2,4-D, has little polar transport (29). Synthetic auxins were originally applied to cuttings at the apical end to conform to the natural downward flow. As a practical matter, it was soon found that basal applications gave better results. Sufficient movement carried the applied auxin into parts of the cutting where it stimulated root production. In tests using radioactive IAA for rooting leafy plum cuttings, IAA was absorbed and distributed throughout leafy cuttings in 24 hours, whether application was at the apex or base (270). However, with basal application, *most* of the radioactivity remained in the basal portion of the cuttings. Leafless cuttings absorbed the same amount of IAA as leafy cuttings, indicating that transpiration "pull" was not the chief cause of absorption and translocation.

In a study comparing auxin uptake of radioactive NAA in cuttings treated by dilute soak, quick-dip, or talc methods, auxin movement occurred in the vascular system of the stem with the talc and aqueous auxin applications (100). In contrast, auxin in a 50 percent alcohol solution entered the stem from the cut surface and the epidermis throughout the area of the stem quick-dipped in solution. Hence, the solvent used for the quick-dip application facilitated auxin movement through the epidermis and the cut surface of the stem.

Mineral Nutrition of Cuttings During Rooting

Optimal nutrition is needed for adventitious rooting and to assure that root development and production of rooted liner plants precedes smoothly. While it is important to maintain stock plants under optimum nutrition prior to the collection of cuttings, it is difficult to quantify the effect of nutrition on root primordia initiation versus root primordia elongation (25, 273). Mobilization studies have been conducted to examine the movement of mineral ions into the base of cuttings during root initiation. The redistribution of nitrogen in stem cuttings during rooting was accelerated by auxin treatment of plum (271). However, N was not mobilized, nor was any redistribution of P, K, Ca, and Mg detected during root initiation in stem cuttings of Japanese holly (23, 24).

There are conflicting reports on mobilization. During root initiation in chrysanthemum cuttings, P, but not N, K, or Ca, was mobilized. Although considered immobile, redistribution of Ca was reported during rooting of Japanese holly. Apparently, Ca was redistributed to support tissue development in the upper cutting sections and not for root growth and development.

The importance of N in root initiation is supported by nutrition studies on rooting of cuttings and the importance of N in nucleic acid and protein synthesis (25). The influence of N on root initiation and development also relates to such factors as carbohydrate availability, C/N ratio, and hormonal interactions.

Zinc can promote the formation of the auxin precursor, tryptophan, and the subsequent formation of auxin (IAA) from tryptophan. Conversely, Mn acts as an activator of the IAA-oxidase enzyme system and B may enhance IAA-oxidase activity, thus regulating endogenous auxin levels (Fig. 9–19) (157, 290). Higher endogenous auxin levels are required for early root initiation than for later root development (see Fig. 9–19 and discussion). If root initiation is related to the relative activity of IAA and IAA-oxidase, then rooting may be correlated with changes in relative Zn, Mn, and B concentration at the site of root initiation during the developmental stages of *de novo* rooting.

In a study with poinsettia where mineral element concentration was analyzed during the developmental

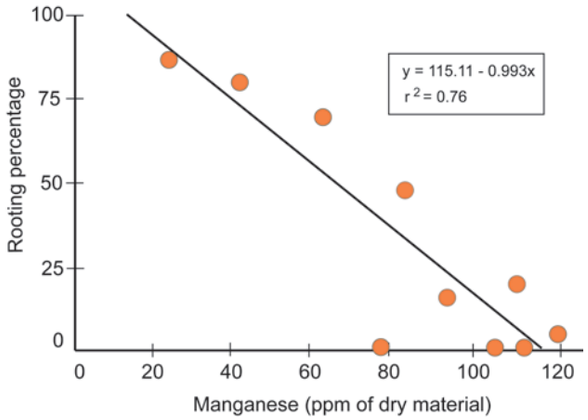


Figure 9–32
Correlation between manganese content of leaves of different avocado clones and rooting percentage. From data of Reuveni and Raviv (234).

stages of rooting, Fe, Cu, and Mo increased in the basal portion of stem cuttings during early root initiation, while P, K, Ca, and Mg decreased (273). During root primordia elongation and root emergence, Fe, Cu, Mo, Mg, Mn, B, and Zn concentration continued to increase at the cutting bases, but P and K concentrations remained low compared to when cuttings were initially inserted into the propagation medium.

High levels of Mn were found (234) in leaves of cuttings taken from difficult-to-root avocado cultivars, whereas cuttings from easy-to-root cultivars had a much lower manganese level (Fig. 9–32). The negative correlation with rooting may be linked to manganese's activating the IAA-oxidase system and lowering endogenous IAA levels (157).

Leaching of Nutrients

The development of intermittent mist revolutionized propagation, but mist can severely leach cuttings of nutrients. This is a particular problem with cuttings of difficult-to-root species, which take a longer time to root under mist. Mineral nutrients such as N, P, K, Ca, and Mg are leached from cuttings while under mist (24).

Nitrogen and Mn are easily leached; Ca, Mg, S, and K are moderately leached; and Fe, Zn, P, and Cl are leached with difficulty (278). Both leaching and mineral nutrient mobilization contribute to foliar deficiencies of cuttings (25). The amount of leaching depends on the growth stage of the cutting material: leafy hardwood cuttings are reported to be more susceptible than softwood or herbaceous cuttings. Apparently, young, growing tissues more quickly tie up nutrients by using them in the synthesis of cell walls and other cell components. Greater leaching occurs with leafy hardwood cuttings, since a greater portion of nutrients is in exchangeable forms.

High leaching rates are avoided by reducing the misting frequency and using mist nozzles that supply smaller volumes of water (247). Foliar nutrition of poinsettia cuttings was significantly reduced during the first week on the mist bench (273, 298)

As a whole, mist application of nutrients has not been a viable technique to maintain cutting nutrition. Nutrient mist application can inhibit rooting (163) and stimulate algae growth, which causes sanitation and media aeration problems (308).

A commercial technique is to apply moderate levels of controlled slow-release macro- and microelements to the propagation media either preincorporated into the media prior to sticking cuttings or by top-dressing (broadcast) during propagation. These supplementary nutrients do not promote root initiation (160) but rather improve root development after root primordia initiation has occurred. Hence, turnover of rooted cuttings occurs more quickly and plant growth is maintained by producing rooted liners that are more nutritionally fit. Optimum levels of fertilization for rooting need to be determined on a species-specific basis (see Chapter 10).

Wounding

Cuttings are naturally wounded when excised from stock plants. Additional basal wounding is beneficial in rooting cuttings of certain species, such as

BOX 9.21 **GETTING MORE IN DEPTH ON THE SUBJECT**

WOUNDING-RELATED COMPOUNDS (WRCS)

- Wounding of cuttings results in destruction of cell compartments (vacuoles, vesicles, per- oxisomes, plastids), which leads to synthesis and/or release of catabolic enzymes (glucanases, peroxidases, phospholipases, lipoxygenases) present in cell organelles.
- Breakdown products of these cell structures are called wounding-related compounds (WRCs).
- WRCs play an important role in rooting and enhance rooting when applied with low auxin concentration (67).

rhododendrons and junipers, especially cuttings with older wood at the base. Following wounding, callus production and root development frequently are heavier along the margins of the wound. Wounded tissues are stimulated into cell division and production of root primordia (Figs. 9–33 and 9–34) (187), due to a natural accumulation of auxins and carbohydrates in the wounded area and to an increase in the respiration rate in the creation of a new "sink area." In addition, injured tissues from wounding produce ethylene, which can indirectly promote adventitious root formation (67, 68, 206, 311). See the schematic on the physiological and biochemical events in severing a cutting for rooting (Fig. 9–21).

It has been proposed that wounding a cutting initiates a chemical signal that induces changes in the metabolism of affected cells (300). A listing of metabolic responses to wounding is given in Table 9–6. Potentially, cells at the base of the cutting influenced by wounding have enhanced receptivity to respond to auxin and other morphogens (nonauxin endogenous compounds) essential to rooting (Fig. 9–21) (300, 301).

Wounding cuttings may also permit greater absorption of applied growth regulators by the tissues at the base of the cuttings. In stem tissue of some species, there is a sclerenchymatic ring of tough fiber cells in the cortex external to the point of origin of adventitious roots. There is evidence in a few species (15) that newly formed roots may have difficulty

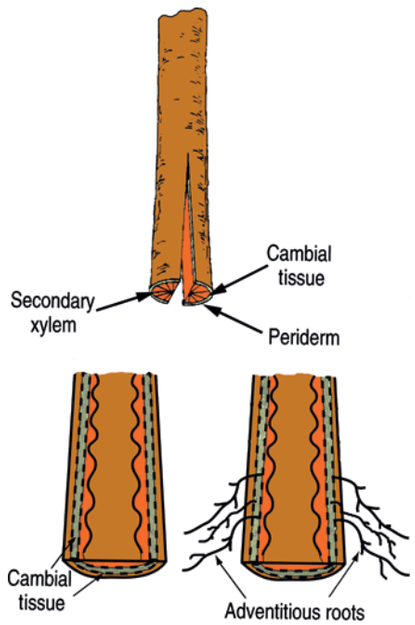
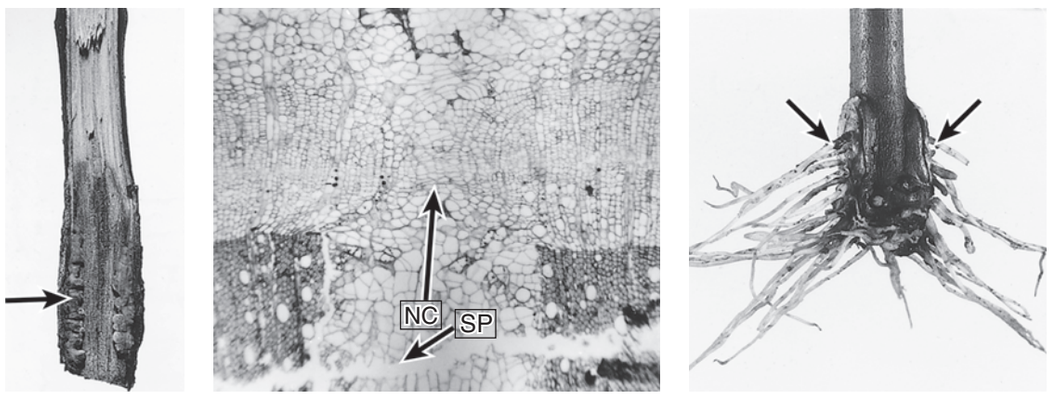


Figure 9–33
(a) Split-base treatment to enhance root initiation in leafless, dormant apple rootstock cuttings. (b) The inner surface of a hardwood cutting wounded by splitting. When split longitudinally, much more cambium is exposed than with a normal cut across the stem base; cambial cells are able to regenerate in response to auxin treatment and produce cambial callus. (c) Roots emerging from cambial callus. Redrawn from MacKenzie et al. (187) and Howard, Horticulture Research International, East Malling, England (150).



(a) (b) (c)

Figure 9–34
(a) Split-base treatment. One-half of the split-stem base has been removed to show the nodular callus (arrow) in the split. (b) Split base—transverse section near the apex of the wound. The split (Sp) here is narrow and, consequently, the new cambium (NC) (tip of arrow) reforms across the split instead of forming callus protrusion and roots as in split base. (c) Note roots emerging in a double rank from the same side of the wound (187).

Table 9–6
SOME PLANT METABOLIC RESPONSES TO WOUNDING

Increase in ascorbic acid	Increase in phenolics
Increase in fatty acids	Evolution of ethylene
Increase in lipids	Increase in terpenoids
Systemic chemical signal	Systemic electrical signal
New membrane synthesis	Peroxidation of membranes
Weakened cell membranes	Induction of cyanide-insensitive pathway
Ion influx into cells	Increased capacity for protein synthesis

Source: Wilson and van Staden (300).

penetrating this band of cells. In those species, a shallow wound would cut through these cells and enhance the emergence of the developing roots.

ENVIRONMENTAL MANIPULATION OF CUTTINGS

Water Relations—Humidity Control

The loss of water from leaves may reduce the water content of the cuttings to such a low level that they do not survive. Propagation systems are designed to maintain:

- **An atmosphere** with low evaporative demand, minimizing transpirational water losses from cuttings and, thereby, avoiding substantial tissue water deficits (cuttings without roots lack effective organs to replace transpired water lost), and cells must maintain adequate *turgor* for the initiation and development of roots (55);
- **Acceptable temperatures** for the regeneration processes occurring at the cutting base, while avoiding the heat stress of leaves; and
- **Light levels** suitable for photosynthesis and carbohydrate production for the maintenance of the cuttings and for use, once root initiation has occurred, without causing water stress (181). See Chapter 3 for the discussion on environmental management and the water relations of propagules.

The water status of cuttings is a balance between transpirational losses and uptake of water. Water absorption through the leaves is *not* the major contributor to water balance in most species. Rather, the cutting base and any foliage immersed in the propagation media are main entry points for water (182). Relative water content is lowest during the first days of sticking poinsettia cuttings and increases with primordia development and root elongation (274, 298). Water uptake of cuttings is directly proportional to volumetric water content of the propagation media, with wetter media improving water uptake (Fig. 9–35) (103, 232). However, excess water reduces media aeration (86) and can lead to anaerobic conditions and the death of cuttings.

Water uptake in cuttings declines after they are initially inserted into propagation media. This decline

Figure 9–35
Water uptake by cuttings is directly proportional to the volumetric water content of the rooting medium. Here, softwood cuttings of Escallonia xexoniensis are inserted in a peat-pumice mix containing 15, 20, 40, and 60 percent by volume of water, and in water (left to right). The degree of wilting relates to the water content. While the cutting in 100 percent water is turgid, most species will not tolerate such an anaerobic environment. Courtesy K. Loach.

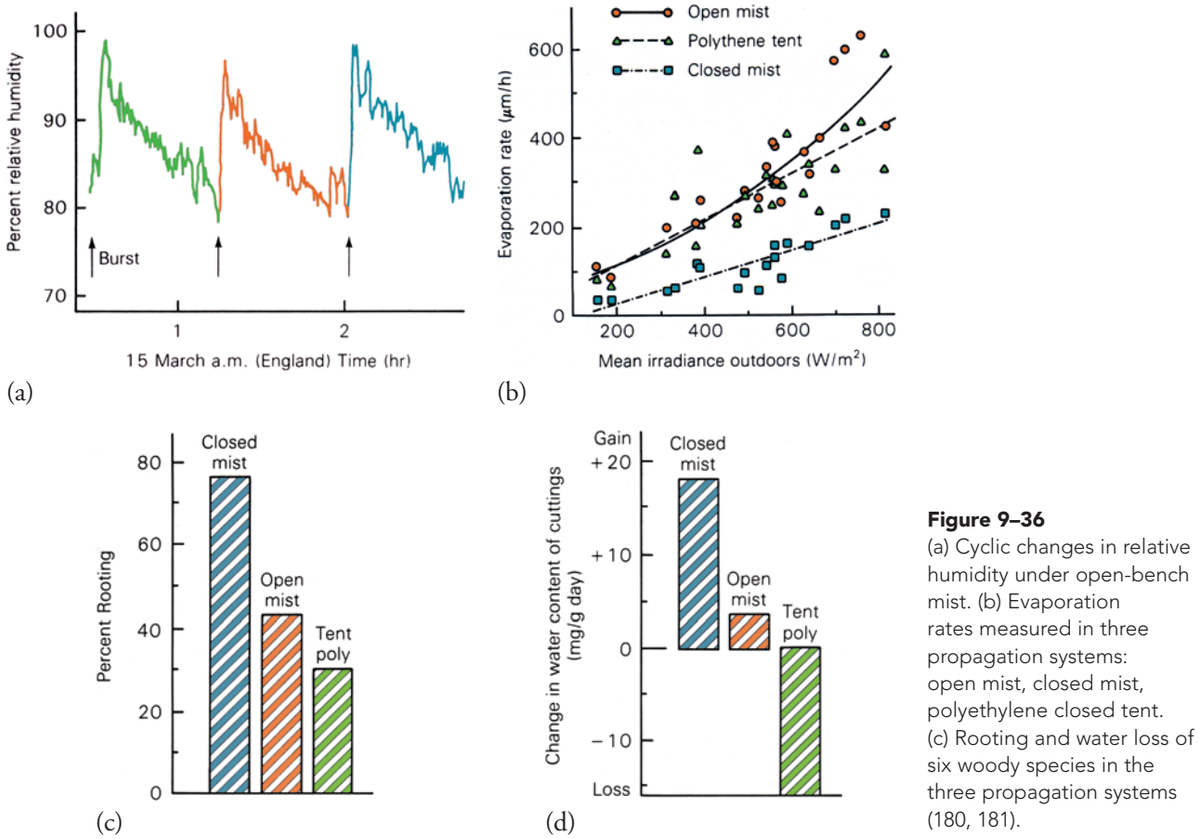


Figure 9–36
(a) Cyclic changes in relative humidity under open-bench mist. (b) Evaporation rates measured in three propagation systems: open mist, closed mist, polyethylene closed tent. (c) Rooting and water loss of six woody species in the three propagation systems (180, 181).

in hydraulic conductivity of cuttings is apparently caused by blockage of xylem vessels and/or collapse of tracheids, which is similar to post-harvest problems observed with cut flowers (153). Another advantage in wounding cuttings is to increase the contact area between the cutting base and propagation medium, thus improving water uptake of cuttings (55, 103, 182). With the formation of functional adventitious roots, new vascular connections occur between the roots and stem. Thus, the hydraulic contact between the propagation medium water and the cutting is maintained.

Degree of stomatal opening can be a useful indicator to determine if a given propagation system is maintaining adequate turgor of cutting leaves. Simpler and more useful systems are to measure evaporation rates directly with evaporimeters (Fig. 9–36) (131, 179, 182) or measuring transpirational loses (298). When water deficits cause stomata to close, CO_2 diffusion into the leaf is restricted, limiting photosynthesis and any subsequent carbohydrate gain in the cuttings. Carbon gain due to photosynthesis is probably more important *after* root initiation has occurred to promote rapid development of roots. It has been reported that translocation of photosynthate from leaves of intact plants continues under moderate or severe stress.

Vapor Pressure Deficit (VPD) Water loss from cuttings is the difference between vapor pressure between the cutting leaf and surrounding air of the mist bed (Figs. 9–37 and 9–38). Water potential of unrooted loblolly pine cuttings has been correlated with VPD, mist application, and rooting percentage (173, 174). Ambient VPD (measurement of general propagation house area) is not dynamic enough to be used as a controlling mechanism. However, VPD determined at the stem-cutting level with temperature and relative humidity probes being misted along with the stem cuttings to provide real-time data of the cuttings is sufficiently sensitive as a dynamic controlling mechanism for misting. If VPD between the cutting and air is high, misting occurs more frequently, and misting is less frequent when there is low VPD (174). Cuttings can tolerate a certain amount of water stress, and moderate stress [-1.0 MPa (-10 bars)] enhances rooting of

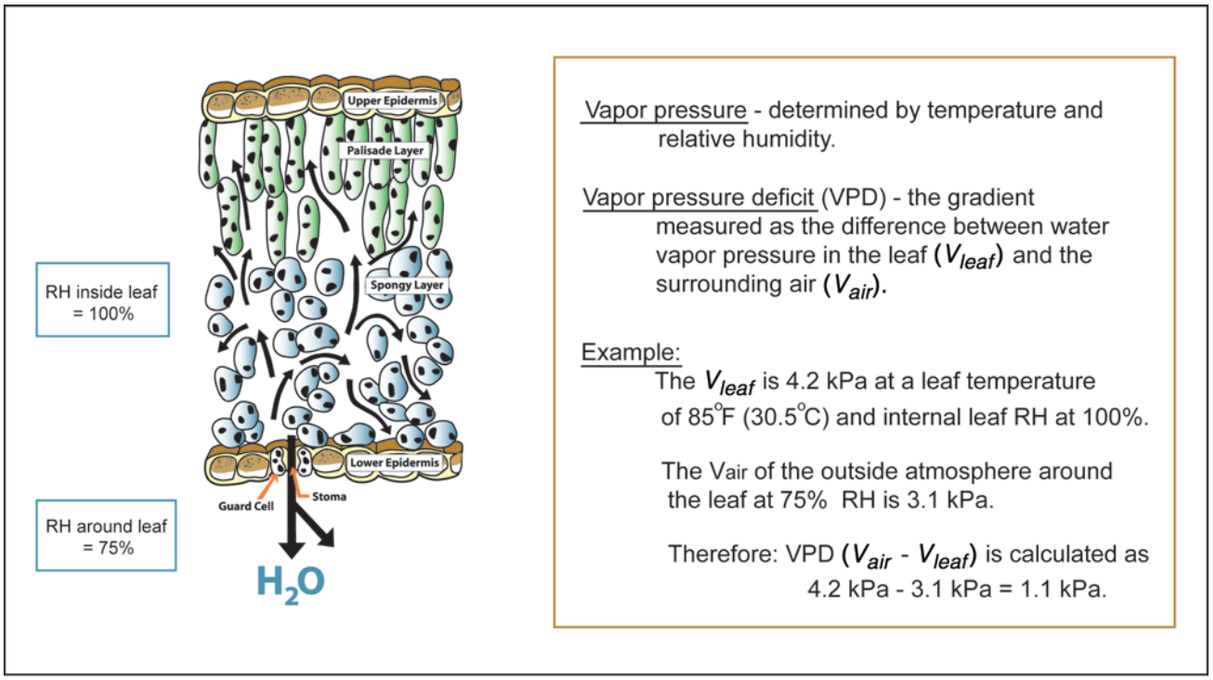


Figure 9–37
Controlling vapor pressure deficit (VPD) during cutting propagation. Leaf cross section with high 100 percent internal relative humidity (RH). Water vapor exits the leaf stomata into the lower RH (lower water potential) of the outside surrounding air.

loblolly pine cuttings (173). While leafy cuttings would not tolerate as low a stress, moderate stress is beneficial (178, 180, 182).

The driving force that determines the rate at which cuttings lose water is the difference in pressure between water vapor in the leaves (V_{leaf}) and that in the surrounding air (V_{air}). Commercial propagation systems aim to minimize this difference either by decreasing V_{leaf} through reducing leaf temperature (e.g., with intermittent mist) and/or by increasing the V_{air} by preventing the escape of water vapor (i.e., with an enclosed polytent). Enclosed systems use humidification since

(a) (b)

Figure 9–38
(a and b) Water loss from cuttings during mist propagation is the difference between the vapor pressure between the leaf and the surrounding environment. This is vapor pressure deficit (VPD). For vapor pressure deficit models, sensors (light, temperature, humidity) send data to a computer that calculates the VPD for the greenhouse environment. Crop models use VPD to estimate water loss from cuttings to initiate misting. Also see Figure 10–42, page 389.

only the V_{air} is increased. Intermittent mist affects primarily V_{leaf} but also provides a modest increase in V_{air}. Methods used to control water loss of leaves (181) are:

- **intermittent mist**—open and enclosed mist systems;
- **nonmisted enclosures**—outdoor propagation under low tunnels or cold frames, or nonmisted enclosures in a glasshouse or polyhouse (shading, tent, and contact polyethylene systems, wet tents); and
- **fogging systems.**

Intermittent Mist. **Intermittent mist** has been used in propagation since the 1940s and 1950s (178). Mist systems minimize V_{leaf}, which lowers the leaf-to-air vapor pressure gradient and slows down leaf transpiration. Mist also lowers ambient air temperature, and the cooler air consequently lowers leaf temperature by **advection,** in addition to cooling occurring through evaporation of the applied film of water (181). Advective cooling occurs only minimally in enclosed nonmist systems. Since intermittent mist lowers medium temperature, suboptimal temperatures can occur, which reduce rooting. A common commercial technique to control the rooting medium temperature is to use bottom (basal) heat both with indoor and outdoor mist systems (see Figs. 3–9 and 10–37). **Enclosed mist** (see Fig. 3–18) utilizes polyethylene-covered structures in glasshouses that reduce the fluctuation in ambient humidity that is common to open-bench mist (Figs. 3–18 and 10–36). Enclosed mist also ensures more uniform wetting of foliage since air currents are reduced. There are advantages to using enclosed mist with difficult-to-root species, compared to **open mist** (Fig. 9–38) or a polytent system without mist (see Fig. 10–36, page 384).

intermittent mist The periodic application of small amounts of water or "mist" to the leaves and shoots of cuttings during propagation.

advection The horizontal movement of a mass of air that causes changes in temperature or in other physical properties of air (i.e., movement of cool air mass).

Major advantages of **enclosed systems** are their simplicity and low cost. The main disadvantage is that they trap heat if light irradiance is high. The trapped heat reduces the relative humidity of the air, and leaf temperature rises to increase the leaf-to-air vapor pressure gradient and, consequently, leaves lose water. Shading must be used with these systems. Polyethylene films have a low permeability to water vapor loss but allow gas exchange. They are used to cover outdoor propagation structures as well as for closed mist systems in greenhouses. Modified polyethylene films are now available with additives of vinyl acetate, aluminum, or magnesium silicates, which increase their opacity to long-wave radiation (i.e., reduce heat buildup). Polyethylene-covered structures have replaced many of the traditional glass-covered cold frames (see Fig. 3–17).

Nonmisted Enclosures. Nonmisted enclosures in a glasshouse or polyhouse can be used for difficult-to-root species and have the advantage of avoiding nutrient-leaching problems of intermittent mist, yet affording greater environmental control than outdoor propagation. The shading system entails applying shading compounds to greenhouse roofs and/or utilizing automatically operating light-regulated shading curtains (see Fig. 3–18). Shading systems are integrated with temperature control by ventilated fogging or pad-and-fan cooling and heating.

Contact Systems. Contact systems entail laying polyethylene, spun-bound polyester, or polypropylene sheets *directly onto cuttings* that are watered-in (see Fig. 10–36). When the irradiance and air temperature can be controlled, leaves tend to be cooler under contact polyethylene because they are in direct contact with the polyethylene and are moistened by condensation forming under the cover. Thus, there is the dual benefit that some evaporative cooling can occur and that water loss from the foliage is reduced since the condensation contributes to the relative humidity of the air rather than solely internal water from the leaf tissue. Hence, there is less internal water stress than with drier leaves in an **indoor polytent system.** Well-managed, enclosed nonmist systems offer a low-tech, cost-effective alternative to mist and fog systems, and may be superior to mist when irradiance and temperature levels are relatively low (207).

Fog Systems. **Fog systems** maximize V_{air} by raising the ambient humidity. Fog generators produce very fine water droplets that average 15 µm in diameter and remain suspended in the air for long periods to maximize evaporation (see Figs. 10–44 and 10–45). Their surface/volume ratio is high (compared with larger water droplets produced from a deflector-type mist nozzle) so that the finely divided mist particle has a larger surface, which also increases evaporation (181). With fog, water passes into the air as a vapor rather than condensing and

fog systems Similar to intermittent mist, except the particle size of the water applied is much finer and water does not condense on the surfaces of the cutting.

wetting leaf surfaces, as mist does. Thus, fog systems avoid foliar leaching and oversaturation of media. Since the propagation media are not saturated and cooled to the same degree as with mist, suboptimal rooting temperatures are avoided and less basal heat is needed. A disadvantage of the fog system is the higher initial cost and maintenance.

There are advantages to using fog over either open or enclosed mist systems, particularly with difficult-to-root plants (129, 130) and with the acclimation and *ex vitro* rooting needs of tissue-culture-produced liners (see Chapter 18). Propagators must decide which is the most cost-effective system for their particular needs.

Temperature

Temperature of the propagation medium can be suboptimal for rooting due to the cooling effect of mist or seasonally related ambient air temperature. It is more satisfactory and cost-effective to manipulate temperature by heating at the propagation bench level rather than by heating the entire propagation house. See Chapters 3 and 10 for heating equipment systems.

The consensus regarding the optimum medium temperature for propagation is 18 to 25°C (65 to 77°F) for temperate-climate species and 7°C (13°F) higher for warm-climate species (75, 166). Daytime air temperatures of about 21 to 27°C (70 to 80°F) with nighttime temperatures about 15°C (60°F) are satisfactory for rooting cuttings of most temperate species, although some root better at lower temperatures. High air temperatures tend to promote bud elongation in advance of root initiation and to increase water loss from the leaves. It is important that adequate moisture status be maintained by the propagation system so that cuttings gain the potential benefit of the higher basal temperature.

Root initiation in cuttings is temperature-driven, but subsequent root growth is strongly dependent on available carbohydrates. This is particularly evident in leafless hardwood cuttings in which excessive root initiation and growth can so deplete stored reserves that there are insufficient available carbohydrates for satisfactory bud growth. The same principle holds true for leafy cuttings (semihardwood, softwood, herbaceous), where shoot growth can divert carbohydrates away from developing root initials and thereby slow root growth (Fig. 9–39).

The optimum air temperature for growing a crop is probably the best for rooting cuttings (220). Bottom heat should be manipulated in two phases, with a higher beginning temperature for **root initiation** and a lower temperature for **root development** and growth (75, 166). Optimum temperature for root initiation in *Forsythia* and *Chrysanthemum* was 30°C (86°F), whereas root development (elongation of primordia and protruding of roots from the stem cutting) was optimum at lower temperatures of 22 to 25°C (72 to 77°F). Respiration is reduced at the lower temperature, which allows more optimum photosynthate accumulation for root development.

A system for predicting rooting stages in poinsettia cuttings was developed using root-zone temperature-based models (299). Optimum rooting for root initiation and elongation stages were 28°C (82°F) and 26°C (79°F), respectively. Rooting did not occur at 20°C (68°F) or less, and was reduced at 32°C (90°F) or more (Fig. 9–40, page 328). See Chapter 3 for the discussion on temperature in propagation.

Figure 9–39
Cuttings Forsythia of Xintermedia 'Lynwood' rooted under open-bench mist (left) and in a misted, polyethylene enclosure (right). Note that cuttings rooted in the warmer, more humid enclosure break bud and grow faster than those under open mist. However, for some species and circumstances, too much top growth can divert carbohydrates away from developing root initials and slow root growth. Courtesy K. Loach.

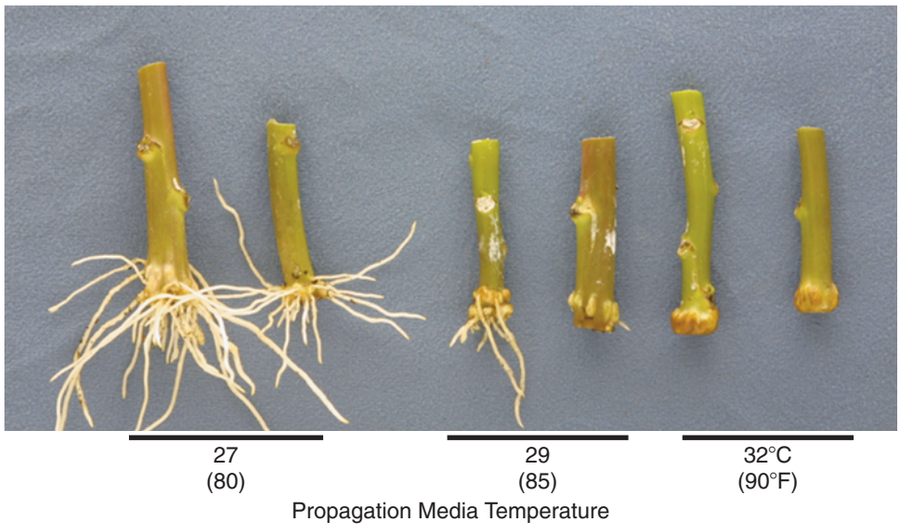


Figure 9–40
Effect of temperature on rooting poinsettia cuttings at 27, 29, and 32°C (80, 85, and 90°F). A temperature of 27°C was optimal. Root induction and initiation temperature is higher than during the later stages of root elongation (300).

Light

As discussed earlier in the stock plant manipulation section and in Chapter 3, light is a contributing factor in the adventitious root and bud formation of cuttings (70, 76).

Irradiance. Cuttings of some woody plant species root best under relatively low irradiance (159, 181). However, cuttings of certain herbaceous plants, such as chrysanthemum, geranium, and poinsettia, root better when the irradiance increased to 116 W/m^2 during trials in winter months. Very high irradiance (174 W/m^2) damaged leaves on the cuttings, delayed rooting, and reduced root growth. With selected temperate species under an English propagation system, acceptable light ranges were 20 to 100 W/m^2 (181). Propagators need to determine irradiance levels to fit their particular production systems. See Chapter 3, page 52 for an explanation of light units.

Most vegetative annuals used for greenhouse and nursery production root within 2 to 3 weeks. Managing light intensity is a key component for successful rooting (183, 184). When light levels are too high, cuttings experience stress and wilt, which delays rooting. When light levels are too low, root formation is delayed, increasing propagation time (Fig. 9–41). Desirable levels of light vary with the stage of root development (77).

Photoperiod. In some species, the photoperiod under which the cuttings are rooted may affect root initiation; long days or continuous illumination are generally more effective than short days (44), although in other species photoperiod has no influence (253).

The relationship of photoperiod and organogenesis is complex, since photoperiod can affect shoot development as well as root initiation. For example, in propagation by leaf cuttings, there must be development of adventitious buds and roots. Using *Begonia* leaf cuttings (135), where the light irradiance was adjusted so that the total light energy was about the same under both long days and short days, it was found that short days and relatively low temperatures promoted adventitious bud formation on the leaf pieces, whereas short days suppressed adventitious root formation. Roots formed best under long days with relatively high temperatures.

In rooting cuttings of 'Andorra' juniper, pronounced variations in rooting occurred during the year, but the same variations took place whether the cuttings were maintained under long days, short days, or natural daylength (171). A number of tests have been made of the effect of photoperiod on root formation in cuttings, but the results are conflicting; hence, it is often difficult to generalize (8, 76, 78, 171).

Herbaceous short-day flowering crops such as poinsettia and chrysanthemum are routinely rooted under long-day conditions to stimulate rooting and inhibit the competing sink of flowering. Once rooted, the plants are switched to short-days to encourage flowering.

In some plants, photoperiod will control growth after the cuttings have been rooted. Certain plants cease active shoot growth in response to natural decreases in daylength. This is the case with spring cuttings of deciduous azaleas and dwarf rhododendrons, which had rooted and were potted in late summer or early fall.

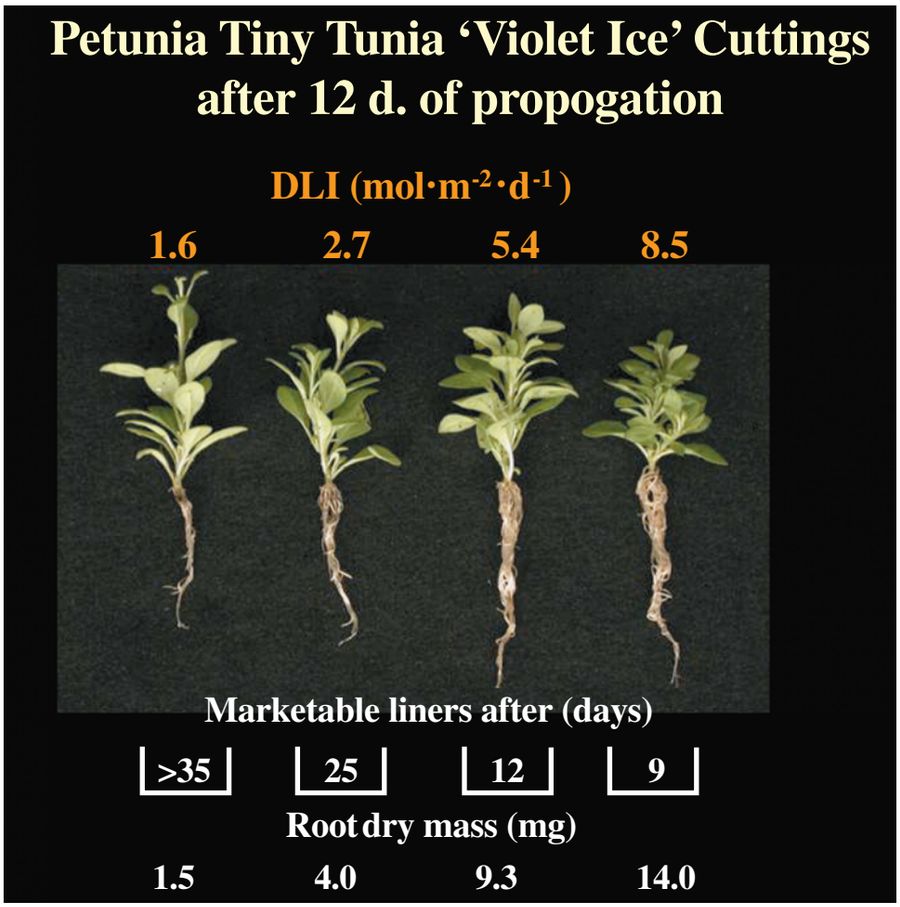


Figure 9–41
Effect of photosynthetic daily light interval (DLI) on producing marketable rooted liners of petunia. The higher the DLI, with sufficient environmental controls to minimize desiccation, the more rapidly rooted liners are produced (183, 184). Photo courtesy R. Lopez & E. Runkel.

Improved growth of such plants was obtained during the winter in the greenhouse if they were placed under continuous supplementary light, in comparison with similar plants subjected only to the normal short winter days. The latter plants, without added daylength, remained in a dormant state until the following spring.

High carbohydrate reserves are important for rooted cuttings (liners), since spring growth in a deciduous plant depends on reserves accumulated during the previous growing season. With red maple (*Acer rubrum*), a night interruption lighting period to extend the natural photoperiod in order to maintain high carbohydrate reserves enhanced growth of rooted liners; however, this was not economically justified, since growth of natural daylength liners was comparable after 2 years of field culture (252).

Light Quality. Lighting that provides more red than far-red light increases rooting in many greenhouse crops (196). It is conceivable with certain plant species that root initiation is regulated by red and far-red light through the phytochrome system. Radiation in the orange-red end of the spectrum seems to favor rooting of cuttings more than that in the blue region, but there are conflicting reports. Using light emitting diodes (LEDs) red light enhance and blue light inhibited *in vitro* rooting of *Tripterospermum* (203). Red shade cloth (e.g., ChromatiNet Red http://www.polysack.com/) that enhances the red and far-red, while reducing the blue, green, and yellow spectra, is being used in mist propagation and tissue culture production to enhance rooting (see Fig. 3–11).

Photosynthesis of Cuttings. Photosynthesis by cuttings is not an absolute requirement for root formation, as has been observed in leafy cuttings forming roots when placed in the dark (61) and with leafless hardwood cuttings that root. Increasing light irradiance has not always promoted rooting, and net photosynthesis of unrooted cuttings is saturated at relatively low PAR (irradiance measured as photosynthetically active radiation) levels (61); hence, high PAR does not enhance photosynthesis and could potentially lead to desiccation of cuttings. Unrooted *Acer rubrum* cuttings are much more prone to drought stress,

BOX 9.22 **GETTING MORE IN DEPTH ON THE SUBJECT**

OPTIMIZING ENVIRONMENTAL CONTROLS IN THE ROOTING OF LEAFY UNROOTED CUTTINGS (URCs)

Over one billion unrooted cuttings (URCs) are produced offshore and sent to greenhouse and nursery operations in the United States. Most vegetative annual URCs can be fully rooted within 2 to 3 weeks—if proper environmental conditions are maintained. While growers have little influence on the stockplant management techniques and the methods employed to harvest, store, and ship these URCs, they can improve how they propagate URCs to reduce rooting time and increase profitability. The critical environmental factors to manage during rooting are:

- controlling light intensity;
- providing adequate mist;
- maintaining high relative humidity;
- maintaining desirable air and media temperatures; and
- limiting air flow around leaves (to minimize desiccation and maintain a low vapor pressure deficit between leaves and surrounding air) (183, 184).

Managing Light Intensity Desirable levels of light vary with the stage of root development.

Stage 1: sticking to callus formation. During the early stages of propagation, maximum recommended light intensity is between 120 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (600 to 1,000 foot-candles) to provide photosynthate for callus formation and root initiation without causing desiccation. In addition, light transmission through the propagation house should be indirect or diffuse via exterior shade or retractable shade curtains.

Stage 2: after initial rooting. Once roots have initiated (generally 5 to 12 days after sticking), maximum light intensity can be increased to 200 to 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (1,000 to 2,500 foot-candles). Light should be diffuse.

Stage 3: after roots fill half the plug. Once cuttings are moderately well rooted into the plug tray or liner (generally 10 to 16 days after sticking), light levels should be increased to near production levels of 500 to 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (2,500 to 4,000 foot-candles) to acclimate plants to the post-propagation environment.

Ideal propagation conditions for rooting and growth of rooted liners for New Guinea impatiens (*Impatiens hawkeri*) and petunia (*Petunia* × *hybrid*) include:

- 8.5 to 5.4 daily light integral (DLI) $\mu mol / m^{-2}$/day (see Fig. 9–41)
- 12- to 13-hour photoperiod *to keep cuttings in vegetative condition*
- maintaining air temperature [20 to 23°C (68 to 73°F)] cooler than media temperature [20 to 23°C (68 to 73°F)], *which retards shoot growth and promotes root development*
- 89 to 85 percent relative humidity (0.3 kPa)
- humid, still air to minimize the vapor pressure deficit and mist frequency
- mist applied minimally to prevent wilting and just long enough so water coats leaf surface, but does not fall off (183, 184).

which lowers photosynthetic rates and stomatal conductance (253).

It has long been thought that the carbohydrate content of cuttings is important to rooting, and carbohydrates do accumulate in the base of cuttings during rooting (119). The amount of carbohydrates accumulated at cutting bases has been correlated with photosynthetic activity (61), but carbohydrates can also accumulate in the upper portion of leafy cuttings until after roots have formed (38). With leafy stem cuttings, the leaf-derived influx of carbohydrates determines the intensity of adventitious root formation (227).

In poinsettia cuttings, stomatal conductance and photosynthetic levels were initially low and remained low until root primordia were first microscopically observed (274); stomatal conductance and photosynthesis increased rapidly as root primordia began to elongate and emerge from the cuttings (Fig. 9–42). Most likely, root primordia were producing phytohormones such as cytokinins, which increased stomatal conductance and subsequently affected photosynthetic rates. In the rooting of cuttings, initial lower light irradiance could be used to hasten root initiation by reducing water stress (181), and light irradiance increased during root primordia emergence to support rapid primordia elongation and root system development.

If a generalization can be made, photosynthesis in cuttings is probably more important *after* root initiation has occurred, and helps aid root development and the more rapid growth of a rooted liner (63) (see Chapter 10).

Accelerated Growth Techniques

Accelerated growth techniques (AGT) were developed by the forestry industry to speed up the production of liners from vegetative propagules and from seed propagation (123). Woody perennial plants undergo cyclic growth, and many tree species experience dormancy. Liners are grown in protective culture facilities where photoperiod is extended and water, temperature, carbon dioxide, nutrition, mycorrhizal fungi (56), and growing

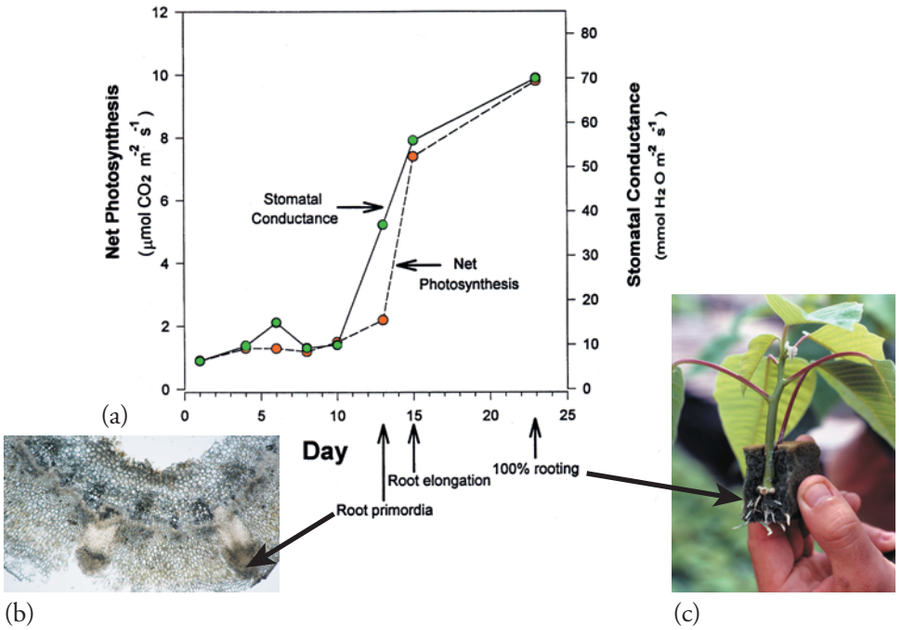


Figure 9–42
(a) Influence of adventitious root formation on gas exchange of poinsettia (Euphorbia pulcherrima cv. Lilo) cuttings. (b) Root primordia were microscopically observed at day 13, when photosynthesis began to increase. (c) Maximum photosynthesis was at 100 percent rooting (274).

media are optimized for each woody species and for each different phase of growth. See Figure 3–30 and the discussion on AGT in Chapter 3.

This concept is also being used in the propagation of horticultural crops where supplementary lighting with high-pressure sodium vapor lamps and injection of CO_2 gas into mist water are used to enhance rooting of holly (*Ilex aquifolium*) (92). The promotive effects on cuttings have been attributed to enhanced photosynthesis. In another study, CO_2 injection into enclosed fog tunnels enhanced root formation of *Chamelaucium* and Australian fuschia (*Correa*). This was attributed, in part, to decreased leaf transpiration and increased water potential of cuttings, implying that the higher CO_2 reduced stomatal conductance and improved water relations of the cuttings (104).

There is a growing trend for **modeling** propagation environments to determine optimal light, temperature, water, CO_2, and nutritional regimes (52, 174, 298, 299). See the discussion in Chapter 10 and earlier discussion on dynamic system models using vapor pressure deficit (VPD), transpiration, and temperature. Computers can be programmed to monitor the propagation environment and adjust environmental conditions as needed through automated environmental control systems (see Figs. 3–13, 3–14, 3–15, and 9–38).

DISCUSSION ITEMS

1. What are the developmental stages of wound-induced *de novo* adventitious roots?
2. What is callus, and how does it contribute to the formation of adventitious roots?
3. What organs must be formed adventitiously in both leaf and root cuttings?
4. How are correlative effects important in the control of adventitious root and bud formation?
5. What is the historical importance of rhizocaline in studies of adventitious root formation?
6. Discuss the most important phytohormones controlling adventitious root and bud formation.
7. What are some advantages of integrating molecular, biochemical, physiological, and anatomical developmental approaches to rooting studies?
8. What are some of the proposed roles of root inhibitors and rooting cofactors in adventitious root formation?
9. How are stock plants manipulated to maximize the rooting of cuttings?
10. How does the physiological age of a stock plant influence the rooting process?
11. How does the type of wood (hardwood, softwood, semihardwood) selected from stock plants influence the rooting process?
12. What is meant by seasonal timing, and why can it be advantageous to collect cuttings of selected plant species during specific times of the year?
13. What are the most effective compounds for stimulating adventitious root formation, and

what hormone (phytohormone) groups are they from?

14. What are the most effective compounds for stimulating adventitious bud and shoot formation, and what hormone (phytohormone) group are they from?
15. How does mineral nutrition affect the rooting of cuttings, and why can leaching of nutrients be a problem during propagation under intermittent mist?
16. What are some of the anatomical and physiological effects of wounding on the rooting of cuttings?
17. How do propagators manipulate the water relations and humidity control of cuttings with intermittent mist, fog, and enclosed propagation systems? In your discussion, include the terminology water potential, turgor potential, leaf and air water vapor pressure.
18. What is the influence of temperature on root initiation and development—how can a propagator manipulate temperature to maximize rooting of stem cuttings?
19. What is the influence of temperature on bud initiation and shoot development, and how can a propagator manipulate temperature to maximize leaf cutting propagation? (When answering the question, remember what organs are formed adventitiously from leaf cuttings.)
20. Give examples of environmental parameters that are manipulated with accelerated growth techniques (AGT) to enhance rooting of cuttings.
21. What is the influence of photosynthesis on the rooting of cuttings, and how does rooting influence photosynthetic rates of cuttings? Based on photosynthetic rates of unrooted and rooted cuttings, how can light irradiance be manipulated to maximize rooting?

REFERENCES

1. Altamura, M. M., F. Capitani, D. Serafini-Fracassini, P. Torigiani, and G. Falasca. 1991. Root histogenesis from tobacco thin cell layers. *Protoplasma* 161:31–42.

2. Altman, A. Y., and Y. Waisel, eds. 1997. *Biology of root formation and development.* New York: Plenum Publishing.

3. Anand, V. K., and G. T. Heberlein. 1975. Seasonal changes in the effects of auxin on rooting in stem cuttings of *Ficus infectoria. Physiol. Plant.* 34:330–34.

4. Arnaud, Y., A Franclet, H. Tranvan, and M. Jacques. 1993. Micropropagation and rejuvenation of *Sequoia sempervirens* (Lamb) Endl: A review. *Ann Sci. For.* 50:273–95.

5. Arteca, R. N., D. S. Tsai, and C. Schlagnhaufer. 1985. Abscisic acid effects on photosynthesis and transpiration in geranium cuttings. *HortScience* 20:370–72.

6. Auer, C. A., V. Motyka, A. Březinová, and M. Kaminek. 1999. Endogenous cytokinin accumulation and cytokinin oxidase activity during shoot organogenesis of *Petunia hybrida. Physiol. Plant.* 105:141–37.

7. Baker, K. F. 1984. The obligation of the plant propagator. *Comb. Proc. Intl. Plant Prop. Soc.* 34:195–203.

8. Baker, R. L., and C. B. Link. 1963. The influence of photoperiod on the rooting of cuttings of some woody ornamental plants. *Proc. Amer. Soc. Hort. Sci.* 82:596–601.

9. Ballester, A., M. C. San-José, N. Vidal, J. L. Fernández-Lorenzo, and A. M. Vieitez. 1999. Anatomical and biochemical events during *in vitro* rooting of microcuttings from juvenile and mature phases of chestnut. *Ann. Bot.* 83:619–29.

10. Barlow, P. W. 1994. The origin, diversity and biology of shoot-borne roots. In T. D. Davis and B. E. Haissig, eds. *Biology of adventitious root formation.* New York: Plenum Press.

11. Bartel, B., S. LeClere, M. Magidin, and B. K. Zolman. 2001. Inputs to the active indole-3-acetic acid pool: *de novo* synthesis, conjugate hydrolysis, and indole-3-butyric acid β-oxidation. *J. Plant Growth Regul.* 20:198–216.

12. Bassil, N. V., W. M. Proebsting, L. W. Moore, and D. A. Lightfoot. 1991. Propagation of hazelnut stem cuttings using *Agrobacterium rhizogenes. HortScience* 26:1058–60.

13. Bassuk, N., and B. Maynard. 1987. Stock plant etiolation. *HortScience* 22:749–50.

14. Basu, R. N., B. N. Roy, and T. K. Bose. 1970. Interaction of abscisic acid and auxins in rooting of cuttings. *Plant Cell Physiol.* 11:681–84.

15. Beakbane, A. B. 1969. Relationships between structure and adventitious rooting. *Comb. Proc. Intl. Plant Prop. Soc.* 19:192–201.

16. Behrens, V. 1988. Storage of unrooted cuttings. In T. D. Davis, B. E. Haissig, and N. Sankhla, eds. *Adventitious root formation in cuttings.* Portland, OR: Dioscorides Press.

17. Bertazza, G., R. Baraldi, and S. Predieri. 2005. Light effects on *in vitro* rooting of pear cultivars of different rhizogenic ability. *Plant Cell. Tiss. Organ Cult.* 41:139–43.

18. Biran, I., and A. H. Halevy. 1973. Endogenous levels of growth regulators and their relationship to the rooting of dahlia cuttings. *Physiol. Plant.* 28:436–42.

19. Biran, I., and A. H. Halevy. 1973. The relationship between rooting of dahlia cuttings and the presence and type of bud. *Physiol. Plant.* 28:244–47.

20. Biricolti, S., A. Fabbri, F. Ferrini, and P. L. Pisani. 1994. Adventitious rooting in chestnut: An anatomical investigation. *Scientia Hort.* 59:197–205.

21. Blakely, L. M., S. J. Rodaway, L. B. Hollen, and S. G. Croker. 1972. Control and kinetics of branch root formation in cultured root segments of *Haplopappus ravenii. Plant Physiol.* 50:35–49.

22. Blazich, F. A., and C. W. Heuser. 1979. A histological study of adventitious root initiation in mung bean cuttings. *J. Amer. Soc. Hort. Sci.* 104:63–7.

23. Blazich, F. A., and R. D. Wright. 1979. Nonmobilization of nutrients during rooting of *Ilex crenata* cv. Convexa stem cuttings. *HortScience* 14:242.

24. Blazich, F. A., R. D. Wright, and H. E. Schaffer. 1983. Mineral nutrient status of 'Convexa' holly cuttings during intermittent mist propagation as influenced by exogenous auxin application. *J. Amer. Soc. Hort. Sci.* 108:425–29.

25. Blazich, F. A. 1988. Mineral nutrition and adventitious rooting. In T. D. Davis, B. E. Haissig, and N. Sankhla, eds. *Adventitious root formation in cuttings.* Portland, OR: Dioscorides Press.

26. Blazich, F. A., and L. E. Hinesley. 1995. Fraser fir. *Amer. Nurs.* 181:54–67.

27. Blazkova, A., B. Sotta, H. Tranvan, R. Maldiney, M. Bonnet, J. Einhorn, L. Kerhoas, and E. Miginiac. 1997. Auxin metabolism and rooting in young and mature clones of *Sequoia sempervirens. Physiol. Plant.* 99:73–80.

28. Bloch, R. 1943. Polarity in plants. *Bot. Rev.* 9:261–310.

29. Blythe, E. K., J. L Sibley, K. M. Tilt, and J. M. Ruter. 2008a. Methods of auxin application in cutting propagation: A review of 70 years of scientific discovery and commercial practice: Part I *North Amer. Plant Prop.* 20:13–25.

30. Blythe, E. K., J. L. Sibley, K. M. Tilt, and J. M. Ruter. 2008b. Methods of auxin application in cutting propagation: A review of 70 years of scientific discovery and commercial practice: Part 2. *North Amer. Plant Prop.* 20:19–27.

31. Boe, A. A., R. B. Steward, and T. J. Banko. 1972. Effects of growth regulators on root and shoot development of Sedum leaf cuttings. *HortScience* 74:404–5.

32. Bollmark, M., and L. Eliasson. 1986. Effects of exogenous cytokinins on root formation in pea cuttings. *Physiol. Plant.* 68:662–66.

33. Bonnett, H. T., Jr., and J. G. Torrey. 1965. Chemical control of organ formation in root segments of *Convolvulus* cultured *in vitro. Plant Physiol.* 40:1228–36.

34. Boot, K. J. M., L. Goosen-deRoo, H. P. Spain, and J. W. Kijne. 2000. Adventitious root formation in stem segments of tobacco. In *Adventitious Root Formation: Third International Symposium on Adventitious Root Formation.* Veldhoven, The Netherlands.

35. Bouillenne, R., and M. Bouillenne-Walrand. 1955. Auxines et bouturage. *Rpt. 14th Inter. Hort. Cong.* 1:231–38.

36. Bouza, L., M. Jacques, B. Sota, and E. Miginiac. 1994. Relations between auxin and cytokinin contents and *in vitro* rooting of tree Peony (*Paeonia suffruticosa* Andr.). *J. Plant Growth Regul.* 15:69–73.

37. Breen, P. J., and T. Muraoka. 1973. Effect of indolebutyric acid on distribution of ^{14}C photosynthate in softwood cuttings of Marianna 2624 plum. *J. Amer. Soc. Hort. Sci.* 98:436–39.

38. Breen, P. J., and T. Muraoka. 1974. Effect of leaves and carbohydrate content and movement of ^{14}C-assimilate in plum cuttings. *J. Amer. Soc. Hort. Sci.* 99:326–32.

39. Brinker, M., L. van Zyl, W. Liu, D. Craig, R. R. Sederoff, D. H. Clapham, and S. von Arnold. 2004. Microarray analyses of gene expression during adventitious root development in *Pinus contorta. Plant Physiol.* 135:1526–39.

40. Caboche, M., J. F. Muller, F. Chanut, G. Aranda, and S. Cirakoglu. 1987. Comparison of the growth-promoting activities and toxicities of various auxin analogs on cells derived from wild type and a non-rooting mutant tobacco. *Plant Physiol.* 83:795–800.

41. Cameron, R. J., and G. V. Thomson. 1969. The vegetative propagation of *Pinus radiata*: Root initiation in cuttings. *Bot. Gaz.* 130:242–51.

42. Carlson, M. C. 1929. Origin of adventitious roots in *Coleus* cuttings. *Bot. Gaz.* 87:119–26.

43. Carlson, M. C. 1950. Nodal adventitious roots in willow stems of different ages. *Amer. J. Bot.* 37:555–67.

44. Carpenter, W. J., G. R. Beck, and G. A. Anderson. 1973. High intensity supplementary lighting during rooting of herbaceous cuttings. *HortScience* 8:338–40.

45. Christiansen, M. V., E. N. Eriksen, and A. S. Andersen. 1980. Interaction of stock plant irradiance

and auxin in the propagation of apple rootstocks by cuttings. *Scientia Hort.* 12:11–7.

46. Christianson, M. L., and D. A. Warnick. 1985. Temporal requirement for phytohormone balance in the control of organogenesis *in vitro*. *In Vitro Cell. Dev. Bio.—Plant.* 112:494–97.

47. Clark, D. G., E. G. Gubrium, J. E. Barrett, T. A. Nell, and H. J. Klee. 1999. Root formation in ethylene-insensitive plants. *Plant Physiol.* 121:53–60.

48. Cline, M. N., and D. Neely. 1983. The histology and histochemistry of the wound healing process in geranium cuttings. *J. Amer. Soc. Hort. Sci.* 108:450–96.

49. Crow, W. D., W. Nicholls, and M. Sterns. 1971. Root inhibitors in *Eucalyptus grandis*: Naturally occurring derivatives of the 2,3-dioxabicyclo (4,4,0) decane system. Tetrahedron Letters 18. London: Pergamon Press. pp. 1353–6.

50. Cuir, P., S. Sulis, F. Mariani, C. F. Van Sumere, A. Marchesini, and M. Dolci. 1993. Influence of endogenous phenols on rootability of *Chamaelaucium uncinatum* Schauer stem cuttings. *Scientia Hort.* 55:303–14.

51. Davies, F. T. 1984. Shoot RNA, cambial activity and indolebutyric acid effectively in seasonal rooting of juvenile and mature *Ficus pumila* cuttings. *Physiol. Plant.* 62:571–75.

52. Davies, F. T. 1985. Plant modeling: Developing an approach. *Comb. Proc. Intl. Plant Prop. Soc.* 35:770–76.

53. Davies, F. T., and H. T. Hartmann. 1988. The physiological basis of adventitious root formation. *Acta Hort.* 227:113–20.

54. Davies, F. T. 1993. What's new in the biology of adventitious root formation. *Comb. Proc. Intl. Plant Prop. Soc.* 43:382–84.

55. Davies, F. T. 2005. Optimizing the water relations of cuttings during propagation. *Comb. Proc. Intl. Plant Prop. Soc.* 55:585–92.

56. Davies, F. T. 2008. How mycorrhizal fungi can benefit nursery propagation and production systems. *Comb. Proc. Intl. Plant Prop. Soc.* 58:539–48.

57. Davies, F. T., and B. C. Moser. 1980. Stimulation of bud and shoot development of Rieger begonia leaf cuttings with cytokinins. *J. Amer. Soc. Hort. Sci.* 105:27–30.

58. Davies, F. T., and J. N. Joiner. 1980. Growth regulator effects on adventitious root formation in leaf bud cuttings of juvenile and mature *Ficus pumila*. *J. Amer. Soc. Hort. Sci.*100:643–46.

59. Davies, F. T., J. E. Lazarte, and J. N. Joiner. 1982. Initiation and development of roots in juvenile and mature leafbud cuttings of *Ficus pumila* L. *Amer. J. Bot.* 69:804–11.

60. Davies, F. T., T. D. Davis, and D. E. Kester. 1994. Commercial importance of adventitious rooting to horticulture. In T. D. Davis and B. E. Haissig, eds. *Biology of adventitious root formation.* New York: Plenum Press.

61. Davis, T. D., and J. R. Potter. 1981. Current photosynthate as a limiting factor in adventitious root formation in leafy pea cuttings. *J. Amer. Soc. Hort. Sci.* 106:278–82.

62. Davis, T. D., and J. R. Potter. 1985. Carbohydrates, water potential and subsequent rooting of stored rhododendron cuttings. *HortScience* 20:292–93.

63. Davis, T. D. 1988. Photosynthesis during adventitious rooting. In T. D. Davis, B. E. Haissig, and N. Sankhla, eds. *Adventitious root formation in cuttings.* Portland, OR: Dioscorides Press.

64. Davis, T. D., and B. E. Haissig. 1990. Chemical control of adventitious root formation in cuttings. *Plant Growth Regul. Soc. Amer. Quart.* 18:1–17.

65. Davis, T. D., and B. E. Haissig. 1994. *Biology of adventitious root formation.* New York: Plenum Press.

66. Davis, T. D., and N. Sankhla. 1989. Effect of shoot growth retardants and inhibitors on adventitious rooting. In T. D. Davis, B. E. Haissig, and N. Sankhla, eds. *Adventitious root formation in cuttings.* Portland, OR: Dioscorides Press.

67. De Klerk, G. J., W. Van der Krieken, and J. C. de Jong. 1999. The formation of adventitious roots: New concepts, new possibilities. *In Vitro Cell. Dev. Bio.—Plant.* 35:189–99.

68. De Klerk, G. J. 2000. Multiple effects of ethylene during rooting of micropropagules. In *Adventitious root formation: Third international symposium on adventitious root formation.* Veldhoven, The Netherlands.

69. Delargy, J. A., and C. E. Wright. 1978. Root formation in cuttings of apple (cv. Bramley's Seedling) in relation to ringbarking and to etiolation. *New Phytol.* 81:117–27.

70. Delargy, J. A., and C. E. Wright. 1979. Root formation in cuttings of apple in relation to auxin application and to etiolation. *New Phytol.* 82:341–47.

71. Drahn, S. R. 2007. Auxin application via foliar sprays. *Comb. Proc. Intl. Plant Prop. Soc.* 57:274–77.

72. Duhamel du Monceau, H. L. 1758. *La physique des arbres.* Paris: Guerin and Delatour.

73. Dunn, D. E., J. C. Cole, and M. W. Smith. 1996. Calendar date, degree days, and morphology

influence rooting of *Pistacia chinensis*. *J. Amer. Soc. Hort. Sci.* 121:269–73.

74. Durzan, D. J. 1988. Rooting in woody perennials: Problems and opportunities with somatic embryos and artificial seeds. *Acta Hort.* 227:121–25.

75. Dykeman, B. 1976. Temperature relationship in root initiation and development of cuttings. *Comb. Proc. Intl. Plant Prop. Soc.* 26:201–7.

76. Economou, A. S., and P. E. Read. 1987. Light treatments to improve efficiency of *in vitro* propagation systems. *HortScience* 22:751–54.

77. Eliasson, L. 1980. Interaction of light and auxin in regulation of rooting in pea stem cuttings. *Physiol. Plant.* 48:78–82.

78. Eliasson, L., and L. Brunes. 1980. Light effects on root formation in aspen and willow cuttings. *Physiol. Plant.* 48:261–65.

79. Ellis, D. D., H. Barczynsha, B. H. McCown, and N. Nelson. 1991. A comparison of BA, zeatin and thidiazuron for adventitious bud formation from *Picea glauca* embryos and epicotyl explants. *Plant Cell Tiss. Organ Cult.* 27:281–87.

80. Emery, A. E. H. 1955. The formation of buds on roots of *Chamaenerion angustifolium* (L.). *Scop. Phytomorphology* 5:139–45.

81. Englert, J. M., B. K. Maynard, and N. L. Bassuk. 1991. Correlation of phenolics with etiolated and light-grown shoots of *Carpinus betulus* stock plants. *Comb. Proc. Intl. Plant Prop. Soc.* 41:290–95.

82. Epstein, E., and J. Ludwig-Müller. 1993. Indole-3-butyric acid in plants: Occurrence, synthesis, metabolism and transport. *Physiol. Plant.* 88:382–89.

83. Eriksen, E. N. 1973. Root formation in pea cuttings. I. Effects of decapitation and disbud-ding at different development stages. *Physiol. Plant.* 28:503–6.

84. Eriksen, E. N. 1974. Root formation in pea cuttings. III. The influence of cytokinin at different development stages. *Physiol. Plant.* 30:163–67.

85. Eriksen, E. N. 1974. Root formation in pea cuttings. II. The influence of indole-3-acetic acid at different development stages. *Physiol. Plant.* 30:158–62.

86. Erstad, J. L. F., and H. R. Gislerød. 1994. Water uptake of cuttings and stem pieces as affected by different anaerobic conditions in the rooting medium. *Scientia Hort.* 58:151–60.

87. Fadl, M. S., and H. T. Hartmann. 1967. Isolation, purification, and characterization of an endogenous root-promoting factor obtained from the basal sections of pear hardwood cuttings. *Physiol. Plant.* 42:541–49.

88. Fadl, M. S., and H. T. Hartmann. 1967. Relationship between seasonal changes in endogenous promoters and inhibitors in pear buds and cutting bases and the rooting of pear hardwood cuttings. *Proc. Amer. Soc. Hort. Sci.* 91:96–112.

89. Faivre-Rampant, O., C. Kevers, and T. Gasper. 2000. IAA-oxidase activity and auxin protectors in nonrooting, rac, mutant shoots of tobacco *in vitro*. *Plant Sci.* 153:73–80.

90. Fischer, P., and J. Hansen. 1977. Rooting of chrysanthemum cuttings: Influence of irradiance during stock plant growth and of decapitation and disbudding of cuttings. *Scientia Hort.* 7:171–78.

91. Fishel, D. W., J. Zaczek, and J. E. Preece. 2003. Positional influence on rooting of shoots forced from the main bole in swamp white oak and northern red oak. *Can. J. Forest. Res.* 33:705–11.

92. French, C. J., and W. C. Lin. 1984. Seasonal variations in the effect of CO_2 mist and supplementary lighting from high pressure sodium lamps on rooting of English holly cuttings. *HortScience* 19:519–21.

93. Flygh, G., R. Grönroos, L. Gulin, and S. Von Arnold. 1993. Early and late root formation in epicotyl cuttings of *Pinus sylvestris* after auxin treatment. *Tree Physiol.* 12:81–92.

94. García-Gomez, C., A. Sanchez-Romero, A. Barcelo-Munoz, A. Heredia, and F. Pliego-Alfaro. 1994. Levels of endogenous indole-3-acetic acid and indole-3-acetyl-aspartic acid during adventitious rooting in avocado microcuttings. *J. Expt. Bot.* 45:865–70.

95. García-Luis, A., Y. Bordón, J. M. Moreira-Dias, R. V. Molina, and J. L. Guardiola. 1999. Explant orientation and polarity determine the morphogenic response of epicotyl segments of Troyer citrange. *Ann. Bot.* 87:715–23.

96. Gasper, T., and M. Hofinger. 1989. Auxin metabolism during rooting. In T. D. Davis, B. E. Haissig, and N. Sankhla, eds. *Adventitious root formation in cuttings.* Portland, OR: Dioscorides Press.

97. Gasper, T., C. Kevers, and J. F. Hausman. 1997. Indissociable chief factors in the inductive phase of adventitious rooting. In A. Altman and Y. Waisel, eds. *Biology of root formation and development.* New York: Plenum Publishing.

98. Geneve, R. L. 1991. Patterns of adventitious root formation in English Ivy. *J. Plant Growth Regul.* 10:215–20.

99. Geneve, R. L., and S. T. Kester. 1991. Polyamines and adventitious root formation in the juvenile and mature phase of English ivy. *J. Exp. Bot.* 42:71–5.

100. Geneve, R. L. 2000. Root formation in relationship to auxin uptake in cuttings treated by the

dilute soak, quick dip and talc methods. *Comb. Proc. Intl. Plant Prop. Soc.* 50:409–12.

101. Ginzburg, C. 1967. Organization of the adventitious root apex in *Tamarix aphylla*. *Amer. J. Bot.* 54:4–8.

102. Girouard, R. M. 1969. Physiological and biochemical studies of adventitious root formation. Extractable rooting co-factors from *Hedera helix Can. J. Bot.* 47(5):287–99.

103. Grange, R. I., and K. Loach. 1983. The water economy of unrooted leafy cuttings. *J. Hort. Sci.* 58:9–17.

104. Grant, W. J. R., H. M. Fan, W. J. S. Downton, and B. R. Loveys. 1992. Effects of CO_2 enrichment on the physiology and propagation of two Australian ornamental plants, *Chamelaucium uncinatum* (Schauer) × *Chamelaucium floriferum* (MS) and *Correa schlechtendalii* (Behr). *Scientia Hort.* 52:337–42.

105. Greenwood, M. S., and G. P. Berlyn. 1973. Sucrose: Indoleacetic acid interactions on root regeneration by *Pinus lambertiana* embryo cuttings. *Amer. J. Bot.* 60:42–7.

106. Greenwood, M. S. 1987. Rejuvenation in forest trees. *J. Plant Growth Regul.* 6:1–12.

107. Greenwood, M. S., C. Diaz-Sala, P. B. Singer, A. Decker, and K. W. Hutchinson. 1997. Differential gene expression during maturation-caused decline in adventitious rooting ability in loblolly pine (*Pinus taeda* L.). In A. Altman and Y. Waisel, eds. *Biology of root formation and development.* New York: Plenum Press.

108. Gupta, P. K., and D. J. Durzan. 1987. Micropropagation and phase specificity in mature, elite Douglas fir. *J. Amer. Soc. Hort. Sci.* 112:969–71.

109. Hackett, W. P. 1970. The influence of auxin, catechol, and methanolic tissue extracts on root initiation in aseptically cultured shoot apices of the juvenile and adult forms of *Hedera helix*. *J. Amer. Soc. Hort. Sci.* 95:398–402.

110. Hackett, W. P. 1985. Juvenility, maturation and rejuvenation in woody plants. *Hort. Rev.* 7:109–15.

111. Hackett, W. P. 1989. Donor plant maturation and adventitious root formation. In T. D. Davis, B. E. Haissig, and N. Sankhla, eds. *Adventitious root formation in cuttings*. Portland, OR: Dioscorides Press.

112. Hackett, W. P. 1997. The use of mutants to understand competence for shoot-borne root initiation. In A. Altman and Y. Waisel, eds. *Biology of root formation and development.* New York: Plenum Publishing.

113. Hagemann, A. 1932. Untersuchungen an Blattstecklingen. *Gartenbauwiss.* 6:69–202.

114. Haines, R. J., T. R. Copley, J. R. Huth, and M. R. Nester. 1992. Shoot selection and the rooting and field performance of tropical pine cuttings. *Forest Sci.* 38:95–101.

115. Haissig, B. E., and T. D. Davis. 1994. A historical evaluation of adventitious rooting research to 1993. In T. D. Davis and B. E. Haissig, eds. *Biology of adventitious root formation.* New York: Plenum Press.

116. Haissig, B. E. 1972. Meristematic activity during adventitious root primordium development. Influences of endogenous auxin and applied gibberellic acid. *Plant Physiol.* 49:886–92.

117. Haissig, B. E. 1974. Influence of auxins and auxin synergists on adventitious root primordium initiation and development. *New Zealand For. Sci.* 4:299–310.

118. Haissig, B. E. 1983. N-phenyl indolyl-3-butyramide and phenyl indole-3-thiolobutyrate enhance adventitious root primordium development. *Physiol. Plant.* 57:435–40.

119. Haissig, B. E. 1984. Carbohydrate accumulation and partitioning in *Pinus banksiana* seedlings and seedling cuttings. *Physiol. Plant.* 61:13–19.

120. Haissig, B. E. 1986. Metabolic processes in adventitious rooting of cuttings. In M. B. Jackson, ed. *New root formation in plants and cuttings*. Dordrecht: Martinus Nijhoff Publishers.

121. Haissig, B. E., T. D. Davis, and D. E. Riemenschneider. 1992. Researching the controls of adventitious root formation. *Physiol. Plant.* 84:310–17.

122. Hambrick, C. E., F. T. Davies, Jr., and H. B. Pemberton. 1991. Seasonal changes in carbohydrate/nitrogen levels during field rooting of *Rosa multiflora* 'Brooks 56' hardwood cuttings. *Scientia Hort.* 46:137–46.

123. Hanover, J. W. 1976. Accelerated-optimal-growth: A new concept in tree production. *Amer. Nurs.* 144(10):11–2, 58, 60, 64–5.

124. Hansen, J., L. H. Strömquist, and A. Ericsson. 1978. Influence of the irradiance on carbohydrate content and rooting of cuttings of pine seedlings (*Pinus sylvestris* L.). *Physiol. Plant.* 61:975–79.

125. Hansen, J. 1988. Influence of gibberellins on adventitious root formation. In T. D. Davis, B. E. Haissig, and N. Sankhla, eds. *Adventitious root formation in cuttings*. Portland, OR: Dioscorides Press.

126. Harbage, J. F., D. P. Stimart, and R. F. Evert. 1994. Anatomy of adventitious root formation in microcuttings of *Malus domestica* Borkh. 'Gala'. *J. Amer. Soc. Hort. Sci.* 118:680–88.

127. Hare, R. C. 1977. Rooting of cuttings from mature water oak (*Quercus nigra*). *Southern J. Appl. For.* 1:24–5.

128. Hare, R. C. 1981. Improved rooting powder for chrysanthemums. *HortScience* 16:90–1.

129. Harrison-Murray, R. S., B. H. Howard, and R. Thompson. 1988. Potential for improved propagation of cuttings through the use of fog. *Acta Hort.* 227:205–10.

130. Harrison-Murray, R. S., and R. Thompson. 1988. In pursuit of a minimum stress environment for rooting leafy cuttings: Comparison of mist and fog. *Acta Hort.* 227:211–16.

131. Harrison-Murray, R. S. 1991. A leaf-model evaporimeter for estimating potential transpiration in propagation environments. *J. Hort. Sci.* 66:131–39.

132. Hausman, J. F. 1993. Changes in peroxidase activity, auxin level and ethylene production during root formation by poplar shoots raised *in vitro. J. Plant Growth Regul.* 13:263–68.

133. Heide, O. M. 1965. Interaction of temperature, auxin, and kinins in the regeneration ability of begonia leaf cuttings. *Physiol. Plant.* 18:891–920.

134. Heide, O. M. 1965. Effects of 6-benzylamino-purine and 1-naphthaleneacetic acid on the epiphyllous bud formation in *Bryophyllum. Planta* 67:281–96.

135. Heide, O. M. 1965. Photoperiodic effects on the regeneration ability of begonia leaf cuttings. *Physiol. Plant.* 18:185–90.

136. Heide, O. M. 1968. Stimulation of adventitious bud formation in begonia leaves by abscisic acid. *Nature* 219:960–61.

137. Heide, O. M. 1968. Auxin level and regeneration of begonia leaves. *Planta* 81:153–59.

138. Heide, O. M. 1969. Non-reversibility of gibberellin-induced inhibition of regeneration in begonia leaves. *Physiol. Plant.* 22:671–79.

139. Henry, P. H., F. A. Blazich, and L. E. Hinesley. 1992. Nitrogen nutrition of containerized eastern red cedar. II. Influence of stock plant fertility on adventitious rooting of stem cuttings. *J. Amer. Soc. Hort. Sci.* 117:568–70.

140. Hess, C. E. 1962. Characterization of the rooting co-factors extracted from *Hedera helix* L. and *Hibiscus rosa-sinensis. L. Proc. 16th Inter. Hort. Cong.*: 382–88.

141. Hess, C. E. 1968. Internal and external factors regulating root initiation. In *Root growth: Proc. 15th Easter School in Agricultural Science.* University of Nottingham. London: Butterworth.

142. Hiller, C. 1951. *A study of the origin and development of callus and root primordia of Taxus cuspidata with reference to the effects of growth regulators.* Master's thesis. Ithaca, NY: Cornell Univ.

143. Hitchcock, A. E., and P. W. Zimmerman. 1942. Root inducing activity of phenoxy compounds in relation to their structure. *Contrib. Boyce. Thomp. Inst.* 12:497–507.

144. Howard, B. H. 1965. Increase during winter in capacity for root regeneration in detached shoots of fruit tree rootstocks. *Nature* 208:912–13.

145. Howard, B. H. 1968. Effects of bud removal and wounding on rooting of hardwood cuttings. *Nature* 220:262–64.

146. Howard, B. H., R. S. Harrison-Murray, J. Vesek, and O. P. Jones. 1988. Techniques to enhance rooting potential before cutting collection. *Acta Hort.* 227:1976–86.

147. Howard, B. H., O. P. Jones, and J. Vasek. 1989. Growth characteristics of apparently rejuvenated plum shoots. *J. Hort. Sci.* 64:157–62.

148. Howard, B. H., O. P. Jones, and J. Vasek. 1989. Long-term improvement in the rooting of plum cuttings following apparent rejuvenation. *J. Hort. Sci.* 64:147–56.

149. Howard, B. H. 1991. Stock plant manipulation for better rooting and growth from cuttings. *Comb. Proc. Intl. Plant Prop. Soc.* 41:127–30.

150. Howard, B. H., and M. S. Ridout. 1992. A mechanism to explain increased rooting in leafy cuttings of *Syringa vulgaris* 'Madame Lemoine' following dark-treatment of the stock plant. *J. Hort. Sci.* 59:131–39.

151. Howard, B. H. 1994. Manipulating rooting potential in stock plants before collecting cuttings. In T. D. Davis and B. E. Haissig, eds. *Biology of adventitious root formation.* New York: Plenum Press.

152. Howard, B. H., and R. S. Harrison-Murray. 1997. Relationships between stock plant management and rooting environments for difficult-to-propagate cuttings. *Comb. Proc. Intl. Plant Prop. Soc.* 47:322–27.

153. Ikeda, T., and T. Suzaki. 1985. Influence of hydraulic conductance of xylem on water status in cuttings. *Can. J. For. Res.* 16:98–102.

154. Jackson, M. B., ed. 1986. *New root formation in plants and cuttings.* Dordrecht: Martinus Nijhoff Publishers.

155. Jain, M. K., and K. K. Nanda. 1972. Effect of temperature and some antimetabolites on the interaction effects of auxin and nutrition in rooting etiolated stem segments of *Salix tetrasperma. Physiol. Plant.* 27:169–72.

156. Jarvis, B. C., S. Yasmin, and M. T. Coleman. 1985. RNA and protein metabolism during

adventitious root formation in stem cuttings of *Phaseolus aureus. Physiol. Plant.* 64:53–9.

157. Jarvis, B. C. 1986. Endogenous control of adventitious rooting in non-woody species. In M. B. Jackson, ed. *New root formation in plants and cuttings.* Dordrecht: Martinus Nijhoff Publishers.

158. Johnson, C. R. 1970. *The nature of flower bud influence on root regeneration in the Rhododendron shoot.* Ph.D. Dissertation. Oreg. State Univ., Corvallis, OR.

159. Johnson, C. R., and A. N. Roberts. 1971. The effect of shading rhododendron stock plants on flowering and rooting. *J. Amer. Soc. Hort. Sci.* 96:166–68.

160. Johnson, C. R., and D. F. Hamilton. 1977. Effects of media and controlled-release fertilizers on rooting and leaf nutrient composition of *Juniperus conferta* and *Ligustrum japonicum* cuttings. *J. Amer. Soc. Hort. Sci.* 102:320–22.

161. Kawase, M. 1964. Centrifugation, rhizocaline, and rooting in *Salix alba. Physiol. Plant.* 17:855–65.

162. Kawase, M. 1971. Causes of centrifugal root promotion. *Physiol. Plant.* 25:64–70.

163. Keever, G. J., and J. H. B. Tukey. 1979. Effect of nutrient mist on the propagation of azaleas. *HortScience* 14:755–56.

164. Keever, G. J., G. S. Cobb, and D. R. Mills. 1987. Propagation of four woody ornamentals from vegetative and reproductive stem cuttings. *Ornamentals Res. Rep. 5,* Alabama Agr. Exp. Sta., Auburn University, Montgomery.

165. Kefford, N. P. 1973. Effect of a hormone antagonist on the rooting of shoot cuttings. *Plant Physiol.* 51:214–16.

166. Kester, D. E. 1970. Temperature and plant propagation. *Comb. Proc. Intl. Plant Prop. Soc.* 20:153–63.

167. Kester, D. E. 1982. The clone in horticulture. *HortScience* 18:831–37.

168. Kling, G. J., J. M. M. Meyer, and D. Seigler. 1988. Rooting co-factors in five *Acer* species. *J. Amer. Soc. Hort. Sci.* 113:252–57.

169. Kraus, E. J., and H. R. Kraybill. 1918. *Vegetation and reproduction with special reference to the tomato. Oreg. Agr. Exp. Sta. Bul.* 149.

170. Krisantini, S., M. Johnston, R. R. Williams, and C. Beveridge. 2006. Adventitious root formation in Grevillea (*Proteaceae*), an Australian native species. *Scientia Hort.* 107:171–75.

171. Lanphear, F. O., and R. P. Meahl. 1961. The effect of various photoperiods on rooting and subsequent growth of selected woody ornamental plants. *Proc. Amer. Soc. Hort. Sci.* 77:620–34.

172. Lanphear, F. O., and R. P. Meahl. 1966. Influence of the stock plant environment on the rooting of *Juniperus horizontalis* 'Plumosa.' *Proc. Amer. Soc. Hort. Sci.* 89:666–71.

173. LeBude, A. V., B. Goldfarb, F. A. Blazich, F. C. Wise, and J. Frampton. 2004. Mist, substrate water potential and cutting water potential influence rooting of stem cuttings of loblolly pine. *Tree Physiol.* 24:823–31.

174. LeBude, A. V., B. Goldfarb, F. A. Blazich, J. Frampton, and F. C. Wise. 2005. Mist level influences vapor pressure deficit and gas exchange during rooting of juvenile stem cuttings of loblolly pine. *HortScience* 40:1448–56.

175. Lek, H. A., and A. van der. 1925. Root development in woody cuttings. *Meded. Landbouwhoogesch. Wageningen.* 38(1).

176. Leopold, A. C. 1964. *The polarity of auxin transport in meristems and differentiation.* Brookhaven Symposia in Biology. Rpt. 16. Upton, NY: Brookhaven Natl. Lab., pp. 218–34.

177. Libby, W. J., A. G. Brown, and J. M. Fielding. 1972. Effect of hedging *radiata pine* on production, rooting, and early growth of cuttings. *New Zealand J. For. Sci.* 2:263–83.

178. Loach, K. 1979. Mist propagation: Past, present, future. *Comb. Proc. Intl. Plant Prop. Soc.* 29:216–29.

179. Loach, K. 1983. Propagation systems in New Zealand: A means of comparing their effectiveness. *Comb. Proc. Intl. Plant Prop. Soc.* 33:291–94.

180. Loach, K. 1987. Mist and fruitfulness. *Horticulture Week.* April 10, 1987:28–9.

181. Loach, K. 1988. Controlling environmental conditions to improve adventitious rooting. In T. D. Davis, B. E. Haissig, and N. Sankhla, eds. *Adventitious root formation in cuttings.* Portland, OR: Dioscorides Press.

182. Loach, K. 1988. Water relations and adventitious rooting. In T. D. Davis, B. E. Haissig, and N. Sankhla, eds. *Adventitious root formation in cuttings.* Portland, OR: Dioscorides Press.

183. Lopez, R. G., and E. S. Runkle. 2008. Photosynthetic daily light integral during propagation influences rooting and growth of cuttings and subsequent development of New Guinea impatiens and petunia. *HortScience* 43:2052–9.

184. Lopez, R. G., and E. S. Runkle. 2005. Managing light during propagation. *Greenhouse Product News* 15(6):1.

185. Lovell, P. H., and J. White. 1986. Anatomical changes during adventitious root formation. In M. B. Jackson, ed. *New root formation in plants and cuttings.* Dordrecht: Martinus Nijhoff Publishers.

186. Ludwig-Müller, J., and E. Epstein. 1994. Indole-3-butyric acid in *Arabidopsis thaliana* III. *In vivo* biosynthesis. *J. Plant Growth Regul.* 14:7–14.

187. MacKenzie, K. A. D., B. H. Howard, and R. S. Harrison-Murray. 1986. The anatomical relationship between cambial regeneration and root initiation in wounded winter cuttings of the apple rootstock M.26. *Ann. Bot.* 58:649–61.

188. Maini, J. S. 1968. The relationship between the origin of adventitious buds and the orientation of *Populus tremuloides* root cuttings. *Bul. Ecol. Soc. Amer.* 49:81–2.

189. Major, J. E., and S. C. Grossnickle. 1990. Chilling units used to determine rooting of stem cuttings of junipers. *J. Environ. Hort.* 8:32–5.

190. Mancuso, S. 1998. Seasonal dynamics of electrical impedance parameters in shoots and leaves relate to rooting ability of olive (*Olea europaea*) cuttings. *Tree Physiol.*19:95–101.

191. Maynard, B. K., and N. L. Bassuk. 1987. Stock plant etiolation and blanching of woody plants prior to cutting propagation. *J. Amer. Soc. Hort. Sci.* 112:273–76.

192. Maynard, B. K., and N. L. Bassuk. 1991. Stock plant etiolation and stem banding effect on the auxin dose-response of rooting in stem cuttings of *Carpinus betulus* L. 'Fastigiata.' *J. Plant Growth Regul.* 10:305–11.

193. Maynard, B. K., and N. L. Bassuk. 1992. Stock plant etiolation, shading, and banding effects on cutting propagation of *Carpinus betulus. J. Amer. Soc. Hort. Sci.* 117:740–44.

194. McAfee, B. J., E. E. White, L. E. Pelcher, and M. S. Lapp. 1993. Root induction in pine (*Pinus*) and larch (*Larix*) spp. using *Agrobacterium rhizogenes. Plant Cell Tiss. Organ. Cult.* 34:53–62.

195. Medina, J. P. 1981. Studies of clonal propagation on pecans at Ica, Peru. *Plant Propagator* 26:11–3.

196. Moe, R., and A. S. Andersen. 1988. Stock plant environment and subsequent adventitious rooting. In T. D. Davis, B. E. Haissig, and N. Sankhla, eds. *Adventitious root formation in cuttings.* Portland, OR: Dioscorides Press.

197. Mohammed, S., and E. N. Eriksen. 1974. Root formation in pea cuttings. IV. Further studies on the influence of indole-3-acetic acid at different development stages. *Physiol. Plant.* 32:94–6.

198. Mohammed, S. 1975. Further investigations on the effects of decapitation and disbudding at different development stages of rooting in pea cuttings. *J. Hort. Sci.* 50:271–73.

199. Mohnen, D. 1994. Novel experimental systems for determining cellular competence and determination. In T. D. Davis and B. E. Haissig, eds. *Biology of adventitious root formation.* New York: Plenum Press.

200. Molitor, H. D., and W. U. von Hentig. 1987. Effect of carbon dioxide enrichment during stock plant cultivation. *HortScience* 22:741–46.

201. Molnar, J. M., and L. J. LaCroix. 1972. Studies of the rooting of cuttings of *Hydrangea macrophylla*: Enzyme changes. *Can. J. Bot.* 50:315–22.

202. Molnar, J. M., and L. J. LaCroix. 1972. Studies of the rooting of cuttings of *Hydrangea macrophylla*: DNA and protein changes. *Can. J. Bot.* 50:387–92.

203. Moon, H. K. 2006. Growth of Tsururindo (*Tripterospermum japonicum*) cultured *in vitro* under various sources of light-emitting diode (LED) irradiation. *J. Plant Biol.* 49:174–79.

204. Moreira-Dias, J. M., R. V. Molina, Y. Bordón, J. L. Guardiola, and A. García-Luis. 2000. Direct and indirect shoot organogenic pathways in epicotyl cuttings of Troyer citrange differ in hormone requirements and in their response to light. *Ann. Bot.* 85:103–10.

205. Morgan, D. L., E. L. McWilliams, and W. C. Parr. 1980. Maintaining juvenility in live oak. *HortScience* 15:493–94.

206. Mudge, K. W. 1988. Effect of ethylene on rooting. In T. D. Davis, B. E. Haissig, and N. Sankhla, eds. *Adventitious root formation in cuttings.* Portland, OR: Dioscorides Press.

207. Mudge, K. W., V. N. Mwaja, F. M. Itulya, and J. Ochieng. 1995. Comparison of four moisture management systems for cutting propagation of bougainvillea, hibiscus and kei apple. *J. Amer. Soc. Hort. Sci.* 120:366–73.

208. Murray, J. R., M. C. Sanchez, A. G. Smith, and W. P. Hackett. 1993. Differential competence for adventitious root formation in histologically similar cell types. In T. D. Davis and B. E. Haissig, eds. *Biology of adventitious root formation.* New York: Plenum Press.

209. Muzik, T .J., and H. J. Cruzado. 1958. Transmission of juvenile rooting ability from seedlings to adults of *Hevea brasiliensis. Nature* 181:1288.

210. Nórgaard, J. V. 1992. Artificial seeds in micropropagation. *Comb. Proc. Intl. Plant Prop. Soc.* 42:182–84.

211. O'Rourke, F. L. 1944. Wood type and original position on shoot with reference to rooting in

hardwood cuttings of blueberry. *Proc. Amer. Soc. Hort. Sci.* 45:195–97.

212. Okoro, O. O., and J. Grace. 1978. The physiology of rooting *Populus* cuttings. II. Cytokinin activity in leafless hardwood cuttings. *Physiol. Plant.* 44:167–70.

213. Oliver, M. J., I. Mukherjee, and D. M. Reid. 1994. Alteration in gene expression in hypocotyls of sunflower (*Helianthus annuus*) seedlings associated with derooting and formation of adventitious root primordia. *Physiol. Plant.* 90:481–89.

214. Patena, L., E. G. Sutter, and A. M. Dandekar. 1988. Root induction by *Agrobacterium rhizogenes* in a difficult-to-root woody species. *Acta Hort.* 227:324–29.

215. Paton, D. M., R. R. Willing, W. Nichols, and L. D. Pryor. 1970. Rooting of stem cuttings of eucalyptus: A rooting inhibitor in adult tissue. *Austral. J. Bot.* 18:175–83.

216. Paton, F., and W. W. Schwabe. 1987. Storage of cuttings of *Pelargonium xhortorum. J. Hort. Sci.* 62:79–87.

217. Pierik, R. L. M., and H. H. M. Steegmans. 1975. Analysis of adventitious root formation in isolated stem explants of Rhododendron. *Scientia Hort.* 3:1–20.

218. Pliego-Alfaro, F., and T. Murashige. 1987. Possible rejuvenation of adult avocado by graftage onto juvenile rootstocks *in vitro. HortScience* 22:1321–4.

219. Plietzsch, A., and H. H. Jesch. 1998. Using *in vitro* propagation to rejuvenate diffficult-to-root woody plants. *Comb. Proc. Intl. Plant Prop. Soc.* 48:171–76.

220. Preece, J. E. 1993. Basics of propagation by cuttings—temperature. *Comb. Proc. Intl. Plant Prop. Soc.* 43:441–43.

221. Preece, J. E. 2003. A century of progress with vegetative plant propagation. *HortScience* 38:1015–25.

222. Preece, J. E. 2008. Stock plant physiological factors affecting growth and morphogenesis. In E. F. George, M. A. Hall, and G. J. De Klerk, eds. *Plant propagation by tissue culture*. Dordrecht: Springer. pp. 403–22.

223. Preece, J. E., and P. Read. 2007. Forcing leafy explants and cuttings from woody species. *Propagation Ornamental. Plants* 7:138–44.

224. Priestley, J. H., and C. F. Swingle. 1929. *Vegetative propagation from the standpoint of plant anatomy*, USDA Tech. Bul.

225. Proebsting, W. M. 1984. Rooting of Douglas-fir stem cuttings: Relative activity of IBA and NAA. *HortScience* 19:854–56.

226. Rajagopal, V., and A. S. Andersen. 1980. Water stress and root formation in pea cuttings. *Physiol. Plant.* 48:114–49.

227. Rapaka, V. K., B. Bessler, M. Schreiner, and U. Druge. 2005. Interplay between initial carbohydrate availability, current photosynthesis, and adventitious root formation in *Pelargonium* cuttings. *Plant Sci.* 168:1547–60.

228. Rashotte, A. M., J. Poupart, C. S. Waddell, and G. K. Muday. 2003. Transport of the two natural auxins, indole-3-butyric acid and indole-3-acetic acid, in *Arabidopsis. Plant Physiol.* 133:761–72.

229. Raskin, I. 1992. Salicylate—a new plant hormone. *Plant Physiol.* 99:799–803.

230. Rasmussen, S., and A. S. Andersen. 1980. Water stress and root formation in pea cuttings. II. Effect of abscisic acid treatment of cuttings from stock plants grown under two levels of irradiance. *Physiol. Plant.* 48:150–54.

231. Read, P. E., and V. C. Hoysler. 1969. Stimulation and retardation of adventitious root formation by application of B-Nine and Cycocel. *J. Amer. Soc. Hort. Sci.* 94:314–16.

232. Rein, W. H., R. D. Wright, and J. R. Seiler. 1991. Propagation medium moisture level influences adventitious rooting of woody stem cuttings. *J. Amer. Soc. Hort. Sci.* 116:632–36.

233. Rein, W. H., R. D. Wright, and D. D. Wolf. 1991. Stock plant nutrition influences the adventitious rooting of 'Rotundifolia' holly stem cuttings. *J. Environ. Hort.* 9:83–5.

234. Reuveni, O., and M. Raviv. 1981. Importance of leaf retention to rooting avocado cuttings. *J. Amer. Soc. Hort. Sci.* 106:127–30.

235. Rey, M., C. Díaz-Sala, and R. Rodríguez. 1994. Exogenous polyamines improve rooting of hazel microshoots. *Plant Cell Tiss. Organ. Cult.* 36:303–8.

236. Ritchie, G. A. 1994. Commercial applications of adventitious rooting to forestry. In T. D. Davis and B. E. Haissig, eds. *Biology of adventitious root formation.* New York: Plenum Press.

237. Robbins, J. A., M. J. Campidonica, and D. W. Burger. 1988. Chemical and biological stability of indole-3-butyric acid (IBA) after long-term storage at selected temperatures and light regimes. *J. Environ. Hort.* 6:33–8.

238. Roberts, A. N., and L. H. Fuchigami. 1973. Seasonal changes in auxin effect on rooting of Douglas-fir stem cuttings as related to bud activity. *Physiol. Plant.* 28:215–21.

239. Robinson, J. C., and W. W. Schwabe. 1977. Studies on the regeneration of apple cultivars from

root cuttings. I. Propagation aspects. *J. Hort. Sci.* 52:205–20.

240. Robinson, J. C., and W. W. Schwabe. 1977. Studies on the regeneration of apple cultivars from root cuttings. II. Carbohydrate and auxin relations. *J. Hort. Sci.* 52:221–33.

241. Rowland, L. J., and E. L. Ogden. 1992. Use of a cytokinin conjugate for efficient shoot regeneration from leaf sections of highbush blueberry. *HortScience* 27:1127–9.

242. Rugini, E., A. Pellegrineschi, M. Mencuccini, and D. Mariotti. 1991. Increase of rooting ability in the woody species kiwi (*Actinidia deliciosa* A. Chev.) by transformation with *Agrobacterium rhizogenes* rol genes. *Plant Cell Rept.* 10:291–95.

243. Rugini, E. 1992. Involvement of polyamines in auxin and *Agrobacterium rhizogenes*-induced rooting of fruit trees *in vitro*. *J. Amer. Soc. Hort. Sci.* 117:532–36.

244. Sachs, J. 1880 and 1882. *Stoff und Form der Pflanzenorgane*. I and II. *Arb. Bot. Inst. Würzburg* 2:450–88; 4:689–718.

245. Sachs, R. M., F. Loreti, and J. DeBie. 1964. Plant rooting studies indicate sclerenchyma tissue is not a restricting factor. *Calif. Agr.* 18:4–5.

246. Sanchez, M. C., A. G. Smith, and W. P. Hackett. 1995. Localized expression of a proline-rich protein gene in juvenile and mature ivy petioles in relation to rooting competence. *Physiol. Plant.* 93:207–16.

247. Santos, K. M., P. R. Fisher, and W. R. Argo. 2008. A survey of water and fertilization management during cutting propagation. *HortTechnology* 18:597–604.

248. Schier, G. A. 1973. Origin and development of aspen root suckers. *Can. J. For. Res.* 3:39–44.

249. Schuerman, P. L., and A. M. Dandekar. 1993. Transformation of temperate woody crops: Progress and potentials. *Scientia Hort.* 55:101–24.

250. Sircar, P. K., and S. K. Chatterjee. 1974. Physiological and biochemical changes associated with adventitious root formation in *Vigna* hypocotyl cuttings. II. Gibberellin effects. *Plant Propagator* 20:15–22.

251. Skoog, F., and C. Tsui. 1948. Chemical control of growth and bud formation in tobacco stem and callus. *Amer. J. Bot.* 35:782–87.

252. Smally, T. J., and M. A. Dirr. 1988. Effect of night interruption photoperiod treatment on subsequent growth of *Acer rubrum* cuttings. *HortScience* 23:172–74.

253. Smally, T. J., M. A. Dirr, A. M. Armitage, B. W. Wood, R. O. Teskey, and R. F. Severson. 1991. Photosynthesis, leaf water, carbohydrate, and hormone status during rooting of stem cuttings of *Acer rubrum*. *J. Amer. Soc. Hort. Sci.* 116:1052–7.

254. Smith, D. L., and N. V. Fedoroff. 1995. LRP1, a gene expressed in lateral and adventitious root primordia of *Arabidopsis*. *Plant Cell* 7:735–45.

255. Smith, D. R., and T. A. Thorpe. 1975a. Root initiation in cuttings of *Pinus radiata* seedlings. I. Developmental sequence. *J. Expt. Bot.* 26:184–92.

256. Smith, D. R., and T. A. Thorpe. 1975b. Root initiation in cuttings of *Pinus radiata* seedlings. II. Growth regulator interactions. *J. Expt. Bot.* 26:193–202.

257. Smith, N. G., and P. F. Wareing. 1972. The distribution of latent root primordia in stems of *Populus xrobusta* and factors affecting emergence of preformed roots from cuttings. *J. Forestry* 45:197–210.

258. Sorin, C., L. Negroni, T. Balliau, H. Corti, M. P. Jacquemot, M. Davanture, G. Sandberg, M. Zivy, and C. Bellini. 2006. Proteomic analysis of different mutant genotypes of *Arabidopsis* led to the identification of 11 proteins correlating with adventitious root development. *Plant Physiol.* 140:349–64.

259. Spano, L., D. Mariotti, M. Cardarelli, C. Branra, and P. Costantino. 1988. Morphogenesis and auxin sensitivity of transgenic tobacco with different complements of Ri T-DNA. *Plant Physiol.* 87:476–83.

260. Stangler, B. B. 1949. An anatomical study of the origin and development of adventitious roots in stem cuttings of *Chrysanthemum morifolium* Bailey, *Dianthus caryophyllus* L., and *Rosa dilecta* Rehd. Ph.D. dissertation. Cornell Univ., Ithaca NY.

261. Steele, M. J., M. M. Yeoman, and M. P. Coutts. 1990. Developmental changes in Sitka spruce as indices of physiological age. II. Rooting of cuttings and callusing of needle explants. *New Phytol.* 114:11–120.

262. Steponkus, P. L., and L. Hogan. 1967. Some effects of photoperiod on the rooting of *Abelia grandiflora* Rehd. 'Prostrata' cuttings. *Proc. Amer. Soc. Hort. Sci.* 91:706–15.

263. Stoltz, L. P., and C. E. Hess. 1966. The effect of girdling upon root initiation carbohydrates and amino acids. *Proc. Amer. Soc. Hort. Sci.* 89:734–43.

264. Stoutemyer, V. T., O. K. Britt, and J. R. Goodin. 1961. The influence of chemical treatments, understocks, and environment on growth phase changes and propagation of *Hedera canariensis*. *Proc. Amer. Soc. Hort. Sci.* 77:552–57.

265. Strobel, G. A., and A. Nachmias. 1988. *Agrobacterium rhizogenes:* A root inducing bacterium. In T. D. Davis, B. E. Hassig, and N. Sankhla, eds.

Adventitious root formation in cuttings. Portland, OR: Dioscorides Press.

266. Strömquist, L., and J. Hansen. 1980. Effects of auxin and irradiance on the rooting of cuttings of *Pinus sylvestris. Physiol. Plant.* 49:346–50.

267. Struve, D. K. 1981. The relationship between carbohydrates, nitrogen and rooting of stem cuttings. *Plant Propagator* 27:6–7.

268. Struve, D. K., and M. A. Arnold. 1986. Aryl esters of IBA increase rooted cutting quality of red maple 'Red Sunset' softwood cuttings. *HortScience* 21:1392–3.

269. Struve, D. K., and R. D. Lineberger. 1988. Restoration of high adventitious root regeneration potential in mature *Betula papyrifera* Marsh. softwood stem cuttings. *Can. J. For. Res.* 18:265–69.

270. Strydom, D. K., and H. T. Hartmann. 1960. Absorption, distribution, and destruction of indoleacetic acid in plum stem cuttings. *Plant Physiol.* 35:435–42.

271. Strydom, D. K., and H. T. Hartmann. 1960. Effect of indolebutyric acid on respiration and nitrogen metabolism in Marianna 2624 plum softwood stem cuttings. *Proc. Amer. Soc. Hort. Sci.* 76:124–33.

272. Sun, W. Q., and N. L. Bassuk. 1993. Auxin-induced ethylene synthesis during rooting and inhibition of bud-break of 'Royalty' rose cuttings. *J. Amer. Soc. Hort. Sci.* 118:638–43.

273. Svenson, S. E., and F. T. Davies, Jr. 1995. Change in tissue elemental concentration during root initiation and development of poinsettia cuttings. *HortScience* 30:617–19.

274. Svenson, S. E., F. T. Davies, Jr., and S. A. Duray. 1995. Gas exchange, water relations, and dry weight partitioning during root initiation and development of poinsettia cuttings. *J. Amer. Soc. Hort. Sci.* 120:454–59.

275. Thimann, K. V., and F. W. Went. 1934. On the chemical nature of the root-forming hormone. *Proc. Kon. Ned. Akad. Wet.* 37:456–59.

276. Thimann, K. V., and J. B. Koepfli. 1935. Identity of the growth-promoting and root-forming substances of plants. *Nature* 135:101–2.

277. Tukey, H. B., and E. L. Green. 1934. Gradient composition of rose shoots from tip to base. *Plant Physiol.* 9:157–63.

278. Tukey, H. B., Jr., H. B. Tukey, and S. H. Wittwer. 1958. Loss of nutrients by foliar leaching as determined by radioisotopes. *Proc. Amer. Soc. Hort. Sci.* 71:496–506.

279. Van Overbeek, J., S. A. Gordon, and L. E. Gregory. 1946. An analysis of the function of the leaf in the process of root formation in cuttings. *Amer. J. Bot.* 33:100–7.

280. Van Sambeek, J. W., J. E. Preece, and M. V. Coggeshall. 2002. Forcing epicormic sprouts on branch segments of adult hardwoods for softwood cuttings. *Comb. Proc. Intl. Plant Prop. Soc.* 52:417–24.

281. Van Staden, J., and A. R. Harty. 1988. Cytokinins and adventitious root formation. In T. D. Davis, B. E. Haissig, and N. Sankhla, eds. *Adventitious root formation in cuttings.* Portland, OR: Dioscorides Press.

282. Vander Krieken, W. M., H. Breteler, M. H. M. Visser, and D. Mavridou. 1993. The role of the conversion of IBA into IAA on root regeneration in apple: Introduction of a test system. *Plant Cell Rpt.* 12:203–6.

283. Veierskov, B. 1988. Relations between carbohydrates and adventitious root formation. In T. D. Davis, B. E. Haissig, and N. Sankhla, eds. *Adventitious root formation in cuttings*. Portland, OR: Dioscorides Press.

284. Venverloo, G. J. 1976. The formation of adventitious organs. III. A comparison of root and shoot formation on *Nautilocalyx* explants. *Z. Pflanzenphysiol.* 80:310–22.

285. Vieitez, J., D. G. I. Kingston, A. Ballester, and E. Vieitez. 1987. Identification of two compounds correlated with lack of rooting capacity of chestnut cuttings. *Tree Physiol.* 3:247–55.

286. Vöchting, H. 1878. *Uber Organbildung in Pflanzenreich*. Bonn: Verlag Max Cohen.

287. Walker, R. I. 1940. Regeneration in the scale leaf of *Lilium candidum* and *L. longiflorum. Amer. J. Bot.* 27:114–17.

288. Wang, Y. T. 1987. Effect of temperature, duration and light during simulated shipping on quality and rooting of croton cuttings. *HortScience* 22:1301–2.

289. Warmke, H. E., and G. L. Warmke. 1950. The role of auxin in the differentiation of root and shoot primordia from root cuttings of *Taraxacum* and *Cichorium. Amer. J. Bot.* 37:272–80.

290. Weiser, C. J., and L. T. Blaney. 1967. The nature of boron stimulation to root initiation and development in beans. *Proc. Amer. Soc. Hort. Sci.* 90:191–99.

291. Welander, M. 1995. Influence of environment, fertilizer and genotype on shoot morphology and subsequent rooting of birch cuttings. *Tree Physiol.* 15:11–8.

292. Went, F. W. 1934. A test method for rhizocaline, the root-forming substance. *Proc. Kon. Ned. Akad. Wet.* 37:445–55.

293. White, J., and P. H. Lovell. 1984a. The anatomy of root initiation in cuttings of *Griselinia littoralis* and *Griselinia lucida. Ann. Bot.* 54:7–20.

294. White, J., and P. H. Lovell. 1984b. Anatomical changes which occur in cuttings of *Agathis australis* (D. Don) Lindl. 1. Wounding responses. *Ann. Bot.* 54:621–32.

295. White, J., and P. H. Lovell. 1984c. Anatomical changes which occur in cuttings of *Agathis australis* (D. Don) Lindl. 2. The initiation of root primordia and early root development. *Ann. Bot.* 54: 633–45.

296. Whitehill, S. J., and W. W. Schwabe. 1975. Vegetative propagation of *Pinus sylvestris. Physiol. Plant.* 35:66–71.

297. Wiesman, Z., J. Riov, and E. Epstein. 1989. Characterization and rooting ability of indole-3-butyric acid conjugates formed during rooting of mung bean cuttings. *Plant Physiol.* 91:1080–4.

298. Wilkerson, E. G., R. S. Gates, S. Zolnier, S. T. Kester, and R. L. Geneve. 2005a. Transpiration capacity in poinsettia cuttings at different stages and the development of a cutting coefficient for scheduling mist. *J. Amer. Soc. Hort. Sci.* 130:295–301.

299. Wilkerson, E. G., R. S. Gates, S. Zolnier, S. T. Kester, and R. L. Geneve. 2005b. Predicting rooting stages in poinsettia cuttings using root zone temperature-based models. *J. Amer. Soc. Hort. Sci.* 130:302–7.

300. Wilson, P. J., and J. Van Staden. 1990. Rhizocaline, rooting co-factors, and the concept of promoters and inhibitors of adventitious rooting—a review. *Ann. Bot.* 66:476–90.

301. Wilson, P. J. 1994. The concept of a limiting rooting morphogen in woody stem cuttings. *J. Hort. Sci.* 69:591–600.

302. Wilson, P. J. 1998. The discipline of forest tree propagation. *South. African For. J.* 183:47–52.

303. Wilson, P. J. 1998. Environmental preferences of *Eucalyptus globulus* stem cuttings in one nursery. *New Zealand J. For. Sci.* 28:293–303.

304. Wilson, P. J. 1999. The growth and form of potted mother plants of *Eucalyptus globulus* Labill. ssp. *globulus* in relation to the rooting ability of stem cuttings. *J. Hort Sci. Biotech.* 74:645–50.

305. Wilson, P. J., and D. K. Struve. 2004. Overwinter mortality in stem cuttings. *J. Hort. Sci. Biotech.* 79:842–49.

306. Woo, H. H., and W. P. Hackett. 1994. Differential expression of a chlorophyll a/b binding protein gene and a proline rich protein gene in juvenile and mature phase English ivy (*Hedera helix*). *Physiol. Plant.* 92:69–78.

307. Woodward, A., and B. Bartel. 2005. Auxin: Regulation, action and interaction. *Ann. Bot.* 95:707–35.

308. Wott, J. A., and J. H. B. Tukey. 1967. Influence of nutrient mist on the propagation of cuttings. *Proc. Amer. Soc. Hort. Sci.* 90:454–61.

309. Yarborough, J. A. 1932. Anatomical and developmental studies of the foliar embryos of *Bryophyllum calycinum. Amer. J. Bot.* 19:443–53.

310. Yarborough, J. A. 1936. Regeneration in the foliage leaf of Sedum. *Amer. J. Bot.* 23:303–7.

311. Zimmerman, P. W. 1933. Initiation and stimulation of adventitious roots caused by unsaturated hydrocarbon gases. *Contrib. Boyce Thomp. Inst.* 5:351–69.

312. Zimmerman, P. W., and F. Wilcoxon. 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. *Contrib. Boyce Thomp. Inst.* 7:209–29.

313. Zimmerman, P. W. 1937. Comparative effectiveness of acids, esters, and salts as growth substances and methods of evaluating them. *Contrib. Boyce Thomp. Inst.* 8:337–50.