Back to the Basics and What's New in Propagation©

Fred T. Davies, Jr.
Department of Horticultural Sciences, Texas A&M University, College Station, Texas 77843-2133
Webpage: http://aggie-horticulture.tamu.edu/faculty/davies/index.html
Email: f-davies@tamu.edu

DEVELOPMENTAL ASPECTS OF ADVENTITIOUS ROOT FORMATION

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 primordial through the cortex and periderm (Hartmann et al., 2011). 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 we know that adventitious root formation of cuttings is a complex process involving many genes. It was recently reported that some 220 genes are differentially expressed during adventitious root development in lodgepole pine (*Pinus contorta*) hypocotyls cuttings (Brinker et al., 2004). Genes were up-regulated (increased gene expression) or down-regulated (decreased expression) during various stages of rooting (Fig. 1). Genes are important because they are expressed through the production of proteins, some of which are enzymes which help drive chemical reactions. 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.

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. 2). 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. 2). Rooting potential is highest from these structures close to the cone of juvenility.

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 1. 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).

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. 3). Using river birch, silver maple, and stem segments of other woody species, epicormic shoots are forced under intermittent mist, and later harvested as softwood cuttings and rooted under mist (Preece and Reed, 2007).

Various layering systems are used to enhance rooting. With Monterey pine *(Pinus radiata*), which is the most important timber species in New Zealand, trench layering is used in clonal propagation systems. For example, stoolbeds are used and mother plants are topped and their shoots pinned down to produce fascicle cuttings.

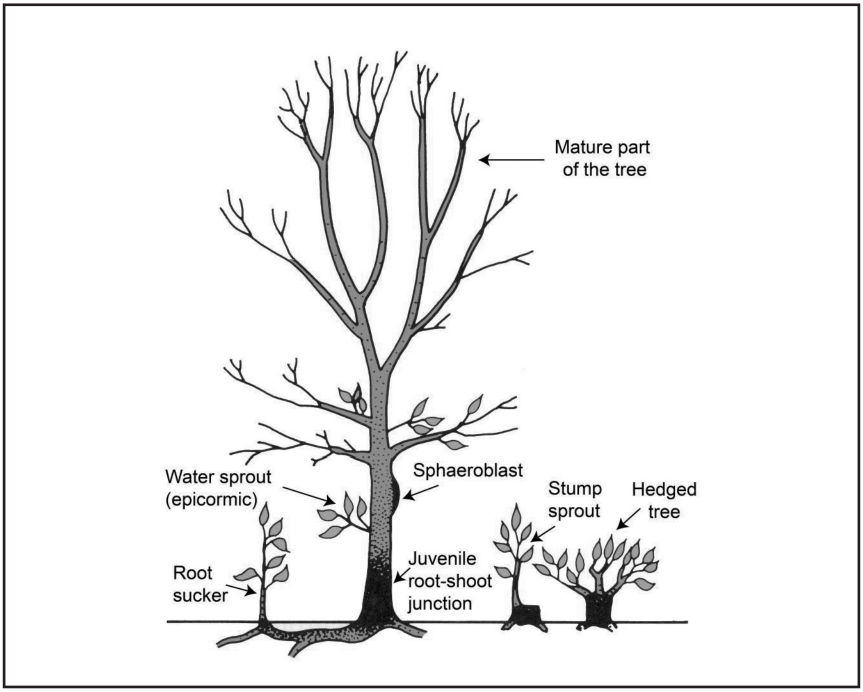

Figure 2. Juvenile-mature gradients occur in seedling trees from the base of the tree to the top. The juvenile root-shoot junction, which is "*physiologically juvenile*" with high rooting potential — even though chronologically it may be decades old. Flowering occurs in the "'*physiologically mature*" 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," water-sprout (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 (Hartmann et al., 2011).

Etiolation or the absence of light can also be used in stock plant manipulation. Etiolation frames are placed over stock plant hedges of lilac (*Syringa vulgaris*) (Fig. 4). *Right:* There is improved rooting following etiolation of *S. vulgaris* 'Madame Lemoine' and *S. vulgaris* 'Charles Joly', compared with cuttings from nonetiolated stock plants which have poor rooting (Fig. 4) (Howard and Harrison-Murray, 1997). Sometimes etiolation of stock plants is used in combination of wrapping black Velcro containing an auxin talc and wrapping it around the base of the etiolated shoots, which are gradually exposed to higher light levels. Enhanced rooting occurs with etiolated Mountain-laurel or spoonwood (*Kalmia latifolia*) 'Ostbo Red' treated with Velcro-impregnated IBA powder. After the shoots green up, they are removed and rooted under mist as softwood cuttings. Highest rooting occurred with Turkish hazel (*Corylus colurna*) shoots that were etiolated and banded with velcro, than made into cuttings and treated with 2000 ppm IBA (Maynard and Bassuk, 1990).

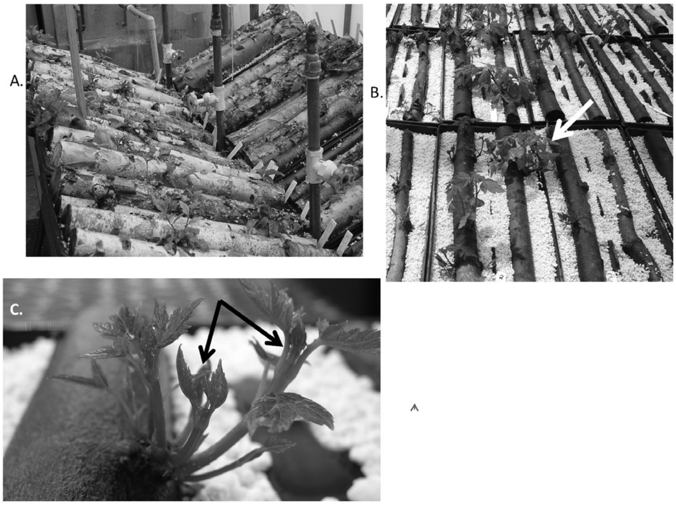

Figure 3. Forcing softwood cuttings from woody stem segments to propagate hardwood species. (a) River birch shoot forcing under intermittent mist, (b) shoot forcing of white ash and silver maple, and (c) epicormic shoots from forced silver maple — will later be harvested as softwood cutting and rooted under mist (Preece and Reed, 2007).

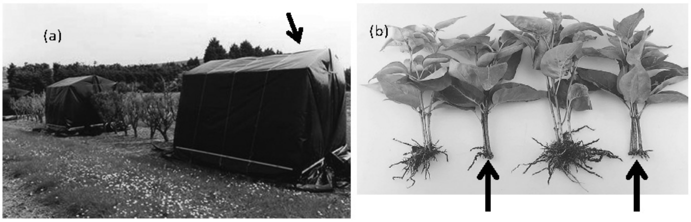

Figure 4. *Left:* Etiolation frames [arrow] in place over stock plant hedges of lilac (*Syringa vulgaris*). *Right:* Improved rooting following etiolation of *S. vulgaris* 'Madame Lemoine' (far left) and *S. vulgaris* 'Charles Joly' (second from right). Cuttings from nonetiolated stock plants have poor rooting (second from left and far right [arrows]) (Howard and Harrison-Murray, 1997).

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. 5). Part of the

Figure 5. (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).

advantage of long cuttings may be the pruning management of the stock plants enhances rejuvenation and rooting (Spethmann, 2007). Long cuttings are propagated using fog systems, rather than intermittent mist systems.

AUXINS AND THEIR APPLICATION

Auxins are used to enhance rooting of cuttings and are typically applied as 1–5 second quick-dips, which entails inserting the cutting base into the auxin solution (Fig. 6). Auxins are also applied 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 (Hartmann et al., 2011). Some common auxin formulations include: (a) Dip'N Grow liquid rooting compound, (b) Woods liquid rooting compound, (c) Seradix and Hormodin rooting powders, and (d) Hortus IBA water-soluble salts. Auxin solutions used to dip cuttings are discarded at the end of the day. Auxin preparations are stained with food dye to denote different concentrations and stored in color-coded containers. Refrigeration of liquid auxin formulations is used to extend their shelf-life.

The auxin, IBA, is a "pesticide" with an LD_{50} of 100, e.g., it is 15-fold more toxic than the insecticide malathion (LD_{50} of 1,500). While no one has been fatally poisoned by IBA, one needs to be careful in handling auxin. Spray applications of

Figure 6. (a, b) Liquid auxin quick-dips of 1 to 5 seconds. (c) Application of auxin by talc. (d) Spray application at end of day reduces exposure of the propagators to auxin (Hartmann et al., 2011).

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). In Holland aqueous IBA is applied to chrysanthemum cuttings in low concentrations (100 ppm) using an overhead boom system (Fig. 7). The trick to using aqueous auxin sprays is to apply it within the first 48 h of sticking the cuttings (Hartmann et al., 2011).

TIMING, SCHEDULING, AND MAINTAINING THE PLANT'S MOMENTUM

It is important to maintain stock plants that are nutritionally fit and under optimal irrigation regimes. Timing and scheduling is important from "maintaining the plant's momentum" to minimize stress, to harvesting the optimum propagation wood — for maximum rooting and reducing the propagation time under mist. Examples are shown in Figure 8 from: (a) harvesting the right kind of cutting wood during the optimum season — i.e., shoot tips of *Nandina* with no brown wood, trimmed to 4 cm, (b) harvesting cuttings early during the day when water status is optimum are plants are stress-free, and (c,d) storing cuttings in cool-moist refrigerated environments until they can be processed and stuck. Storage under low light, high RH and cooler temperatures helps to control vapor pressure deficit (VPD). Processed cuttings are covered with moist burlap until stuck to minimize plant stress.

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

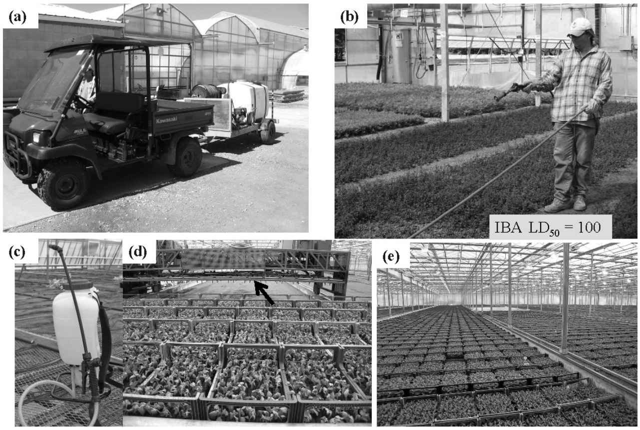

Figure 7. (a, b) Applying aqueous sprays of auxin to cuttings with a high pressure system [the applicator will wear a protective suit, glasses, gloves and respirator during application] (Drahm, 2007). (c) Spray application of auxin can also be applied with back-pack sprayer, or (d,e) with a spray boom applying IBA at 100 ppm in a chrysanthemum propagation house (courtesy Kees Eigenraam and Joel Kroin).

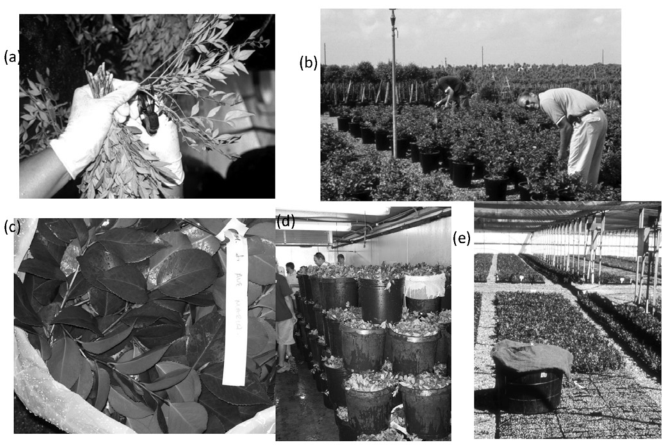

Figure 8. Timing and Scheduling: "Maintaining the plant's momentum" to minimize stress. (a) Harvesting the right kind of cutting wood during the optimum season — i.e., shoot tips of *Nandina* with no brown wood, trimmed to 4 cm. (b) Harvesting cuttings early during the day when plants are stress-free. (c,d) Storing cuttings in cool-moist refrigerated environments until they can be processed and stuck. (e) Processed cuttings covered with moist burlap until stuck.

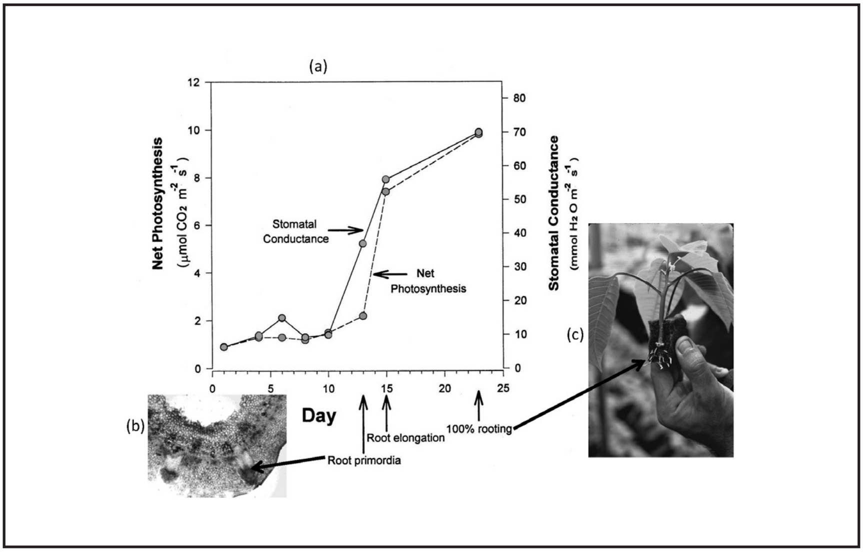

Figure 9. (a) Influence of adventitious root formation on gas exchange of poinsettia (*Euphorbia pulcherrima* cv. Lilo) cuttings. (b) Root primordia were microscopically observed at day 13, when photosynthesis began to increase. (c) Maximum photosynthesis was at 100% rooting (Svenson et al., 1995).

to form root primordia and adventitious roots first became visible did stomatal conductance and net photosynthesis start to increase (Fig. 9). The take home message is that prior to visible roots — keep light levels low to reduce VPD. 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.

WATER MANAGEMENT OF CUTTINGS

Proper water management is critical for maintaining proper tissue moisture — if cuttings are to survive, successfully form adventitious roots and develop into commercially acceptable rooted liners. About 1%–2% of water utilized is needed for photosynthesis and plant growth, while the remaining 98% of water is lost to transpiration and the subsequent cooling of leaves. Evaporative cooling occurs during transpiration as water passes from a liquid to gaseous phase (vapor). Transpiration is the "engine" that pulls (lifts) water up from the roots. Unlike people, who can move and find a more comfortable location, a cutting lacks mobility so it needs to do its best to reduce the heat load, which it does through transpiration.

There are three environmental factors that effect transpiration: light, temperature, and humidity. Light causes plants to transpire more rapidly, stimulates the opening of the stomata and warms the leaf. Temperature increases transpiration since water evaporates more quickly. Humidity affects the diffusion of water as a vapor from the leaf through the stomata into the surrounding drier air. Water travels from a high potential (saturated internal leaf cavities) to a lower potential (unsaturated, drier) surrounding air outside the leaf (Davies, 2005).

WATER RELATIONS OF CUTTINGS

The water relations of cuttings is a balance between transpirational losses and the uptake of water. Water travels from the soil (propagation media) through the roots into the stems and into the leaves where photosynthesis and transpiration occurs. Cells must maintain adequate turgor for growth and for initiation and development of adventitious roots. Root meristematic areas also produce a phytohormone, ABA or abscisic acid, which is a chemical signal for drought. As the surrounding soil (medium) dries, ABA travels through the xylem from the roots to leaves and causes the guard cells to collapse, which closes the stomata and helps to regulate the loss of water.

THE PROBLEM

Since cuttings initially do not have roots, they cannot produce ABA to control water loss, and lack effective organs to replace transpired water lost. Cuttings take up water poorly through the base of the stem — until adventitious roots are formed. The cutting base and any foliage immersed in the propagation media are main entry points of water until adventitious roots form. Water absorption through leaves is not a major source/contributor of water balance. It is Important to maintain hydraulic contact between the cutting base and propagation media — thus improving water uptake of cuttings. Wounding increases the contact area between the cutting base and propagation medium for more optimum water uptake of cuttings.

CONTROL OF WATER LOSS IN CUTTINGS (WILKERSON ET AL., 2005)

Intermittent Mist is the most common system for propagating cuttings. Mist is composed of water droplets that average >50 µm, and have a size range of 50 to 100 µm (the diameter of a human hair strand is around 100 µm). The mist condenses and forms a film of water on the leaf surface. Water evaporates from the leaf surface rather than from internal water in leaf tissue. Fog systems produces fine water droplets that average around 15 µm. Fog has a high surface to volume ratio that allows it to remain suspended in air as a vapor (gas) to maximize evaporation. Fog does not condense, which avoids the over-saturation of media and foliar leaching that occurs with mist. Problems with fog systems include high costs and high maintenance requirements — including clogging and wearing out of nozzles. Filtration/deionizing systems are required to remove any salts from the water supply. Contact systems and nonmisted enclosures reduce water loss from foliage and the condensation increases the relative humidity of the air. These systems are simple, inexpensive, and cost-effective. There is minimal condensing, which avoids the over-saturation of media and foliar leaching that occurs with mist. This system works well with hardwood and semi-hardwood cuttings of difficult-to-root species that require longer propagation times. While inherently cheaper, there are problems with contact systems/nonmisted enclosures. It is critical to control irradiance and subsequent heat load via shade and temperature control. The system easily traps heat via light irradiance, which adversely can increase the VPD by reducing RH of air and increasing the air and leaf temperature.

SUMMARY OF OPTIMIZING WATER RELATIONS OF CUTTINGS (DAVIES, 2005)

- Maintain the plant's momentum by propagating during optimum rooting periods, collecting cuttings early in the day and minimizing plant stress.
- Control stress—light, temperature, and humidity (RH) to reduce the vapor pressure deficit (VPD), i.e. an atmosphere of low evaporative demand decreases transpirational losses from cuttings.
- Do not increase light until cuttings start to form adventitious roots.
- Apply just enough mist to form a thin film of water on leaf surface.
- Use a loose propagation media for proper aeration.
- Group cuttings in propagation by species requirement for moisture, i.e. *Zelkova* and Chinese elm have a lower tolerance for mist and saturated propagation media than River Birch.

SYSTEMS FOR STICKING CUTTINGS — DIRECT ROOTING

Systems for sticking cuttings. More cuttings can be rooted per unit area in a conventional plastic rooting flat, but additional labor is needed to initially transplant rooted cuttings into small liner pots, and then transplant into larger containers or produce as a field-grown crop. Direct sticking (direct rooting) allows cuttings to be rooted directly into small liner pots which saves labor and avoids transplant shock to the root system (Fig. 10). With some species it is possible to direct root into large 3.8-liter (1-gal) containers with no transplanting steps (Fig. 10).

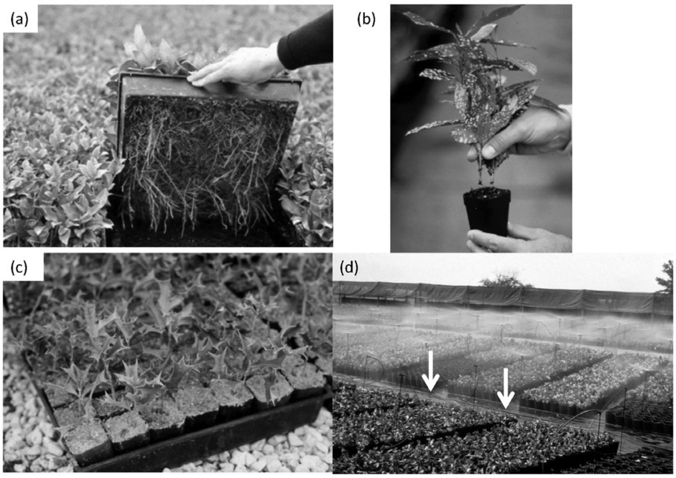

Figure 10. Systems for sticking cuttings. (a) More cuttings can be rooted per unit area in a conventional plastic rooting flat, but additional labor is needed to initially transplant rooted cuttings into small liner pots, and then transplant into larger containers or produce as a field-grown crop. (b and c) Direct sticking (direct rooting) allows cuttings to be rooted directly into small liner pots which saves labor and avoids transplant shock to the root system. (d) Direct sticking into large 3.8-liter (1-gal) containers with no transplanting steps. Notice sloped incline (arrow) for better drainage.

DO NOT PROPAGATE ALL ITEMS IN YOUR INVENTORY

It is not always cost-effective to propagate all inventory items in a nursery or greenhouse operation. It may be more cost-effective to buy in rooted liners, seedling plugs, tissue culture-produced liners, and/or grafted or budded liner trees. Custom propagators can be more efficient and effective in producing selected liner plants. A good example of this is the millions of unrooted cuttings being produced and shipped internationally from Central America to U.S.A. nursery and greenhouse companies to root and finish off the plants. Propagators need to be diligent in their handling, storing, processing, and sticking.

SANITATION & DISEASE CONTROL

Good sanitation and disease control are part of best management practices (BMPs) to minimize plant stress, production costs, and enhance plant quality. Some correct and incorrect ways to propagate are illustrated in Fig. 11. Poor sanitation with algae build-up (arrow) — can harbor disease, insects and creates a poor propagation and work environment for personnel. (b) Good cultural & chemical practices: Sanitizing concrete pads before starting the next propagation crop. Some common

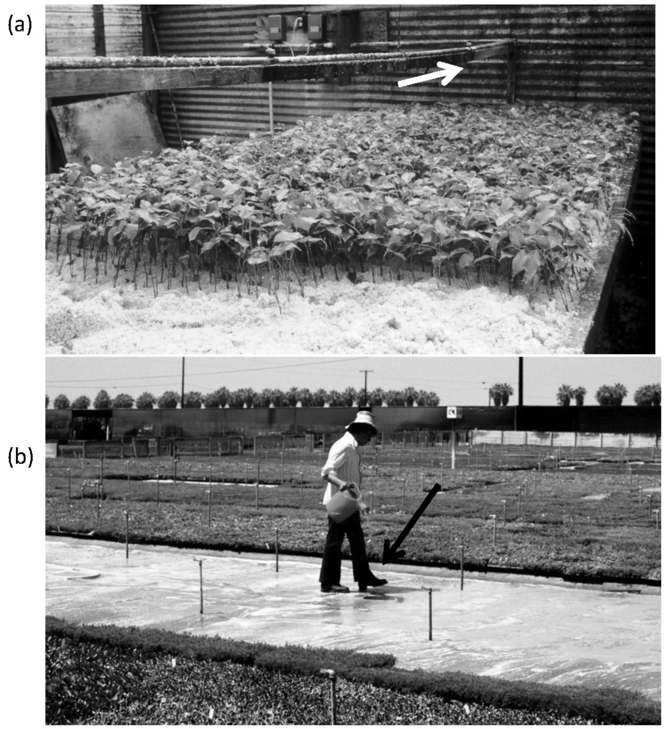

Figure 11. Some correct and incorrect ways to propagate. (a) Poor sanitation with algae build-up (arrow) — can harbor disease, insects, and creates a poor propagation and work environment for personnel. (b) Good cultural and chemical practices: Sanitizing concrete pads before starting the next propagation crop.

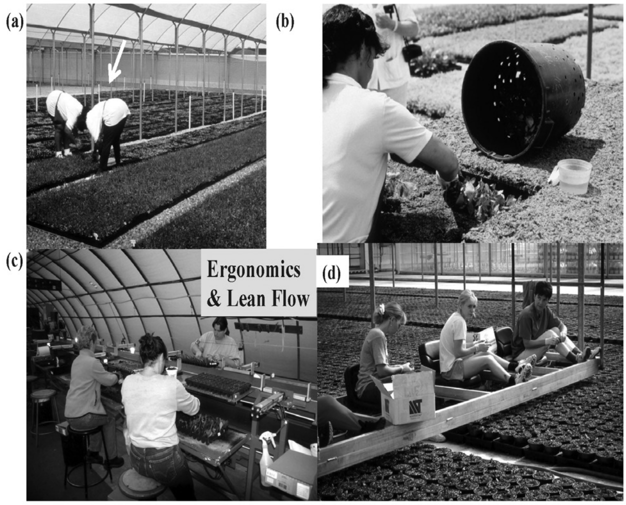

Figure 12. The importance of ergonomics and efficiency of movement. (a) Poor ergonomics with uncomfortable back posture, compared to (b,c) good ergonomics with correct posture, close proximity of materials and economy of movement which enhances worker efficiency. (d) Ultimate of efficiency with propagators "floating" about the containers sitting in a trellis system for direct sticking in a flood floor system (Hartmann et al., 2011).

chemicals for disinfecting propagation facilities and propagules are: (a) Benzylkonium chloride, (b) hydrogen dioxide, (c) bromine and (d) diluted sodium hypochlorite solution (household bleach) can be used for (d) disinfesting both propagation facilities and propagules. Diluted household vinegar can control algae and moss along walkways. Always follow directions and try small trials first.

ERGONOMICS AND LEAN FLOW (EPPS, 2009)

Figure 12 depicts the importance of ergonomics and efficiency of movement. (a) Poor ergonomics with uncomfortable back posture, compared to (b,c) good ergonomics with correct posture, close proximity of materials, and economy of movement which enhances worker efficiency. (d) Ultimate of efficiency with propagators "floating" about the containers sitting in a trellis system for direct sticking in a flood floor system.

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