

## What's New in the Biology of Cutting Propagation<sup>©</sup>

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### DEVELOPMENTAL ASPECTS OF ADVENTITIOUS ROOT FORMATION

The formation of adventitious roots is critical for successfully propagating cuttings. Cuttings with preformed adventitious roots, common to willow (*Salix*) or vines such as English ivy or creeping fig (*Ficus pumila*) - are easy to root (**Fig. 1**). *De novo* adventitious roots are formed “anew” by creating a new meristematic area which is stimulated by removing a shoot or leaf-bud cutting from the stock plant (**Fig. 1**). Wound-induced *de novo* adventitious roots are easy to form in herbaceous plants, such as chrysanthemum – and can be extremely difficult in woody species, such as oaks (*Quercus*) – particularly when cuttings are taken from physiologically mature stock plants (Davies et al., 2018).

*De novo* adventitious root formation is composed of four stages: 1) dedifferentiation of parenchyma cells in the phloem ray area, 2) formation of root initials, 3) formation of a fully developed meristematic area — the root primordia, and 4) elongation of the root primordia through the cortex and periderm (Davies et al., 2018).

What separates out an easy vs. difficult to root species is the ability to complete the first two stages: dedifferentiation and root initial formation (early organization of the root primordia). If a cutting can complete these first two steps, it will successfully root — provided the proper environmental conditions are maintained.

While we have gotten to be pretty good at manipulating stock plants, using auxins

and controlling environmental conditions to maximize commercial rooting of cuttings — there are still many woody plant species that are too difficult to root in acceptable numbers. It would be great if we could manipulate a single gene to enhance rooting, but adventitious root formation of cuttings is a complex process involving many genes.

Genes are important because they are expressed through the production of proteins, some of which are enzymes which help drive chemical reactions. Genes can be upregulated (turned on) or down regulated (turned off). During adventitious root development in lodgepole pine (*Pinus contorta*) hypocotyls cuttings - there are some 220 genes are differentially expressed (**Fig. 2**) (Brinker et al., 2004). In rooting easy-to-root petunia cuttings – there are some 1,354 genes that were induced (upregulated) and some 607 proteins identified during various stages of rooting (Ahkami et al. 2014). Why is this relevant? Well, differences between easy- and difficult-to-root species are because of gene expression. Hence, a mature, difficult-to-root plant species has certain genes that are being turned off or on, whereas the juvenile, more easy-to-root form of the same species differs in its gene expression, even though the genome (gene composition) is the same between the two. Bottom line: we still have a long way to go in understanding and utilizing the molecular biology of rooting.

### **AUXINS, PHYTOHORMONES, CROSS-TALK AND ROOTING**

For commercial rooting of cuttings: the auxins - IBA and NAA or in combination - are applied. However there are other phytohormones involved in the rooting process. And they communicate with each other – through phytohormone interactions and cross talk during the four stages of rooting. There is signaling (cross-talk) among hormones that impacts gene and protein expression. While auxin is considered the “master regulator” of rooting - higher endogenous

levels stimulate the early events of rooting (Stage I), while lower auxin is desirable for root initial formation through the elongation of roots (Stages II, III and IV) (**Fig. 3**).

Phytohormones such as cytokinins and ethylene, which inhibit the early events of rooting - enhance rooting during later events (**Fig. 3**). There is cross- talk with a high auxin/low cytokinin ratio favoring the early events of adventitious root formation, while a low auxin/high cytokinin ratio favors elongation of roots and adventitious bud formation (of leaf cuttings). However, in commercial practice - only auxins are used to stimulate rootig.

### **AUXINS AND THEIR APPLICATION**

To enhance rooting of stem cuttings, auxins are typically applied as 1–5 second quick-dips, which entails inserting the cutting base into the auxin solution. Auxins are also applied as spray applications to foilage or as talc powder applications to the base of the cutting, and sometimes combinations of a liquid quick-dip followed by a powder application are used with more difficult-to-root species (Davies et al., 2018).

Spray applications of auxins can be applied at the end of the day with the mist system turned off, or early morning prior to turning on the mist system. This avoids worker contact with auxin, since just the protected applicator applies the auxin as spray. Some commercial nurseries use auxin spray applications of Hortus Water soluble Salts from 500 to 1,500 ppm (Drahn, 2007). It is best to apply aqueous auxin sprays within the first 48 hours of sticking the cuttings (Davies et al., 2018).

In a study of difficult-to-root, mature *Ficus pumila* – applying spray applications of auxin (IBA) within the first nine days enhanced rooting, but delaying to 15 days after sticking cuttings – greatly reduced rooting response (**Fig. 4**). The optimum window to apply auxin was lost – in part because of the loss of cell receptivity/ sensitivity to respond to auxin (Davies et al., 2018).

Auxins can speed-up rooting of easy-to-root species, but are not required (**Fig. 5**). They are essential in propagating moderately, easy-to-root plants such as Camelia – but have little effect on recalcitrant species such as pawpaw (*Asimina*). Easy-to-root—plants that have all the essential endogenous substances (root morphogens) plus auxin needed for rooting. Auxin is needed to enhance rooting of moderately easy-to-root plants in which the naturally occurring root morphogen(s) are present in ample amounts - but endogenous auxin is limited. Difficult-to-root (recalcitrant)—plants 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. Hence, external application of auxin gives little or no rooting enhancement (**Fig. 5**).

Wilson (1994) proposed that a rooting morphogen can be assumed to induce roots in woody stem cuttings. The interaction between a rooting morphogen(s) of vascular origin and potential sites for root initiation is likely to be dynamic and variable. Rooting of difficult-to-root species is complicated. 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). Generally, cuttings that do not root are considered deficient in rooting promoters, including hormones.

## **CHRONOLOGICAL VS. PHYSIOLOGICAL AGE AND MANIPULATION OF STOCK PLANTS**

Juvenile-mature gradients occur in seedling trees from the base of the tree to the top. The juvenile root-shoot junction, which is “*physiologically juvenile*” has a high rooting potential — even though chronologically it may be decades old (**Fig. 6**).

Flowering occurs in the “*physiologically mature*” part of tree at the apical part, even though some of the flowering shoots may be *chronologically* only several months old; shoots taken from this region generally have low rooting potential. Juvenile

structures arising from the “*cone of juvenility*” [dark area] near the base (crown) of the tree include: adventitious root “suckers,” watersprouts (epicormic shoots), and sphaeroblasts. Stump sprouts result from severe pruning, and shoots from heavily pruned or hedged bushes (**Fig. 6**). Rooting potential is highest from these structures closest to the cone of juvenility.

### **MANIPULATING STOCK PLANTS TO ENHANCE ROOTING**

Stock plants can be manipulated to enhance rooting of woody plant species. One technique is to force softwood cuttings (epicormic shoots) from woody stem segments to propagate hardwood species (**Fig. 7**). Using river birch, silver maple, and stem segments of other woody species - epicormic shoots were forced under intermittent mist, later harvested as softwood cuttings - and rooted under mist (Preece and Reed, 2007).

### **LONG CUTTINGS**

The majority of cuttings are typically 5–20 cm (2–8 in.) long. However, long cuttings of 50–152 cm (20–60 in.) are used to propagate ornamental and fruit crops with enhanced rooting success (Spethmann, 2007). Successful rooting with long, semi-hardwood cuttings has been done with rose (*Rosa* ‘Pfaenders’) rootstock, elm (*Ulmus* ‘Regal’), sycamore maple (*Acer pseudoplatanus*), pear (*Pyrus* ‘Williams Christ’), *Tilia cordata* (linden), and English oak (*Quercus robur*) (**Fig. 8**). Part of the advantage of long cuttings may be that pruning management of the stock plants - enhances rejuvenation and rooting (Spethmann, 2007). Long cuttings are also propagated using fog systems, rather than intermittent mist systems.

### **ENVIRONMENTAL CONTROLS TO ENHANCE ROOTING**

Besides the selection and manipulation of stock plants - optimizing environmental conditions can enhance propagation success and transplanting of rooted liner plants. Plants can sense changes in

light and temperature, which are often referred to as “Environmental Cues”. As an example, moderately high temperature (and long-day conditions) can stimulate auxin production and decrease cytokinin production, whereas cooler temperatures (less than 20°C/ 68°F) (and short-day conditions) reduce auxin and increase cytokinin production (Davies et al., 2018). For rooting of stem cuttings - it would be optimal to expose cuttings to moderately high temperature to stimulate auxin, whereas cooler temperatures are ideal for leaf cuttings to stimulate cytokinins and subsequent adventitious bud formation.

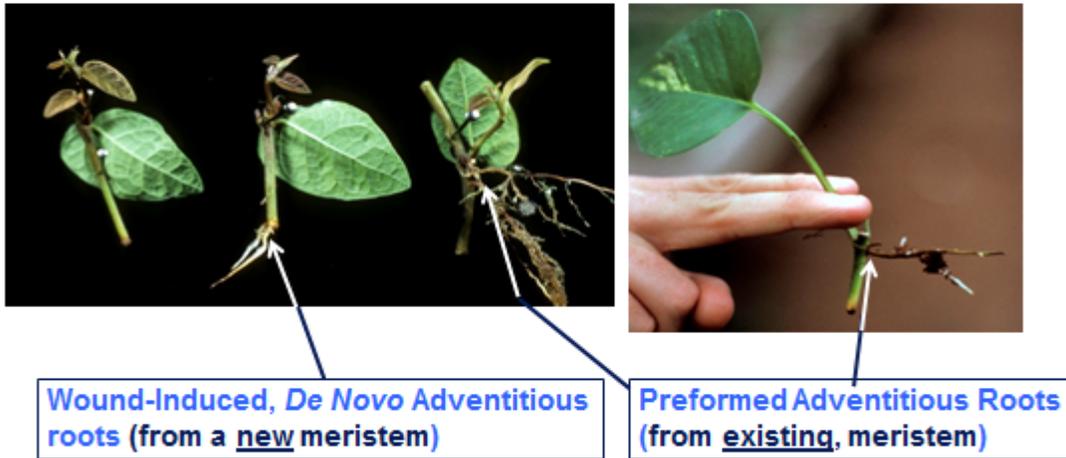
Often overlooked are the secondary causes of poor rooting. Many leafy woody (and herbaceous) cuttings have major limitations affecting their survival (i.e., they are quite susceptible to drought and temperature stress prior to developing roots) - and require good management to avoid mortality. In rooting of poinsettia cuttings, a temperature of 27°C (80°F) was optimal, whereas 32°C (90°F) depressed rooting (Wilkerson et al. 2005) (**Fig. 9**). Auxin requirements are lower during the later stages of root elongation. Hence, to reduce potential desiccation stress and maximize rooting - it would be best to maintain cuttings at 27°C (80°F).

During the initial week or two of cutting propagation, it is not necessary to maintain high light conditions under mist. In a study with poinsettia, relative water content, xylem water potential, net photosynthesis and stomatal conductance were initially low with unrooted cuttings (Svenson et al., 1995). Only when cuttings started to form root primordia and adventitious roots first became visible did stomatal conductance and net photosynthesis start to increase (**Fig. 10**). The take home message is that prior to visible roots — keep light levels low to reduce vapor pressure deficit (VPD) – drought stress. When roots start to form, increase the light so plants can take advantage of higher photosynthetic rates to improve root development and production of rooted liners.

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## Adventitious Roots



**Figure 1.** Preformed adventitious roots on *Ficus pumila* and philodendron and *de novo* adventitious roots that are formed from a new meristem after cuttings have been taken.

Days	Phase of Development	Up ↑ or Down ↓ Gene Regulation
0 to 3	<b>Dedifferentiation</b>	<ul style="list-style-type: none"> <li>↑ Cell replication</li> <li>↑ Cell wall weakening</li> <li>↑ Water stress</li> <hr/> <li>↓ Cell wall synthesis</li> <li>↓ Auxin transport</li> <li>↓ Photosynthesis</li> </ul>
3 to 6	<b>Root Initiation</b>	<ul style="list-style-type: none"> <li>↑ Flavonoid pathway enzymes</li> </ul>
6 to 9	<b>Root Primordia</b>	<ul style="list-style-type: none"> <li>↑ Auxin transport</li> <li>↑ Auxin responsive</li> <li>↑ Cell wall synthesis</li> <li>↑ Hypersensitive response proteins</li> <li>↑ Pathogenesis proteins</li> <hr/> <li>↓ Cell wall weakening</li> <li>↓ Cell wall modification</li> <li>↓ Water stress</li> </ul>
9 to 12	<b>Root</b>	<ul style="list-style-type: none"> <li>↑ Auxin transport</li> </ul>
12 to 33	<b>Elongation</b>	<ul style="list-style-type: none"> <li>↓ Water stress</li> <li>↓ Cell replication</li> </ul>

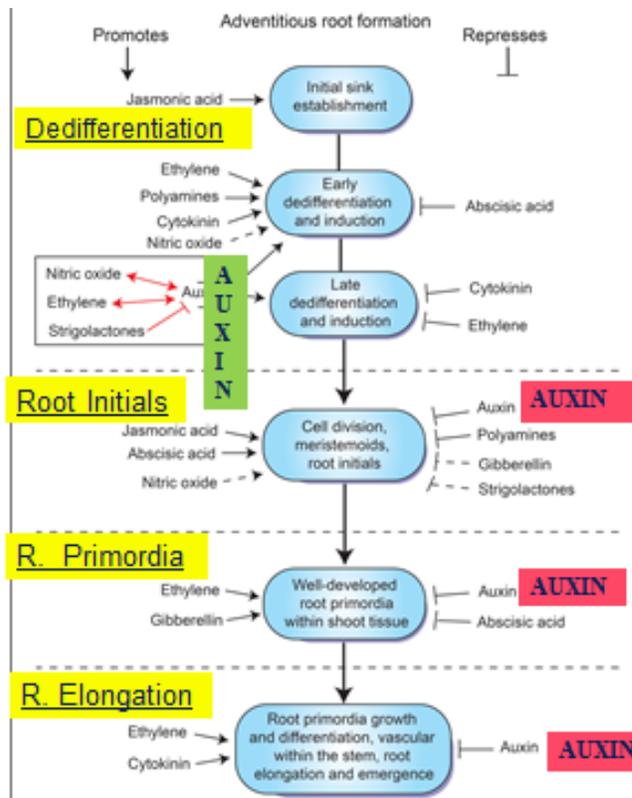
[Gene expression during the different stages of Rooting](#)  
Lodgepole pine (*Pinus contorta*) hypocotyl cuttings.

Gene – segment of DNA – proteins – enzymes – drive chemical reactions

220 genes & encoding proteins were up-regulated (↑increased expression) or down-regulated (↓decreased)

[Rooting of petunia](#)  
1354 genes induced/ upregulated; 607 proteins produced during rooting

**Figure 2.** 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) (Brinker et al., 2004).



### [Phytohormone Interactions and Cross Talk during 4 Stages of Rooting](#)

**Cross Talk:** Signaling among hormones – impacts gene & protein expression.

***Auxin is the Master Regulator of Rooting:***  
commercially use: IBA, NAA

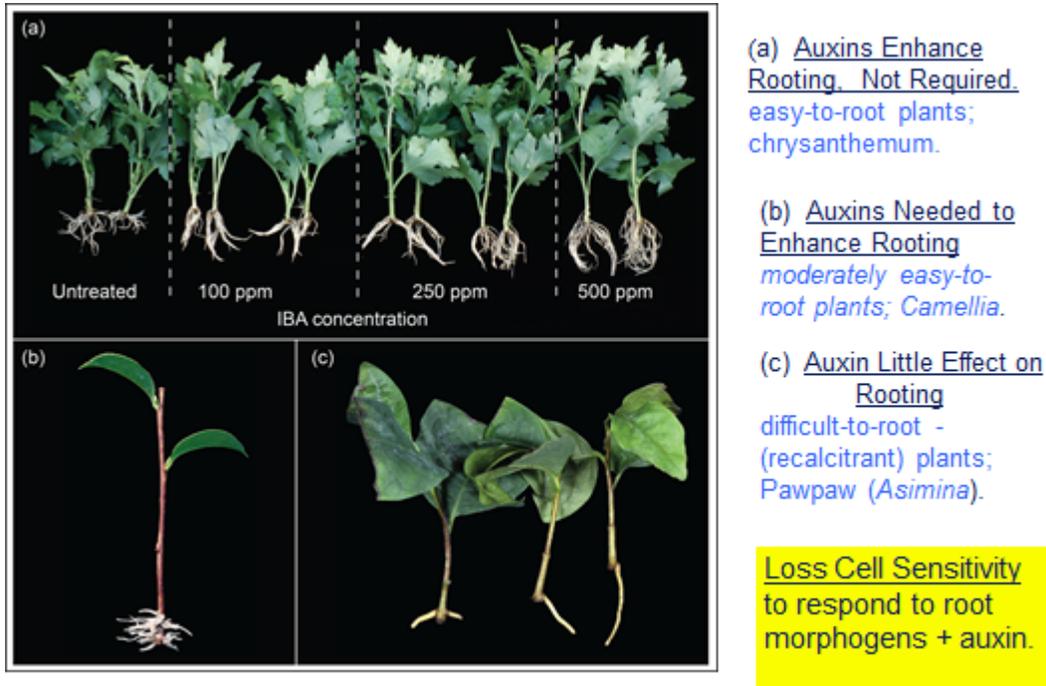
**Auxin Promote Rooting Stages**

**Auxin Inhibit Rooting Stages**

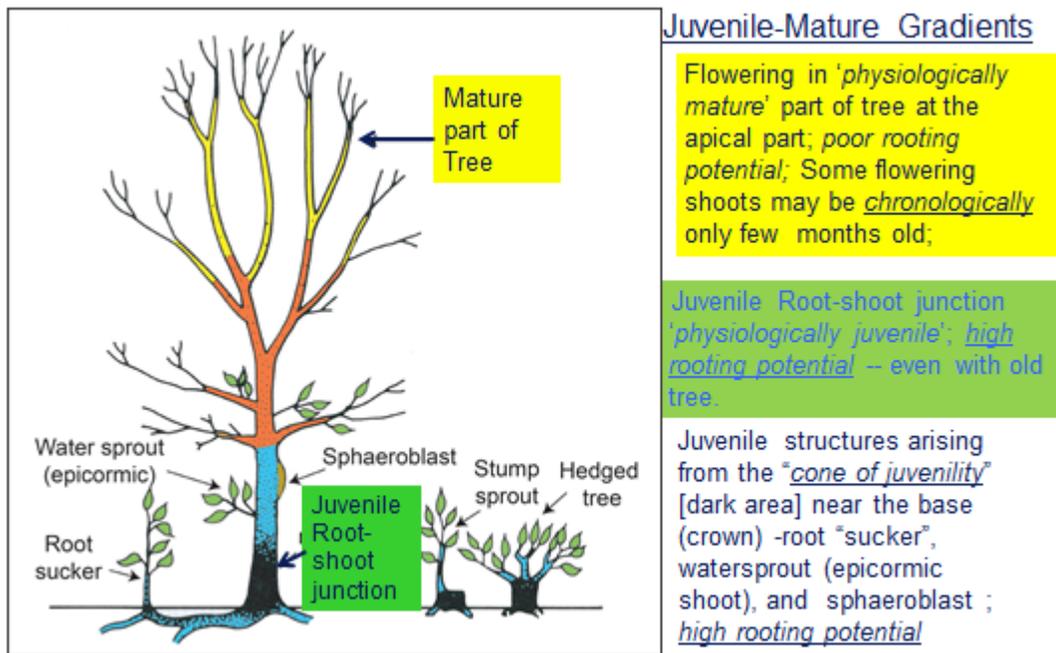
**Figure 3.** Phytohormone interactions and cross-talk during the four stages of rooting. There is signaling among hormones that impacts gene and protein expression. While auxin is considered the “master regulator” of rooting - higher endogenous levels stimulate the early events of rooting, while reduced auxin is desirable for root initial formation through the elongation of roots (da Costa et al., 2013 and Steffens and Rasmussen, 2016).



**Figure 4.** The effect of applying auxin as a foliar spray application to mature, difficult-to-root *Ficus pumila* leaf-bud cuttings. Delaying auxin (IBA) application to 15 days after sticking cuttings dramatically reduced rooting (Davies et al., 2018).



**Figure 5.** Auxins can (a) speed-up rooting of easy-to-root species, but are not required; (b) are needed to enhance rooting of moderately, easy-to-root species; or (c) have little effect on rooting difficult-to-root species.



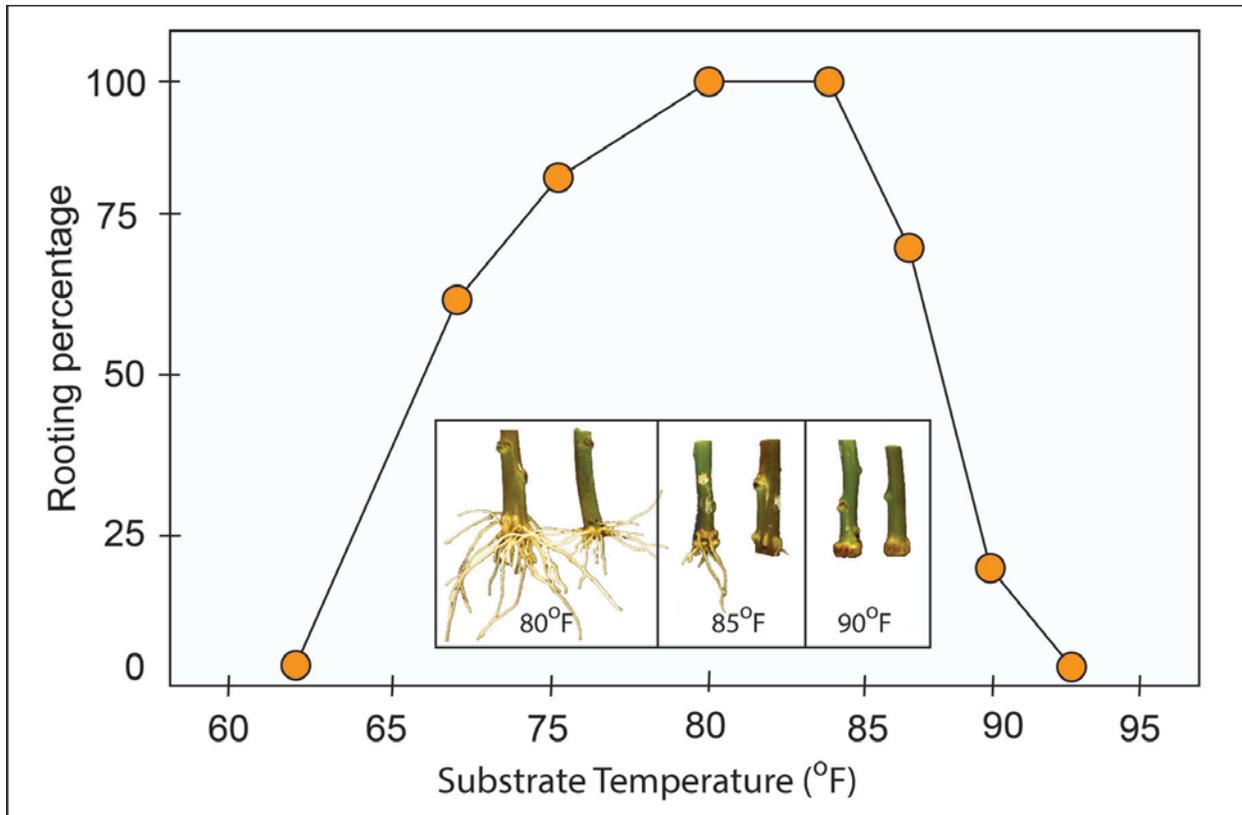
**Figure 6.** Juvenile-mature gradients occur in seedling trees from the base of the tree to the top. The juvenile root-shoot junction, which is "*physiologically juvenile*" with high rooting potential — even though chronologically it may be decades old. Flowering occurs in the "*physiologically mature*" part of tree at the apical part, even though some of the flowering shoots may be *chronologically* only several months old; shoots taken from this region generally have low rooting potential. Juvenile structures arising from the "*cone of juvenility*" [dark area] near the base (crown) of the tree include: adventitious root "sucker," watersprout (epicormic shoot), and sphaeroblast. Stump sprout from severe pruning, and shoots from heavily pruned or hedged bush. Rooting potential is highest from these structures close to the cone of juvenility (Davies et al., 2018).



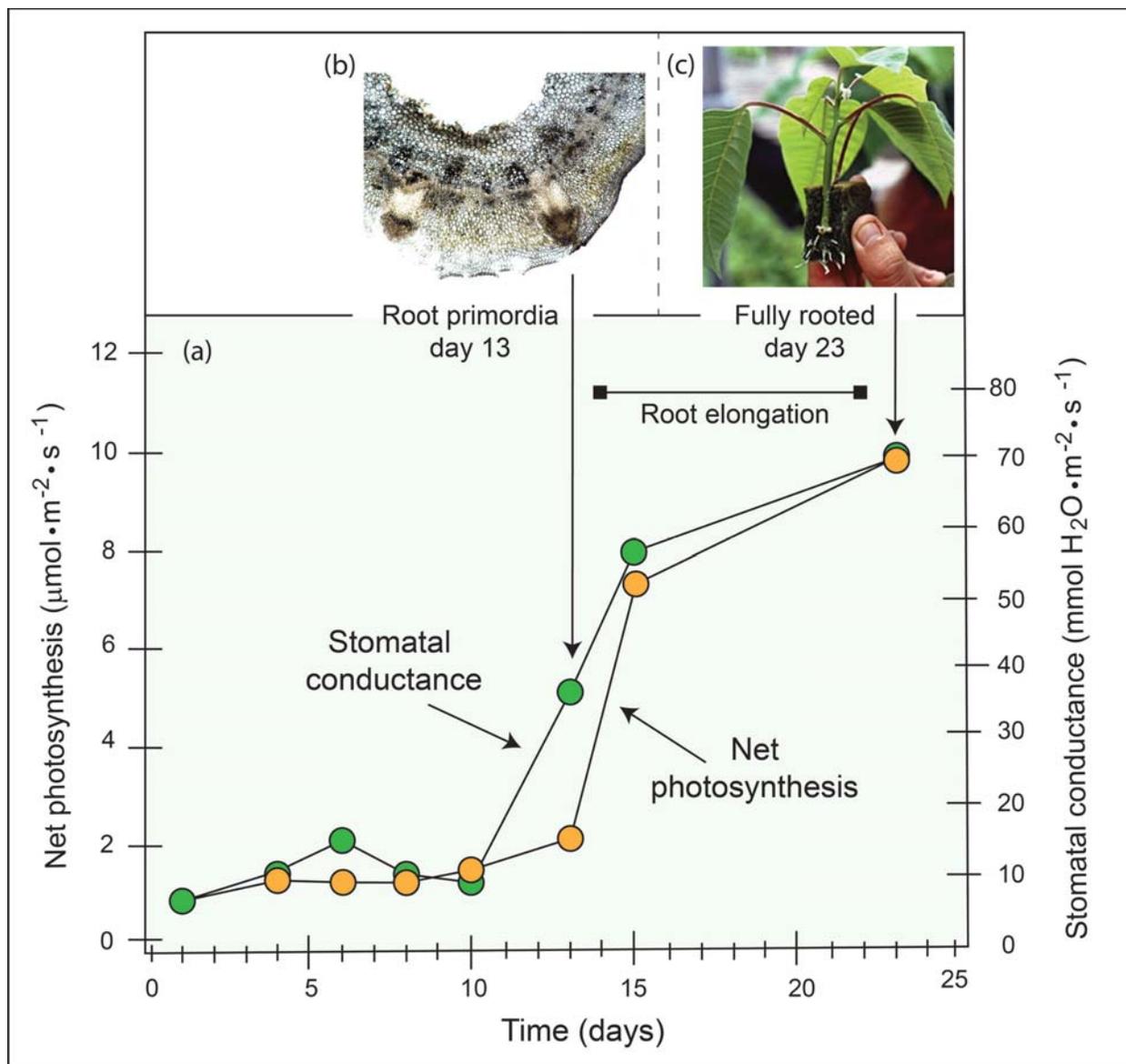
**Figure 7.** Forcing softwood cuttings from woody stem segments to propagate hardwood species. (a) River birch shoot forcing under intermittent mist, (b) shoot forcing of white ash and silver maple, and (c) epicormic shoots from forced silver maple — will later be harvested as softwood cutting and rooted under mist (Preece and Reed, 2007).



**Figure 8.** (a) A majority of cuttings are 5–20 cm (2–8 in.) long. However, long cuttings of 50–152 cm (20–60 in.) are used to propagate ornamental and fruit crops. (b) Long, rooted semi-hardwood cuttings of rose (*Rosa* 'Pfaenders' rootstock for standard roses) in a greenhouse propagation bed. (c) 9-month-old rooted liners of elm (*Ulmus* 'Regal'), sycamore maple (*Acer pseudoplatanus*), pear (*Pyrus* 'Williams Christ'), (Linden) *Tilia cordata*, and English oak (*Quercus robur*) propagated from long cuttings. Part of the advantage of long cuttings may be the pruning management of the stock plants enhances rejuvenation and rooting (Spethmann, 2007).



**Figure 9.** 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 that during the later stages of root elongation (Wilkerson et al., 2005).



**Figure 10.** (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% rooting (Svenson et al., 1995).