The Container Tree Nursery Manual

Volume Six
Seedling Propagation

Chapter 3
Vegetative Propagation

6.3.1 Introduction

Vegetative propagation is defined as the production of new plants that contain the *exact* genetic characteristics of the parent plant. This is possible because the nucleus of each living cell contains all the genetic information that is necessary to reproduce another identical plant, a concept known as "totipotency." Because only one parent is required and no genetic recombination is involved, vegetative propagation is also known as **asexual propagation.** By avoiding the genetic recombination inherent in sexual reproduction and seed development, nursery managers can produce multiple "carbon copies" of the parent plant.

6.3.1.1 Objectives and resources affect propagation method

In nature, some plants spread naturally by vegetative propagation, but most plants propagate by seed. This is also the case in forest and conservation nurseries, where well over 95% of the species are produced from seed. In ornamental nurseries, where producing many identical plants from specific "cultivars" (cultivated varieties) is the objective (figure 6.3.1 A), vegetative propagation is the most common method. One technique in particular, rooted cuttings, constitutes from 70 to 90% of the total production (Davies 1994). Some ornamental plant cultivars have been specifically selected for their ability to root easily.

As stressed in chapter 1, the choice of propagation method depends on the management objectives and the characteristics of the plant species. If the objective is to generate a large number of plants that have been genetically selected for a particular characteristic (such as fast growth for use in plantation forestry, for example), then vegetative propagation is a logical choice (figure 6.3.1 B). On the other hand, if the objective is to produce plants that maintain the broad range of genetic diversity that is found in nature, then seed propagation makes more sense.

Special circumstances, however, may make vegetative propagation the best or only option (table 6.3.1). Some plants just do not produce seed in sufficient quantities or frequently enough, and so they must be propagated vegetatively. In other situations, pests have so damaged the flowers or seeds that propagation by seed is not an option. For example, when a spruce budworm epidemic destroyed the seed crop of black spruce in Nova Scotia for over 10 years, nurseries there had to propagate this species vegetatively from cuttings (Levy 1983). Many

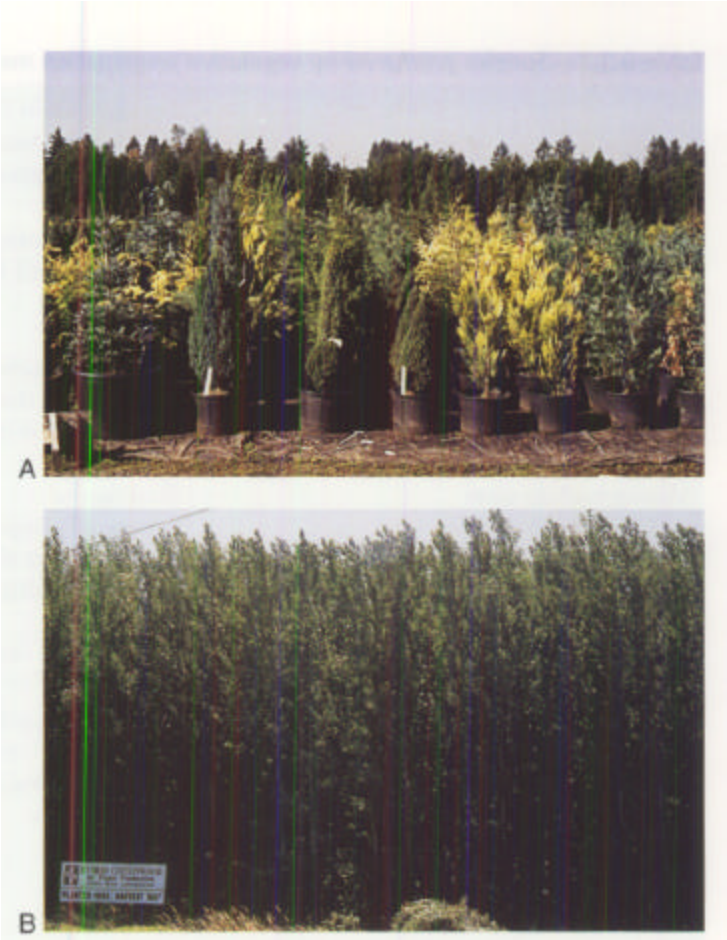


Figure 6.3.1—*Vegetative propagation is common in ornamental nurseries where numerous copies of cultivars with desirable form and color are desired* (**A**). *In plantation forestry, vegetative propagation is used to produce large numbers of trees that were genetically selected for fast growth* (**B**).

seeds of forest and conservation species have complex dormancy requirements that make vegetative propagation more practical, especially when time is critical. This often is the situation for restoration projects at high elevations. Rooting cuttings collected during the summer were found to have many advantages over seed propagation (Scianna and others 1998). Plants that have unique genetic properties, such as resistance to an insect or disease, can be propagated vegetatively so that the desired genes are not lost in sexual recombination (figure 6.3.2A). Vegetative propagation is also used to maintain or even increase populations of rare and endangered species (figure 6.3.213). One exciting recent development is the successful production of American chestnut plantlets through micropropagation. This tree was once

Table 6.3.1—*Species produced by vegetative propagation methods at the University of Idaho's Forest Research Nursery*

Species	Rationale for vegetative propagation	Propagation method
Western larch	Infrequent cone crops and poor seed quality	Softwood cuttings root within 6 to 8 weeks with up to 80% success
Rocky Mountain juniper	Slow and erratic germination due to complex seed dormancy	Softwood cuttings root in 2 to 6 months with up to 68% success
Western white pine	Shortage of genetically improved seed resistant to white pine blister rust fungus *(Cronartium ribicola)*	Softwood cuttings root in 3 to 6 months with 50 to 90% success; micropropagated plantlets ready for outplanting after 18 months
Scouler willow	Traditional hardwood cuttings are hard to root and many die from stem cankers	Softwood cuttings treated with hormones and rooted under fog performed significantly better; micropropagated plantlets rooted with 92% success and had less disease than cuttings
Antelope bitterbrush (rare ecotypes)	Low seed yields and difficulty with direct seeding; conventional cuttings are hard to root	Micropropagated shoots root with 92% success in 4 to 6 weeks, and flower after 1 year in greenhouse

Source: Edson (1995).

the most important broadleaved forest tree in the eastern United States but was essentially eliminated earlier this century by a fungal blight. Scientists believe that they can produce disease-resistant chestnuts through genetic engineering and micropropagation (Carraway and Merkle 1997). Lastly, tree improvement programs can benefit greatly from vegetative propagation techniques. For example, it takes about 21 years for a seedling seed orchard to become fully productive whereas one established from cuttings can produce improved seed in as little as 9 years (Greenwood and others 1991).

Micropropagation, the newest type of vegetative propagation, is often attractive to prospective nursery developers or new customers because it is so modern and "high-tech." The idea of being able to produce thousands of new plants from a small amount of plant tissue is certainly attractive. However, micropropagation has many of the same limitations as the other vegetative propagation techniques and is by far the most expensive. So again, the choice of propagation method should be determined by the objectives and resources of the nursery.

A B

Figure 6.3.2—*Vegetative propagation can also be used for conservation objectives such as restoration of ecologically important western white pine* (**A**) *or increasing the populations of threatened or endangered species, such as this rare ecotype of Pacific dogwood* (**B**).

6.3.1.2 Advantages and disadvantages of vegetative propagation

The interest and activity in vegetative propagation has increased dramatically in recent years because of its many advantages:

- Rapid multiplication of selected plant material in a short time
- High degree of crop quality and uniformity (Jones and others 1996)
- No complex seed dormancy problems
- Ability to overcome low seed availability and seasonality
- Earlier and more frequent flowering than plants grown from seed (Jones and others 1996)

On the other hand, vegetative propagation has certain limitations:

- Cost-vegetatively produced plant material can be several times as expensive as seedlings (table 6.3.2)
- Propagation environments-sophisticated structures and equipment are required, particularly for micropropagation
- Labor intensive-all vegetative propagation methods require more hand labor, which often exceeds 80% of total costs (Davies 1994)
- Reduced vigor-some vegetatively propagated plants are less vigorous than those raised from seed
- Loss of genetic diversity -vegetative propagation usu ally means less natural variation
- Possibility of plagiotropism-vegetatively propagated plants may lose apical dominance

6.3.1.3 Basic concepts and terminology

A clone is defined as a group of genetically uniform individuals that were originally derived from a single parent by asexual propagation. **Clonal forestry** is a term used to

Table 6.3.2—*Production costs of radiata pine stock types*

Stock type	Source of plant material	Cost ($)/ 1,000 plants
Seedlings	Open-pollinated seed orchard	76
Seedlings	Control-pollinated seed orchard	317
Cuttings	Stool beds in nursery	138
Cuttings	Field collections	255
Plantlets	Micropropagation	483

Source: Menzies (1995).

describe the silvicultural system based on regeneration with 'vegetatively propagated plants, which are usually outplanted in intensively managed plantations. Horticulturists refer to the parent as the "mother" plant and the progeny as "daughter" plants whereas forest geneticists refer to the original plant as the "ortet" and each its progeny as a "ramet" (Hartmann and others 1997). **Stock plant** is another term for the mother plant and will be the preferred term in this volume. For collections in the wild, cuttings are collected from a **donor plant.**

Juvenility. The concept of juvenility, which is critical to successful vegetative propagation of woody plants, is difficult for many people to understand because it differs between plants and animals (Geneve 1995). Each organism goes through a normal developmental and aging process that begins with an embryo, continues through juvenility, and then develops into a mature stage in which it is capable of sexual reproduction. The difference between animals and plants is that chronological age does not equal biological age in plants like it does for animals, because **different parts of a plant can be at different stages of maturity at the same time.** The biologically youngest (most juvenile) but chronologically oldest part of a tree is located at the junction between the root and shoot (figure 6.3.3 A-E). The juvenile phase is characterized by the inability to produce flowers under conducive environmental conditions and often can be identified by specific morphological and physiological traits including leaf shape, thorniness, vigor, and disease resistance. Of interest to propagators, however, is the fact that cuttings taken from juvenile plant tissue regenerate roots easily. For example, black spruce cuttings taken from the bottom third of the crown of 9-year-old saplings rooted almost twice as easily as those collected from the top third (Tousignant and others 1995).

On the other hand, cuttings or buds collected from the upper parts of the crown are sexually mature, which is advantageous when the object of propagation is to collect mature grafting material that has the ability to flower and produce seeds. These "scions" are then grafted on rootstocks (figure 6.3.4). and outplanted in clonal seed orchards.

Growers can manipulate plants, either culturally or chemically, to retain juvenility for a longer period.

- By repeatedly cutting a plant back to the base, it is possible to produce stump sprouts (figure 6.3.3E), which are then maintained in the nursery to provide a steady supply of cuttings. Pruning the stock plant all the way back to the root collar often is most effective. For example, the rootability of cuttings from American elm stump sprouts increased from 38% near the top to 83% at the base (Schriebner and Kawase 1975).

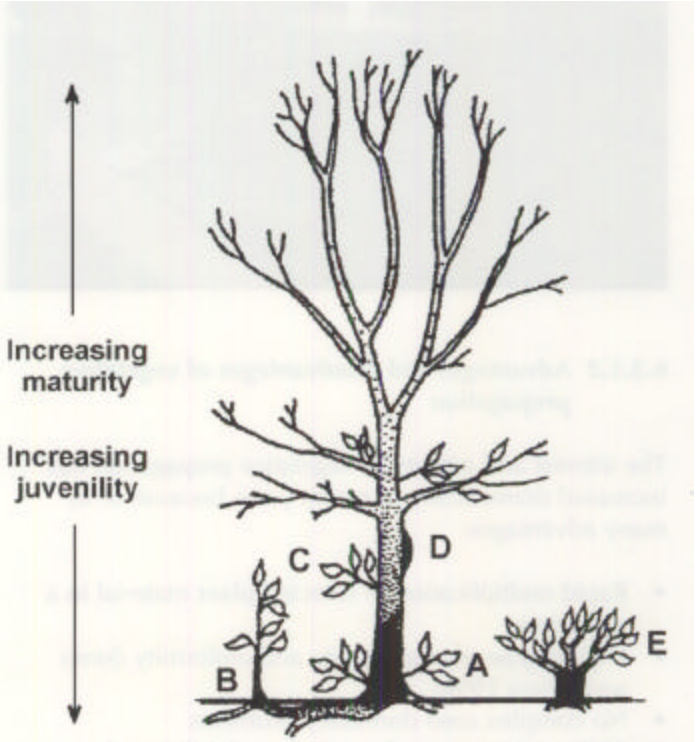


Figure 6.3.3—*Plant structures near the base of a tree and the root system remain juvenile and provide growers with a source of propagation material:* **A**—*water sprouts,* **B**—*root suckers,* **C**—*epicormic branches,* **D**—*sphaeroblasts. Propagators can maintain tissue juvenility and increase their supply of plant material through cultural techniques such as hedging* (**E**) *(modified from Bonga 1982).*

- **Hedging** consists of regularly cutting stock plants back to a predetermined height and is particularly useful for species that do not produce stump sprouts. Hedging is an efficient means of increasing the yields of cuttings from a limited number of stock plants.
- The maturity process can be chemically retarded through the use of hormones. juvenile plant material typically produces roots naturally, but more mature cuttings often can be stimulated to produce roots with hormone treatments. Cuttings that fail to root, even when treated with hormones, are termed **recalcitrant** (Geneve 1995).

Adventitious rooting. The definition of an adventitious plant structure is one that does not develop from a normal meristem or bud (Hartmann and others *1997).* Adventi tious shoots can develop in burl-like structures called sphaeroblasts on the stem (figure 6.3.3D), or adventitious roots can be induced in juvenile plant material. Because rooted cuttings are the most common vegetative propagation technique, the ability to culturally induce adventitious roots is of major interest in horticulture. Adventitious rooting is genetically controlled and the responsible genes are active during juvenility but, as the plant tissue ages, they gradually are switched off.

Figure 6.3.4—*Vegetatively mature cuttings, called "scions," are collected from the upper crown of specially selected older trees and grafted onto younger rootstocks to form clonal seed orchards.*

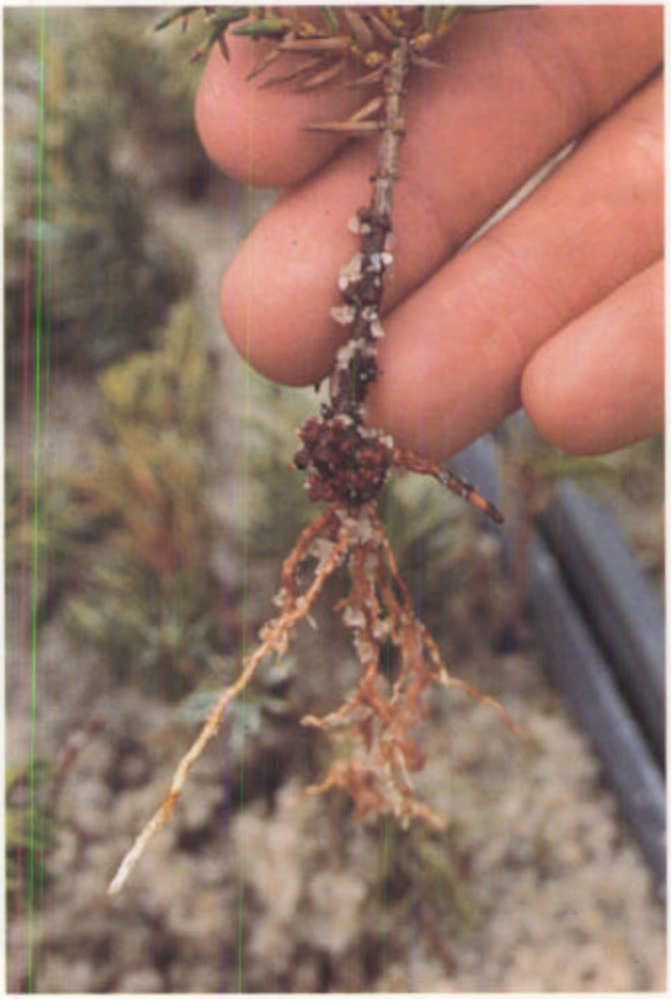


Figure 6.3.5—*Callus cells develop from cambial cells on the cut surface of the cutting, followed by the development and emergence of wound roots.*

New biotechnology techniques may soon be able to manipulate the juvenility genes to increase their sensitivity to hormones or even switch them back on and reverse the maturity process (Davies 1994). At the present time, however, growers still use cultural techniques to induce adventitious rooting.

There are two types of adventitious roots: **preformed roots**, and **wound roots.** The former develop naturally at the base of the stem and the latter form only after the cutting is made. Some woody plants, such as poplars, willows, and gooseberries, have latent root bud primordia that are preformed in their inner bark. These buds lie dormant as long as the stems remain attached to the stock plants but develop into roots after cuttings are taken. Wound roots form out of the mass of callus cells that develops as part of the wounding response (figure 6.3.5). Although it varies with the type of plant, adventi tious roots generally develop around the central core of vascular tissue. There is a general belief that callus tissue is a precursor to the development of adventitious roots, but some recalcitrant species form callus without subsequent root formation (Hartmann and others 1997).

6.3.1.4 Vegetative propagation systems

Once the decision has been made to propagate a plant vegetatively, the best method to use depends on several factors, including: species characteristics, the type of propagation environment, and the skill of the propagator. Several different vegetative propagation methods are being used in forest and conservation nurseries (table 6.3.3). The following sections discuss the basics of each technique. For a more detailed discussion on basic vegetative propagation concepts and procedures, the reader is referred to horticultural texts including Dirr and Heuser (1987), Hartmann and others (1997), and Macdonald (1986).

Table 6.3.3—*Comparison of vegetative propagation methods*

Vegetative propagation method	Labor skills	Relative cost/plant	Required facilities	Uses in forest & conservation nurseries
Stem cuttings	Low	Low	Rooting chamber	Problems with seed quality or availability; genetic improvement
Root cuttings	Low	Medium	Rooting chamber	Limited to a few species
Layering	Low	High	None	Limited to a few species
Division	Low	Medium	None	Bulbs & grass-like species
Grafting	High	High	Greenhouse	Seed orchards
Micropropagation	High	High	Transfer hood, culture room, & greenhouse	Genetic improvement; threatened & endangered species

6.3.2 Stem Cuttings

Rooted stem cuttings—**steckling**—are the most widely used vegetative propagation technique for forest and conservation species. Some of these species can also be produced from seed, but growers turn to vegetative propagation for certain specific reasons:

- **Ease of propagation**--Poplars and willows have traditionally been produced from hardwood stem cuttings because their very small seeds are hard to handle, do not store well, and are covered with fine hairs that resist water imbibition. Most poplars and willows root well without hormones, although some recalcitrant species and clones require them. For example, hardwood cuttings of Scouler willow rooted and survived significantly better when treated with 0.3% IBA (indole-3-butyric acid) (Edson and others 1995).
- **Lack of seed**--Rooted cuttings have been the principal method of reforesting Alaska-cedar in British Columbia for the past 15 years, and currently over a half-million stecklings are produced annually. The original purpose of this program was to supplement seed that was in short supply, but it has recently been expanded to include genetic improvement, with over 1,000 clones being evaluated in a cutting orchard (Russell 1993).
- **Genetic improvement**--Rooted cuttings are becoming increasingly popular in plantation forestry for the **bulking** or **cloning** of genetically improved plant material (Ritchie 1996). Bulking is done at the family level and involves making a relatively few copies of a large number of genotypes, the offspring (progeny) of which have been tested to verify the genetic gain. Bulking is most common with conifers and is particularly popular for expanding the number of propagules from control-pollinated seed orchards (figure 6.3.6). Cloning is primarily done with broadleafed trees and shrubs and consists of making a relatively large number of copies of a few elite genotypes. Although clones have been phenotypically selected, sometimes over several generations, their offspring have not been progeny-tested.

6.3.2.1 Importance of source

For forest and conservation purposes, knowledge of the origin of cuttings is just as important as with seeds to insure that the nursery stock is well-adapted to its outplanting environment. For restoration projects, cuttings should always be collected from donor plants on or near the outplanting site. One Intermountain nursery collects from at least 50 different plants from a specific project

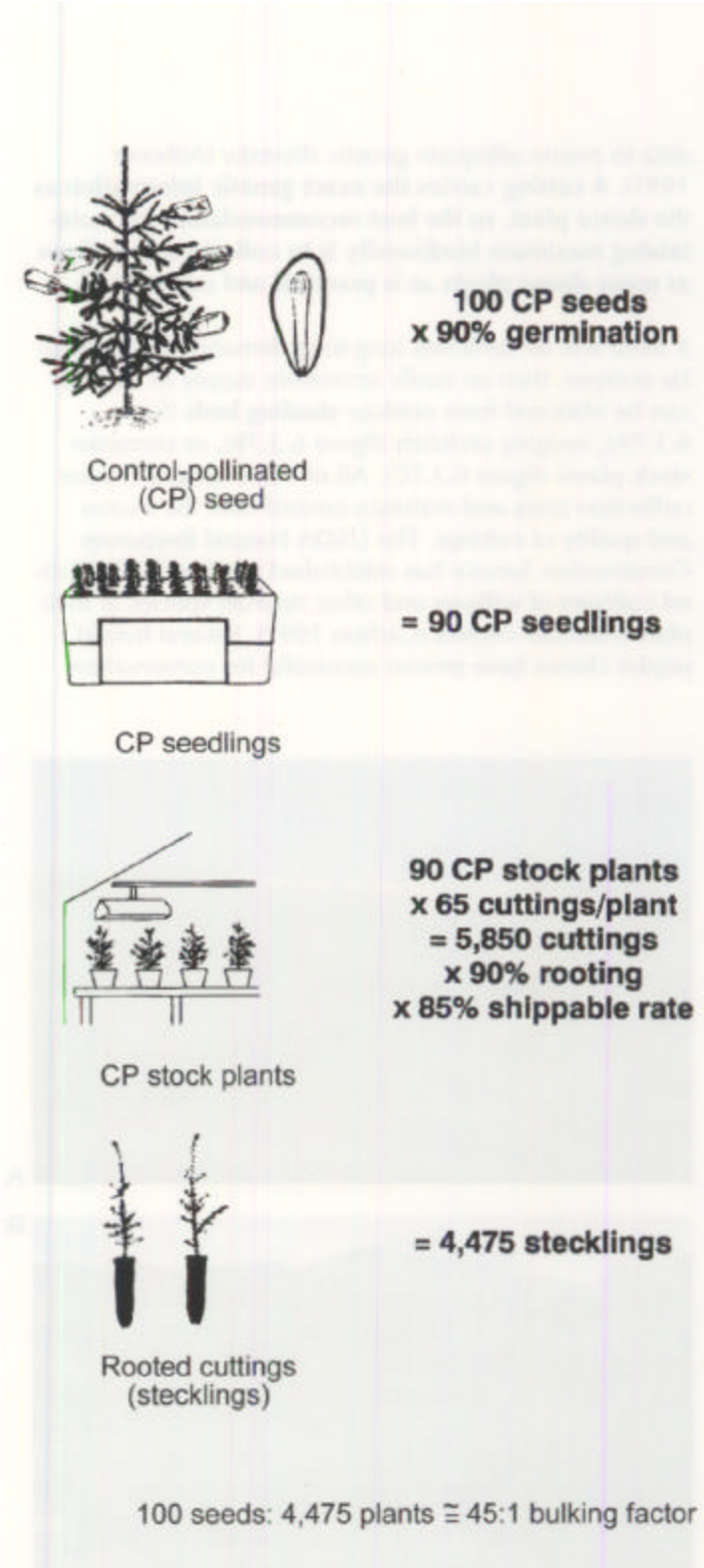


Figure 6.3.6—*Bulking is used to multiply the limited number of control-pollinated seed by establishing stock plants and starting rooted cuttings; in this example, the ratio of stecklings to seeds produces a 45:1 bulking factor (modified from Russell and Ferguson 1990).*

area to ensure adequate genetic diversity (Atthowe 1993). **A cutting carries the exact genetic information as the donor plant, so the best recommendation for maintaining maximum biodiversity is to collect cuttings from as many donor plants as is practical and economical.**

If there will be sufficient long-term demand for a particular ecotype, then an easily accessible supply of cuttings can be obtained from outdoor **stooling beds** (figure 6.3.7A1, hedging orchards (figure 6.3.713), or container stock plants (figure 6.3.7C). All of these options reduce collection costs and maintain control over the source and quality of cuttings. The USDA Natural Resources Conservation Service has established stool beds of selected cultivars of willows and other riparian species at their plant materials centers (Carlson 1992). Several hybrid poplar clones have proven successful for conservation outplantings in the Great Plains and they are maintained as stooling beds in regional nurseries (USDA SCS 1991). Most government nurseries have established stooling beds or stock plants of the species and ecotypes that are adapted to their local area and thus can be a potential source of cutting material for private growers.

6.3.2.2 Types of stem cuttings

In horticulture, cuttings are divided into categories depending on the type of and maturity of tissue (Hartmann and others 1997; Dirr and Heuser 1987).

Hardwood cuttings. Hardwood cuttings come from either broadleaf or conifer species, consist of mature woody tissue of last season's growth, and typically are collected during the dormant period (figure 6.3.8A).

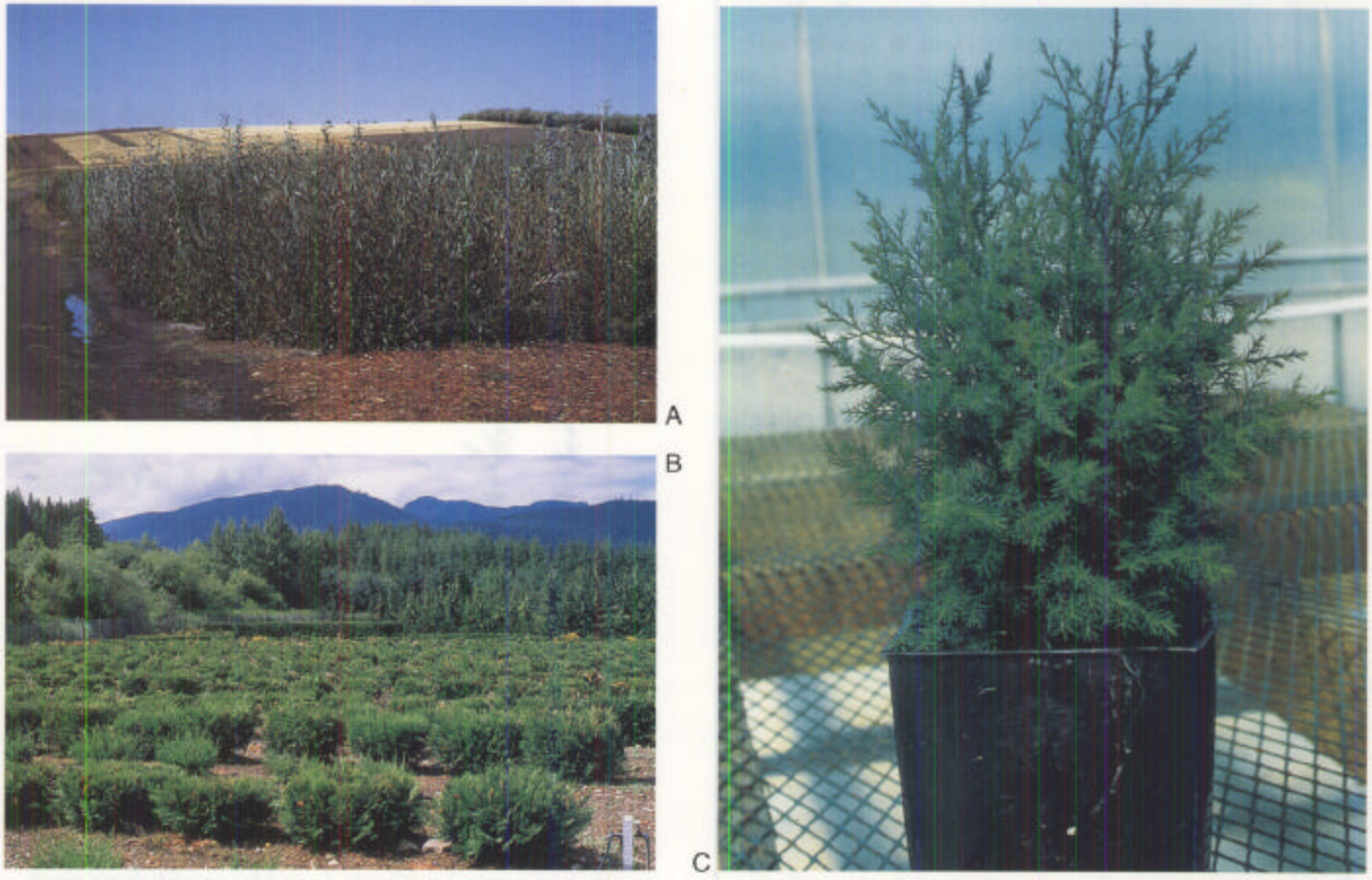


Figure 6.3.7—*Stooling beds (**A**), hedging orchards (**B**), or container stock plants (**C**) can be established in the nursery when there is a continuing demand for rooted cuttings of certain ecotypes or cultivars (B & C, courtesy of J. Russell, British Columbia Ministry of Forests).*

Figure 6.3.8—*In direct sticking, hardwood cuttings of poplars and willows, which root relatively easily, are often stuck directly into the final growth container without pretreatment* (**A**). *Conifers are propagated with semi-hardwood cuttings* (**B**), *which must be treated with rooting hormones* (**C & D**), *which stimulate root formation* (**E**). *(B, courtesy of G. Dunsworth; D, courtesy of J. Russell; E, courtesy of Don Carson, British Columbia Ministry of Forests.)*

Hardwood cuttings are easy to prepare, store, ship and handle, and sometimes can be rooted under normal irrigation without relatively few special cultural treatments.

Semi-hardwood cuttings. Semi-hardwood cuttings are collected from the semi-lignified tissue of actively growing plants (figure 6.3.813). Because they consist of partially mature tissue, semihardwood cuttings will bend but not break when flexed. They cannot be stored for more than a few days and require specialized propagation environments, such as a greenhouse with mist or a rooting chamber.

Softwood cuttings. Softwood cuttings are collected from the soft succulent new shoots during the growing season, and suitable cuttings will snap or break cleanly when flexed. They cannot be stored and must be carefully handled. Because softwood cuttings are very sensitive to desiccation, a mist bed or rooting chamber and treatment with hormones (figure 6.3.8C-E) usually are necessary.

This traditional nomenclature works well when collecting from stock plants or stooling beds in the nursery. Propagating plants for many conservation and restoration projects, however, requires collecting cutting material on remote project sites where access to donor plants is a severe limitation. For example, hardwood cuttings may need to be collected from remote, high-elevation donor plants during the summer or fall-most subalpine and alpine plants are low in stature and buried by snow during the winter and early spring. Repeated browsing often eliminates the type of plant tissue that can be collected at field locations, so it may be useful to distinguish between first-year tissue and second-year hardwood tissue. For example, cuttings from first-year hardwood of gooseberries showed 61 % rooting compared with 21 from second-year wood. Therefore, **a more practical terminology for cuttings from forest and conservation plants would include the season of collection along with the type and dormancy status of the tissue (for example, late-fall dormant hardwood, summer softwood)** (Scianna and others 1998).

6.3.2.3 Collecting and processing stem cuttings

The best type of cutting will depend on the species, genotype, and season of collection. For example, hardwood cuttings of willows are generally best, but there are some exceptions. Scouler willow does not root easily, so softwood cuttings were more successful (Edson and others 1994). Rootability of stem cuttings also varies greatly with the season of the year, and the best season for collection also varies by species and even genotype. With red ironbark eucalyptus, the rootability of two genotypes varied during the year, whereas another was completely recalcitrant to rooting (table 6.3.4).

Table 6.3.4—*The rootability of red ironbark eucalyptus varies considerably between genotypes and time of collection*

	Rooting percentage		
Month & year	Genotype #1	Genotype #2	Genotype #3
July 1984	0	70	90
September 1984	0	50	90
November 1984	0	57	77
March 1985	0	64	88
May 1985	0	36	48

Source: Burger and Lee (1987).

Condition of donor or stock plants. Because the physiological condition of donor or stock plants affects rooting success, cuttings typically are collected from healthy donor plants that are growing in the full sun or from well-fertilized stock plants in the nursery. It is a good idea to visit collection sites a year in advance if possible to locate and label suitable donor plants (Scianna and others 1998). To minimize moisture stress, wild collections should always be made as early in the day as possible and on days with little or no wind. Cloudy or foggy days would be ideal. Container stock plants should be irrigated before cuttings are taken and moved to a shady area for several days prior to collection if possible. Moe and Andersen (1988) present a comprehensive discussion of the maintenance of stock plants and cultural treatments that increase rootability of cuttings.

Collection equipment and supplies. Proper equipment and supplies greatly increase ease of collection and rooting success. High-quality pruning shears and a sharp knife are essential. Two types of plastic bags will keep cuttings together and prevent desiccation: smaller plastic zipper-lock bags for cuttings from individual plants and larger white trash bags for bulking collections. For wild collections, a list of supplies should include spray bottles with extra water, permanent labels and marking pens, work gloves, and white portable coolers with blue ice to keep the cuttings cool and protected (Scianna and others 1998).

Size and orientation of cuttings. The best size of cutting to collect depends on species, type of plant tissue available, and size of the growth container. In nursery stooling beds, willow and poplar are collected as long whips that are then cut into the proper length. If collected by hand, the basal cut is typically made just below a node where roots form more readily. When large numbers of cuttings have to be made, then bundles of whips are cut with a band saw. Hardwood poplar cuttings are collected relatively large, ranging from 10 to 25 cm (4 to 10 in.) in length and 0.5 to 2 cm (0.2 to 0.8 in.) in diameter. The bundles of cuttings are then secured with a rubber band and stored under refrigeration at 0 to 4.5 °C (32 to 40 °F) to keep them dormant until they are needed.

Semi-hardwood and softwood cuttings are generally smaller than hardwood cuttings. Semi-hardwood and softwood cuttings from a variety of native donor plants should be from 7.6 to 15.2 cm in length (3 to 6 in.) and around 6.4 mm (0.25 in.) in diameter (Scianna and others 1998). Softwood cuttings of Alaska-cedar range from 3 to 8 crn (1 .1 to 3.2 in.) in length (Russell and Ferguson 1990). Pruning shears, especially the anvil type, crush the softer tissues of semi-hardwood or softwood cuttings so each cutting should be recut at an angle with a sharp knife when back at the nursery. This removes any damaged tissue and improves uptake of water and rooting hormones (Scianna and others 1998).

Stem cuttings have an inherent **polarity** and will always produce shoots at the distal end (nearest the bud) and roots at the proximal end (nearest the main stem or root system). 'To distinguish between the top and bottom of hardwood cuttings, the bottoms are cut at an angle, which not only ensures that the cuttings are planted right side up but makes them easier to stick (Hartmann and others 1997). Cuttings from lateral branches of conifers may exhibit **plagiotropism,** which is the term for a horizontal growth habit that some lateral cuttings maintain after they are rooted (figure 6.3.9). Plagiotropism is much more troublesome in some species than in others. About one-third of western larch stecklings grown in greenhouses exhibited plagiotropism during the first growing season. This and other growth abnormalities makes the greenhouse production of larch stecklings unpractical for the present time (Edson and others 1996). Propagators have developed innovative cultural procedures to overcome plagiotropism. For example, some nurseries have found that transplanting their Douglas-fir rooted cuttings in bareroot beds as plug+one transplants helps eliminate plagiotropism (Ritchie 1996).

Figure 6.3.9—*Plagiotropism is a morphological condition in which cuttings collected from lateral branches, such as the Rocky Mountain juniper steckling on the right, retain their horizontal growth habit (courtesy of J. Edson).*

Sanitation. The wounding that is inherent in collecting cuttings leaves them vulnerable to decay, making sanitation critical. The epidermis or bark of a plant functions like human skin in preventing infection by fungi or bacteria, but this protection is lost when cuttings are made. For example, cottonwoods and willows are very susceptible to a canker fungus (*Cytospora* spp.) that can normally be found on the outside of branches. Because of the frequent wounding, this fungus builds up in stooling beds to the extent that the beds must be periodically ripped out and replaced with new healthy plant material.

To prevent the spread of decay when collecting cuttings, pruning shears and other tools should be kept clean and

disinfected regularly. Several different disinfectants have been used in cutting preparation (table 6.3.5). Household bleach (sodium hypochlorite) is the most common disinfectant because it is cheap and readily available but may pose environmental risks. Bleach contains hypochlorite ions that react to form very stable organochlorine compounds that accumulate in animal tissue and may cause health problems. Although they are more expensive, benzalkonium chlorides and hydrogen peroxide are just as effective and have no potentially damaging breakdown products (McClelland and Smith 1994).

Cuttings can also be treated at the nursery. Some nurseries soak their willow and cottonwood cuttings in a surface sterilant or fungicide to retard storage molds and decay of the cut surfaces. Cuttings from donor plants in the wild can be soaked in a broad-spectrum fungicide such as thiram and the rooting medium drenched with fungicide (Scianna and others 1998).

***Rooting* hormones.** Treating stem cuttings with hormones greatly increases the speed and uniformity of root development for many species, especially those that are hard to root. Many different rooting hormones are commercially available (figure 6.3.8C). IAA (indole-3-acetic acid) was the first natural auxin discovered but is not used commercially nowadays because it is relatively unstable. Two synthetic auxins-IBA (indole-3-butryic acid) and NAA (naphthaleneacetic acid)-are the active ingredients found in commercial rooting compounds at rates ranging from 1,000 to 20,000 parts per million (ppm) (Van Dellen 1998). Rooting compounds contain varying concentrations of IBA and NAA as well as other supplemental materials such as fungicides and vitamins (table 6.3.6). This can be confusing to the novice because there is no standard nomenclature for the contents or their concentration. For example, the numbering of the Hormex® line of products indicates the concentration whereas that of Hormodin® compounds does not. Van Dellen (1998) provides an excellent discussion of the commercial rooting compounds that are currently on the market.

Powder formulations are easier to use because the hormone concentration is preset and the powder sticks to the cut surface. Liquid products are typically formulated with ethyl or isopropyl alcohols, and some must be diluted to the proper strength (Dirr and Heuser 1987). Some growers prefer liquids because they penetrate the plant tissues better than powders (Van Dellen 1998). Care should be taken during dilution, as this process provides opportunity for error. For nurseries without easy access to laboratory chemicals, radiator antifreeze (~ 95% propylene glycol) and windshield washer fluid (~50% methanol) proved to be safe and effective dilutants (Chong and Hamersma 1995). As with any new practice, it would be wise to test these products before using them on an operational scale.

Table 6.3.5—*Properties of common disinfectants used to collect and process cuttings*

Product/ trade name	Chemical formula	Efficacy	Economics	Safety & environmental risks
Bleach	NaClO $Ca(ClO)_2$	Controls fungi & bacteria	Cheap & available	Fumes irritating; organochlorides may pose risks
Isopropyl alcohol	CH_3 (CH-OH) CH_3	Doesn't control all bacteria	Inexpensive	Dangerous to breathe
Physan®, Green-Shield®, & Triathalon®	Benzalkonium chlorides	Controls fungi & bacteria	Economical	None: inert by-products
Hydrogen peroxide	H_2O_2	Controls fungi & bacteria	Inexpensive	None: breaks down into water & oxygen

Source: Modified from McClelland and Smith (1994).

Table 6.3.6—*Composition of commercial rooting compounds containing the chemicals IBA (indole-3-butryic acid) and NAA (naphthaleneacetic acid)*

Product	Formulation	Types of hormone & concentration	Other chemicals
Dip'N Grow®	Liquid	IBA & NAA (depends on dilution rate)	None
Hormodin 1®	Powder	0.1% IBA	None
Hormodin 2®	Powder	0.3 % IBA	None
Hormodin 3®	Powder	0.8 % IBA	None
Hormex No. 1®	Powder	0.1 % IBA	None
Hormex No. 3®	Powder	0.3 % IBA	None
Hormex No. 8®	Powder	0.8 % IBA	None
Hormex Concentrate®	Liquid	NAA, IBA (depends on dilution rate)	Vitamin B_1
Rootone®	Powder	0.1% NAA & 0.06% IBA	Thiram fungicide

Sources: Modified from Hummert (1997); a complete listing can be found in Blazich (1988).

Rooting hormones are applied to cuttings in three ways (Blazich 1988):

- **Dipping in hormone powder**--This method is quick and easy for single cuttings but it is difficult to control the application rate because the amount that adheres to the cutting depends on size, surface texture, and moisture content (figure 6.3.8D).
- **Dipping in liquid formulations for a few seconds ("quick dip")**--This technique is simple and fast and provides uniform results when cuttings are bundled during treatment. It is recommended for more concentrated alcohol-based products. The duration of the dip should be kept constant to ensure a uniform application rate and minimize the possibility of phytotoxicity.
- **Soaking in liquid formulations for 2 to 24 hours**--This method is slower but provides a more uniform application rate per cutting. Best for water-based formulations of lower hormone concentration.

***Cultural treatments*.** Growers use several other cultural techniques at the nursery to improve rooting.

Warm-temperature callusing is achieved by treating hardwood cuttings with rooting hormones and storing them under relatively warm, moist conditions (18 to 21 °C (65 to 70 °F) for 3 to 5 weeks. Bottom heat is becoming a standard practice but is particularly valuable for difficult-to-root species. Nurseries that process many cuttings have special rooting benches with bottom heat, but less expensive heating mats are commercially available. The temperature difference between the roots and shoots needs only to be a few degrees, as excessive temperatures or callusing time can weaken stored photosynthate reserves and lower survival after planting (Hartmann and others 1997). Bundles of willow or cottonwood cutting can be stored upright in moist sawdust or wood shavings while in the cooler, which keeps the bottoms warmer than the tops thus promoting callusing and speeding-up root initiation.

Basal wounding involves removing the outer bark or making incisions at the bottom of the cutting (Hartmann and others 1997). This not only exposes the cambial tissue to the rooting hormone but encourages the formation of callus tissue that is often the precursor of root initials. Removing approximately 2 to 5 cm (1 to 2 in.) of bark tissue was found to be beneficial for encouraging root development of native plants (Scianna and others 1998).

Clipping, which includes removal of shoot tips or basal leaves, has been shown to improve rooting success with semi-hardwood or softwood cuttings (Hartmann and others 1997). To minimize transpiration, plants with large leaves should be pruned to remove 25 to 50% of the surface area of each leaf (Scianna and others 1998).

6.3.2.4 Planting stem cuttings

Cuttings can be **stuck** or **set** into containers in two ways that differ not only in technique but also between types of cuttings and the necessary propagation facilities.

***Direct sticking*.** This technique involves planting cuttings directly into the growth containers and then moving them immediately to the propagation area, where they will grow to shippable size (figure 6.3.8A-E). Direct sticking is commonly used with hardwood cuttings of willows and cottonwoods, which root easily, but also can be done with semi-hardwood or softwood cuttings of species that require treatment with rooting hormones. Hardwood cuttings stick easily into the filled containers but less-woody cuttings need to have the container prepared with a hole made by a dibble or a template.

Direct-stick cuttings have relatively simple cultural needs, requiring only frequent irrigation or misting to minimize transpiration demand. The height of the container is very important to good drainage because the perched water table that exists at the bottom of all containers will be proportionately less in taller containers (see section 4.2.3.3 in volume four of this series for more specifics). Growing media for direct sticking should be porous enough to prevent waterlogging while promoting good aeration around the developing roots. This is especially critical under mist irrigation, where growers include high concentrations of perlite or pumice into a standard peat moss-vermiculite mix. Coir is a relatively new media component that is made from coconut husks and coir-based media have been shown to greatly improve rooting success with some species.

***Prerooting*.** This is recommended for semi-hardwood and softwood cuttings that are more difficult to root, and therefore treatment with rooting hormones and a special propagation environment are essential (figure 6.3.10 A-D). Prerooting consists of planting treated cuttings into a shallow tray or small container until they begin to form roots. Depending on the species, rooting can take from several weeks to many months. Root development can be determined by gently tugging the cutting-any resistance means that roots have formed and the cutting can be transplanted. Rooted cuttings are transplanted into growth containers where they are grown to shippable size. Newly formed roots are very tender and susceptible to breakage, and so rooted cuttings must be carefully removed from trays and transplanted into their final growth containers. Just as with other types of transplants, prerooted cuttings must be carefully planted to avoid root injury or the formation of a "J-root." Making a hole in the medium in the growth container with a pointed dibble protects the tender roots and the medium can be pressed around the stem. Some growers use a forked tool to position the roots. Transplanted cuttings should be irrigated frequently in the weeks following transplanting, and fertilization can begin as soon as the plants have rooted in the growth container. Established cuttings are treated like normal seedlings and hardened-off before outplanting.

The height of the rooting trays and the moisture-holding and aeration characteristics of the medium are critical to rooting success. Just as with growth containers, taller trays will provide better drainage than shallower ones. The rooting medium should provide four functions: prevent disease, hold the cutting in place, supply water as fast as it is lost through transpiration, and permit easy penetration of air to the bottom of the cuttings. Cuttings are particularly susceptible to rot fungi and therefore the growing medium should be sterilized. Rooting trays are prepared with a sterile but well-aerated medium that will hold the cuttings upright yet drain easily. Fine sand or mixtures of perlite, vermiculite, or coir have been used (Hartmann and others 1997). The choice of medium affects both the speed of rooting and the type of roots produced. Some species produce very fine, fragile roots in a well-drained medium, such as sand, and these roots often break-off during transplanting.

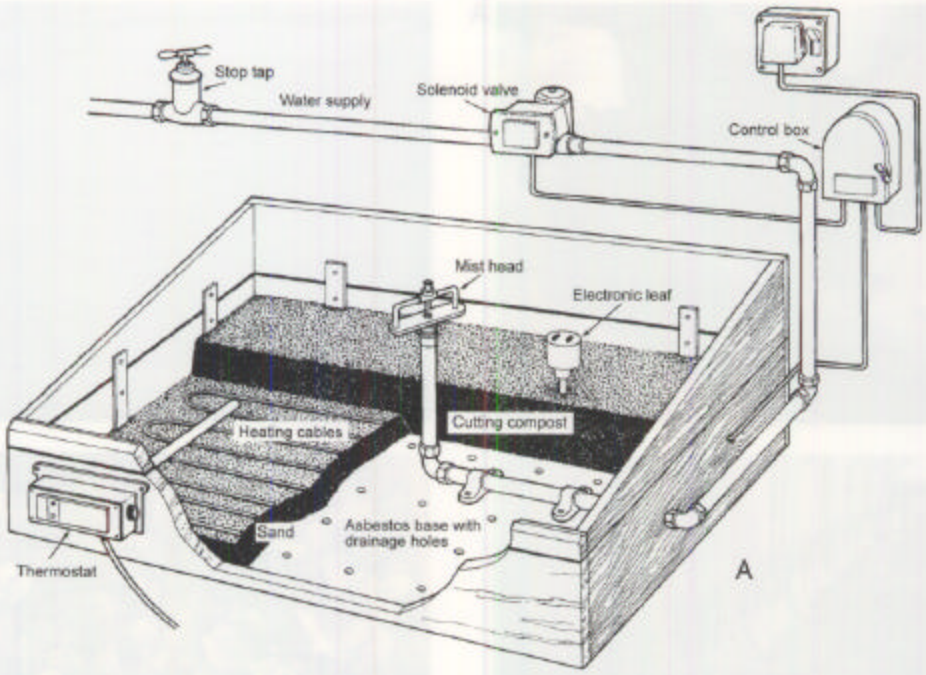
***Propagation environments*.** Cuttings must be carefully cultured in special propagation environments until they can form new roots. Some growers construct relatively simple rooting beds (figure 6.3.11 A) or chambers (figure 6.3.11 B) that feature bottom heat and humidity controls. Nurseries that produce a large number of cuttings use sophisticated propagation facilities that have completely controlled environments including sophisticated fogging systems. The Quebec Ministry of Natural Resources has developed an innovative rack propagation system that consists of enclosed shelves that hold 4 "mini-greenhouses" equipped with fluorescent lights and boom irrigation systems (figure 6.3.11C/D). The 24 mini-greenhouses can hold up to 51,000 cuttings each, for a total capacity of over 1.1 million cuttings per propagation structure. The facilities can be used year-round for up to 5 production cycles. The mini-greenhouses must be installed in an air-conditioned room to prevent heat build-up. The temperature within the mini-greenhouses is maintained at 20 °C (68 °F), the relative humidity at 90 to 95%, and the light

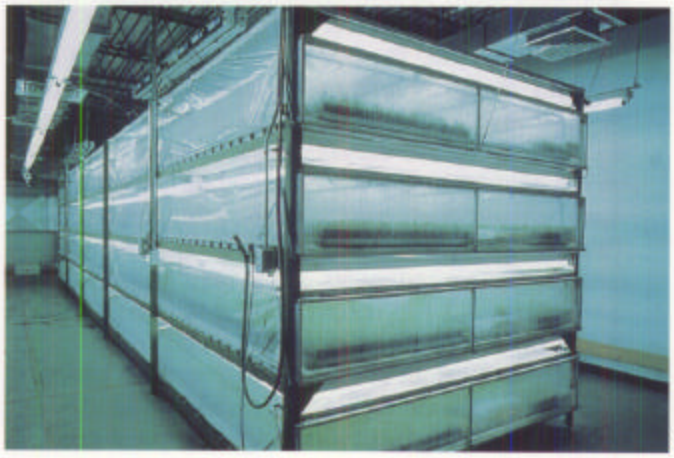
Figure 6.3.10—*In pre-rooting, semi-hardwood or softwood cuttings must be rooted first in beds or trays* (**A**) *with hormones and special propagation environments* (**B**). *Misting or fogging* (**C**) *is crucial to minimizing transpirational water loss in cuttings, but only the highest quality irrigation water should be used to avoid the formation of crusts on leaves and surfaces* (**D**).

at 20 µmol/m²/s for a 18- to 20-hour photoperiod (Tousignant and others 1996).

Growers should pay special attention to the following potentially limiting factors in the propagation environment. The reader is referred to Hartmann and others (1997), Macdonald (1986), and the other general references for a more detailed discussion.

- **Irrigation**--Water loss is the single most important limiting factor because newly harvested cuttings do not have any roots to replenish moisture lost through transpiration. Some nurseries have specially constructed enclosures that generate a periodic mist or fog that keeps the relative humidity at almost 100% (figure 6.3.1 OC). Many automated misting systems require sophisticated electrical controls, which can be quite expensive. However, less expensive systems can be constructed using time clocks. Some species root more easily in subirrigated trays, although success varies greatly between species (Regan and Henderson 1996). The quality of irrigation water is critically important with mist nozzles or fog systems, as water high in dissolved salts (high electrical conductivity) will leave damaging and unsightly deposits on the cuttings (figure 6.3.1 OD). "Hard" irrigation water is particularly troublesome in this regard (Read 1995). In addition, all irrigation sources must be filtered to remove sediments which may plug nozzles. (See section 4.2.4 in volume four of this series for more information on irrigation water quality.)
- **Temperature**--Both air and rooting medium temperatures are very important to successful rooting. The relationship between day and night temperatures is





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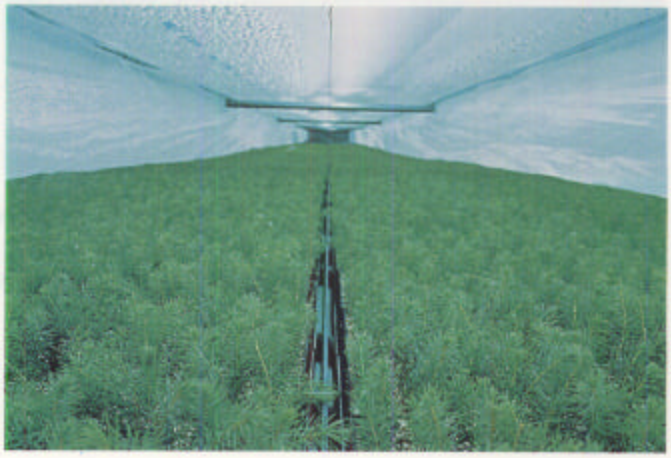

Figure 6.3.11—*Cuttings root easier in specialized propagation beds* (**A**) *or chambers* (**B**) *that feature misting or fogging to maintain almost 100% relative humidity, and electrical cables or steam pipes to keep the root systems warmer than the shoots. Nurseries that produce large numbers of cuttings have developed specialized propagation facilities* (**C & D**) *(A, modified from Wright and Titchmarsh 1981; C & D, courtesy of D. Tousignant, Quebec Ministry of Natural Resources).*

critical. For most species, daytime air temperatures of 18 to 32 °C (64 to 90 °F) with nighttime temperatures about 5 °C (10 °F) lower are recommended (Preece 1995). Actually, the temperature of the rooting medium is even more critical. Root initiation and development have different optima, however, and so growers should lower root zone temperatures after roots emerge. Although it must be determined for each species, a rooting medium target of 30 °C (86 °F) for root initiation and 20 °C (68 °F) for root development is a good place to start. Bottom heat is even more important under mist irrigation because the frequent irrigation keeps the temperature of the growing medum low through evaporative cooling (Read 1995).

- **Light**--Effects of light on the rooting of cuttings is very complex, as some species respond to different daylengths (photoperiods), intensity of light, and light quality. Traditionally, leafy cuttings have been placed under 50% shadecloth, as much to minimize transpiration as to improve rooting (Maynard 1993). Maximum photosynthesis of black spruce cuttings occurred at light intensities of 200 to 300 Nmol/s/m2 which is only about 10 to 15% of full sunlight. These studies also revealed a strong relationship between light and air temperature, however, as successful rooting at 30 °C (86 °F) required three times the amount of light as those rooted at 10 °C (50 °F)(Yue and Margolis 1993). The effect of long days on the rooting of cuttings is less clear: many growers believe that anything that stimulates shoot growth decreases root growth. On the other hand, photoperiod lights would be beneficial in finishing cuttings after roots have formed. The influence of light quality on the rooting of forest and conservation crops has not received much study although root initiation, like many other growth processes, is probably regulated by the phytochrome process. Crop lighting that gives more red than far-red light appears to stimulate rooting in many greenhouse crops (Moe and Andersen 1988).

With some species, stock plant **etiolation** (figure 6.3.12) has greatly improved rooting success (Maynard and Bassuk 1988). The process consists of forcing stock plants to produce new shoot growth under heavy shade or complete darkness and then using that portion of the stem as the cutting. Banding or wrapping the bottom of stems with Velcro® strips to exclude light is a practical application of the technique. Etiolation and banding have been used successfully with many woody plants, including maples, oaks, and pines (Maynard and Bassuk 1988).

Fertilization--Most species do not generally require mineral nutrients until they are transplanted, and there is no reason to fertilize cuttings until they have rooted. Frequent misting, however, can leach nutrients directly from leafy cuttings and from the growing medium. Some growers incorporate slow-release fertilizer into the growing medium, but research has shown that this is quickly depleted. A weekly fertigation of 300 to 400 ppm nitrogen may be a better option during root formation (Argo and others 1995).

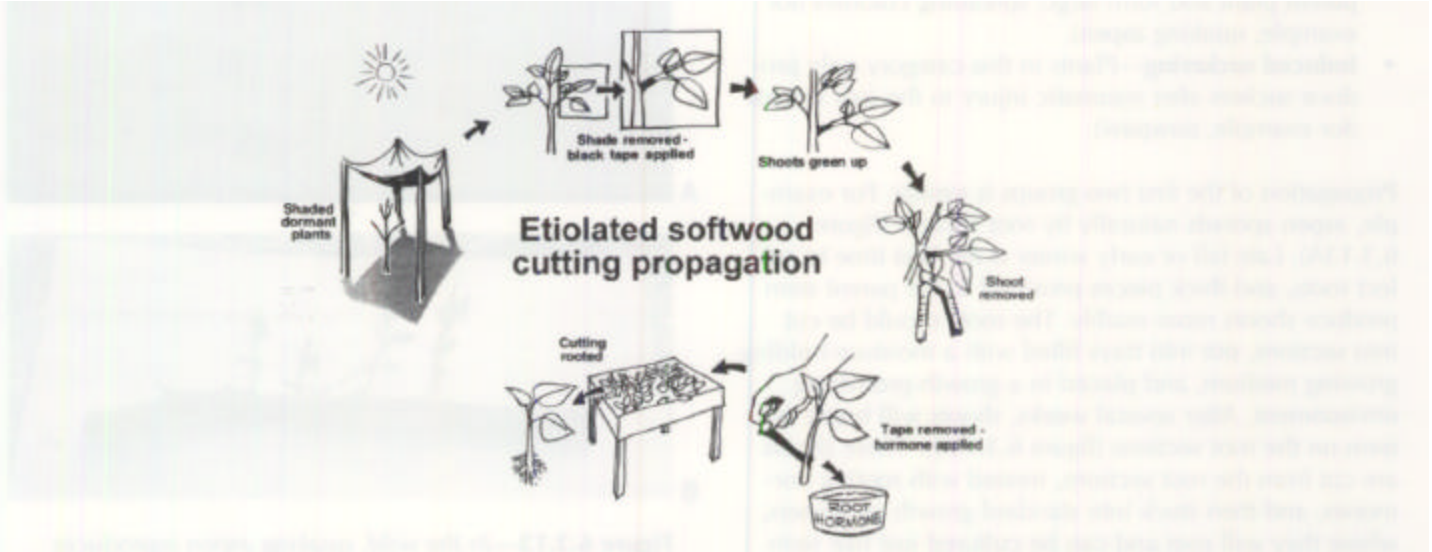

Figure 6.3.12—*Stock plants can be given special cultural techniques to improve rooting. Banding is a form of etiolation that uses exclusion of light as a stock plant pretreatment for difficult-to-root species (modified from Maynard and Bassuk 1988).*

6.3.3 Root Cuttings

Although not commonly used in forest and conservation nurseries, propagation by root cuttings is an ancient practice that was first described by the Greek philosopher Theophrastus (371-287 BC). Root cuttings are not common today because of the greater ease of rooting stem cuttings, especially with hormones, bottom heat, and intermittent mist. Nevertheless, several hard-to-grow native plants can be propagated by root cuttings, including sumacs, blackberries, and hawthorns. Root cuttings can also be used to meet a specific management objective: for example, quaking aspen can be grown from seed but root cuttings can be used when seed is difficult to obtain or to maintain certain genotypes. A complete list of trees and shrubs that can be produced with root cuttings can be found in Del Tredici (1996), who also does an excellent job of summarizing the literature.

Root cuttings have their own terminology. The **proximal end** of the root is the end nearest the main stem, whereas **distal end** refers to the end farthest from the stem. This distinction is important because, when root cuttings form buds, they are typically found at the proximal end. There are three basic groups of plants that form root suckers (Hudson 1956):

- **Natural suckering without division**--This group includes species that naturally produce root suckers near the parent plant, often forming dense thickets (for example, hawthorn).
- **Natural suckering without division**--These species naturally produce suckers at greater distance from the parent plant and form large, spreading colonies (for example, quaking aspen).
- **Induced suckering--**Plants in this category only produce suckers after traumatic injury to the root system (for example, pawpaw).

Propagation of the first two groups is similar. For example, aspen spreads naturally by root suckers (figure 6.3.13A). Late fall or early winter is the best time to collect roots, and thick pieces proximal to the parent stem produce shoots more readily. The roots should be cut into sections, put into trays filled with a moisture-holding growing medium, and placed in a growth-promoting environment. After several weeks, shoots will begin to form on the root sections (figure 6.3.1313). These shoots are cut from the root sections, treated with rooting hormones, and then stuck into standard growth containers, where they will root and can be cultured just like stem cuttings (Schier 1978). Just recently, aspen has been propagated by sticking root cuttings directly in growth containers. The cuttings were collected from container stock plants, treated with captan fungicide and stored in damp peat moss. When the cuttings were deeply stuck, with the proper polarity and the top of the cutting just below the surface of the growing medium, rooting success ranged from 42 to 91 % (Dreesen and Harrington 1997). Paulownia is propagated similarly: sections of the primary and lateral roots of 1- or 2-year-old seedlings are used as propagules and planted directly in the growth container. Because these succulent root cuttings are subject to rotting, the sections should be air-dried for 10 days and then dipped into a fungicide solution before planting (Stringer 1994).

Root cuttings from plants in the third category will not produce shoots and thus the plant must be propagated in place. In late fall, the roots should be severed in the wild with a spade starting about 15 to 25 cm (6 to 10 in) from the main stem and moving out in concentric circles. Leaving the severed roots in the ground stimulates shoots to develop with their own root systems. These separate plants can then be harvested the following year. In addition to pawpaw, this in *situ* technique will work for other shrubs and small trees, including sumac and aralia. (Del Tredici 1996).

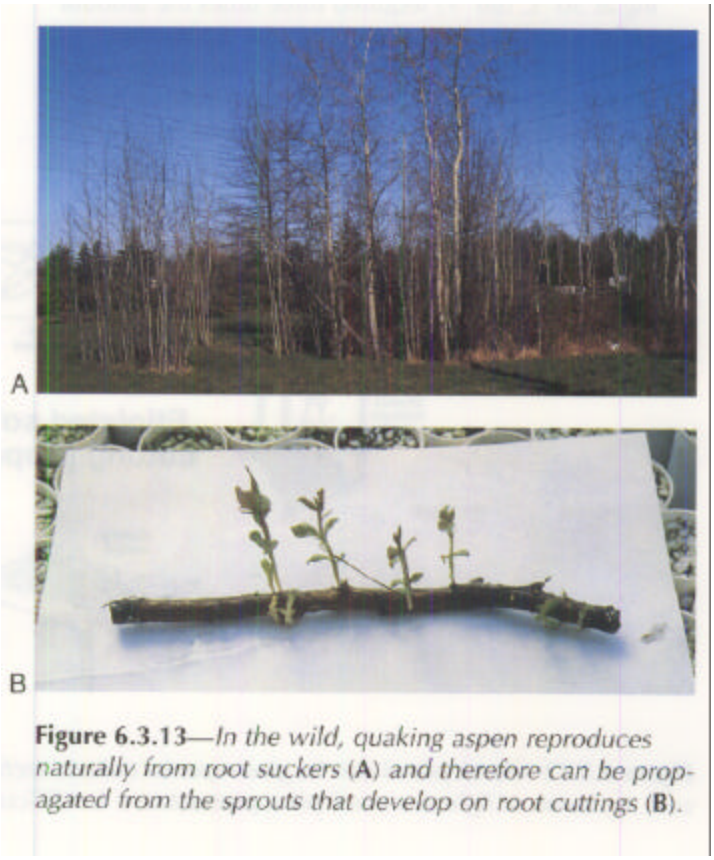

Figure 6.3.13—*In the wild, quaking aspen reproduces naturally from root suckers* (**A**) *and therefore can be propagated from the sprouts that develop on root cuttings* (**B**).

6.3.4 Layering

This traditional technique was developed in bareroot nurseries but can be adapted to container culture for species that cannot be propagated otherwise. Layering involves forcing a part of the stem to form adventitious roots while still attached to the parent plant: the method has several biological advantages. Layering is a low-stress propagation method because there is minimal physical trauma and the donor plant provides a steady supply of water and nutrients while roots are being formed. This is especially valuable when propagating threatened and endangered plants because there is little risk to the donor (figure 6.3.2B). Layering also produces a relatively large plant in a short time. Because it is so labor-intensive and the multiplication factor is low, layering is very expensive and can only be justified for special propagation projects. However, layering can be very productive in nurseries with minimal propagation facilities. See Hartmann and others (1997) for more details on the many types of layering used in horticulture. In forest and conservation nurseries, three types have been used.

6.3.4.1 Tip layering

This traditional technique consists of bending a side shoot or branch over until it can be held in place and covered with growing medium or mulch (figure 6.3.14A). Rooting of the buried section is naturally stimulated by

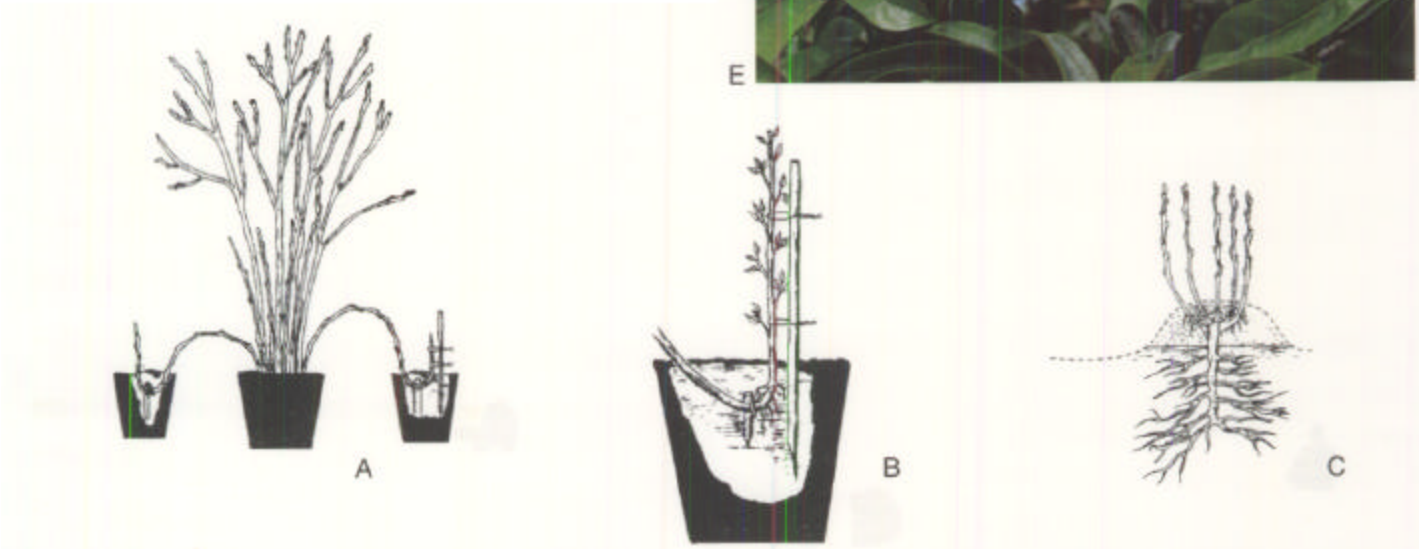

Figure 6.3.14—*Layering is a traditional propagation technique in which rooting is induced on stems or branches by covering them with growing medium or soil. In tip layering, roots are induced on a lateral branch tip* (**A&B**) *whereas in mound layering, shoot sprouts from a decapitated plant are covered with soil* (**C**) *and then transplanted to containers after roots are formed* (**D**). *Air layering* (**E**) *consists of wrapping a branch section with moisture-retentive material until roots form (A–C, modified from Hartmann and others 1997).*

the interruption of the normal basipetal translocation of photosynthates that accumulate near the bend and by the exclusion of light. Cultural procedures that encourage rooting in stem cuttings, such as the use of hormones and wounding, also hasten the formation of roots in the buried stem section (figure 6.3.14B).

6.3.4.2 Mound layering

Another type of layering involves inducing roots to form on stern or root sprouts. Mound or "stool" layering consists of planting a rooted cutting and allowing it to become established. Before growth starts the following spring, the top of the shoot is severed just above the ground line, stimulating new sprouts to form. When the sprouts reach 8 to 13 cm (3 to 5 in.), sawdust or soil is mounded over them and they are kept moist until the end of the season. By this time, roots have formed on the sprouts (figure 6.3.14C), which are then cut off as close as possible to the base and used as rooted cuttings (Hartmann and others 1997). Mound layering has recently been modified for large container production of Arizona sycamore and other riparian trees in New Mexico (Dreesen and Harrington 1997). Although these species can be grown from seed, mound layering quickly produces the large-l9- to 76-liter (5- to 20-gal) container plants that are needed for riparian restoration projects in this relatively stressful environment (figure 6.3.14 D).

6.3.4.3 Air layering

With this technique, the bark is wounded or completely stripped from a section of stem or a lateral branch of the donor plant (figure 6.3.14E). The best success occurs with stems of the previous season that still have mature leaves. The wounded section is treated with rooting hormone and immediately covered with damp peat moss or other moisture-holding material that is secured with a clear plastic wrap. The air layer is then covered with aluminum foil or other reflective wrapping to exclude light and moderate the inside temperature. The protective ,wrapping can be removed periodically to check for root development and to rewet the peat moss. The new plant is ready to be cut off the parent and transplanted in about 2 to 3 months; however, difficult-to-propagate species may require up to two seasons. Air layering has been used to propagate many tropical and subtropical plants, notably Citrus spp., as well as some pines for tree improvement purposes.

6.3.5 Division

Some plants naturally spread laterally by forming new shoots, rhizomes (modified stems), or bulbs, and so they can be easily propagated by separation (removing naturally detachable structures) or division (cutting the plant into sections). Although Hartmann and others (1997) distinguish between these two methods, we see little operational difference and so will refer to them both as division.

In forest and conservation nurseries, division is used primarily for propagating wetland species such as sedges, rushes, and bulrushes. that are used for restoration or constructed wetland projects. The initial collections are made from native stands but then stock plants are started in large containers or trays in the nursery. When they have grown large enough, the plants can be divided into sections (figure 6.3.15A) and transplanted to new containers. With some species, even the smallest section will root but the size of the divisions is critical with some species; for example, divisions of soft rush must have at least 5 stems or they are not viable (Street 1994). Division can be repeated several times during the growing season and the new transplants are then given normal culture until they are ready for harvest (Beagle and Justin 1993). During harvesting, new plantets can be collected and used to start the next crop (figure 6.3.1513). Like other vegetative propagation techniques, division is relatively labor-intensive but provides a quick and sure way to produce wetland species.

Figure 6.3.15—*Wetland plants such as sedges are propagated by division. Donor or stock plants are separated physically into sections* (**A**)*, which are transplanted back into containers* (**B**).

6.3.6 Grafting

Grafting is the art of propagating plants that are difficult or virtually impossible to raise by other vegetative techniques. There are several types of grafts but all involve physically binding two plant parts together so that they will bond and grow into a single individual. The **scion,** the upper portion of the graft that will develop into the new shoot system, is bonded to the **rootstock**, which is an established plant of the same or a closely related species (figure 6.3.16A). Although it is commonly used to propagate ornamental and fruit trees, grafting is only used for genetic improvement purposes in forest and conservation nurseries. Commercial forest tree species require many years to produce seed naturally and so tree improvement specialists use grafting to establish clonal seed orchards that can become productive in only a few year.

Scions are collected from the tops of specially selected "plus trees" in the forest and then grafted onto seedling rootstocks in the nursery. Because these scions are vegetatively mature (see section 6.3.1.3), they will flower and produce seed much sooner than would trees in a seed orchard produced from seedlings (figure 6.3.5). Grafts are subject to desiccation and physical damage so container grafting is often preferred because the plants can be protected and moisture loss can be controlled. In some nurseries, grafted container plants are being raised in greenhouses or other sheltered propagation environments to further accelerate seed production (figure 6.3.1613). Grafting is a very precise technique requiring considerable training and experience, and so the reader is referred to Hartmann and others (1997) and Macdonald (1986) for more specific information.

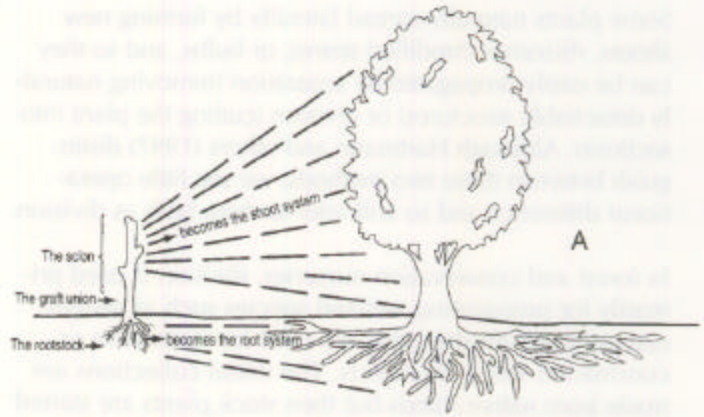

Figure 6.3.16—*Grafting is the art of physically joining the scion and rootstock from two different individuals into a single plant* (**A**). *In forest nurseries, grafted container plants are being raised in greenhouses or other sheltered propagation environments to further accelerate seed production* (**B**). *(A, modified from Hartmann and others 1997.)*

6.3.7 Micropropagation

This application of tissue culture techniques to propagation involves very small plant parts grown under aseptic laboratory conditions. Although there are several different types of micropropagation, they all start with excising a small piece of plant tissue, cleansing it of microorganisms, and culturing it in an artificial medium in a test tube or small laboratory vessel. The excised plant part that serves as the initial propagule is known as the **explant.** By manipulating laboratory environment and supplying specific hormones and vitamins at the proper stage of development, these explants multiply and develop into numerous miniature **plantlets.** Obviously, the micropropagation process is quite complicated and so the reader is referred to Dirr and Heuser (1987), Kyte and Kleyn (1996), and Hartmann and others (1997) for a complete and thorough discussion.

Micropropagation has many advantages (Suttle 1995):

- **Ease of propagation**--Poor rooting percentages and graft incompatibility are common problems that can be overcome with micropropagation; some plants, such as redbuds and lilacs, are just very difficult to propagate.
- **Juvenility**--Micropropagated plants retain their juvenility longer and so can be used as stock plants for a source of easy-to-root cuttings.
- **Clean plant material**--Micropropagated plantlets are inherently free of pathogens, especially viruses that can easily be spread by other vegetative propagation techniques.
- **Low space requirement**--Large stool beds or blocks of container stock plants are expensive to maintain, whereas cultures of micropropagated stock plants require minimal space.
- **Year-round production**--Because micropropagation is not subject to seasonal cycles, plants can be propagated all year, which spreads out the workload.

Operational implementation of micropropagation, however, poses several potential economic and technical problems (Zimmerman 1985):

- **Unique cultural requirements**--Each new species requires empirical testing to adapt existing procedures, and it may be impossible to economically propagate some plants.
- **High labor costs**--Production costs will remain high because specialized labor accounts for 60 to 80% of the Iota] costs of micropropagation.
- **Scaling up**--Although the ability to produce tens of thousands of plants from a single explant is theoretically correct, the costs of the facilities and labor required to expand to an economical scale are often prohibitive.
- **Culture contamination--**The control of microorganisms, especially bacteria, and mites is a continual problem and requires sterile facilities and rigorous sanitation procedures.
- **Field performance and phenotypic stability**--With forest crops, the long time needed to evaluate the performance of micropropagated plants is an expensive but necessary process. Field testing of micropropagated hardwood trees has shown some undesirable phenotypic variation (Charest 1996).

Micropropagation is commercially viable for many ornamental plants because of their intrinsic high value. For example, Colorado blue spruce trees with deep blue foliage are in great demand for landscape plantings, but the best cultivars do not grow true-to-seed. Only a very low percentage of the seedlings grown from the seeds of a blue parent retain the desirable foliage color, and so vegetative propagation is required. Rooted cuttings are expensive to produce and can remain plagiotropic (making them misshapen), whereas grafting is effective but expensive. Micropropagation, however, has tremendous potential for high-volume production of desirable cultivars of this valuable landscape tree (Cervelli and Webster 1995).

There are several different types of micropropagation, but only two are currently being used in forest and conservation nurseries.

6.3.7.1 Organogenesis (microcuttings)

Organogenesis, the most popular micropropagation process, can be divided into four sequential cultural operations (Dirr and Heuser 1987):

- **Establishment of an aseptic culture**--This step begins with surgically excising the desired plant tissue and establishing and maintaining the explant on artificial media under sterile laboratory conditions (figure 6.3.17A).
- **Multiplication of the explant**--The objective of this stage is to rapidly increase the number of propagules by chemically coaxing the stock cultures to divide and elongate into miniature shoots that can be harvested as **microcuttings.**

A

B

C

D

Figure 6.3.17—*Microcuttings are established in artificial cultures* (**A**)*, which require highly trained workers and specialized sterile facilities including laminar-flow transfer hoods* (**B**) *and climate-controlled culture rooms. For example, shoot tips of a rare plant, showy stickseed, were induced to proliferate and then root in tissue culture* (**C**)*, grown into hardened plantlets that were then cultured like normal seedlings* (**D**) *(A, C, & D, courtesy of J. Edson).*

- **Rooting of the microcuttings-The** microcuttings are collected under aseptic conditions and transferred to another vessel with a different media containing hormones that induce rooting (figure 6.3.1713).
- **Transplanting and acclimating the plantlets.-When** the microcuttings have developed enough roots (figure 6.3.17C), they can be transplanted from the culture vessels to typical growth containers and moved to the normal propagation area. They are gradually acclimated in several stages and then grown to shippable size by normal cultural operations (figure 6.3.17D).

The exact propagation protocol of hormone types, concentrations, and timing of the various stages varies between species and even cultivars. One of the real challenges of this technique has been to develop routine procedures that can be used for operational production.

High production costs make microcuttings impractical for most forest and conservation species at the present time. However, the Simpson Timber Company in Korbel, California, has an operational micropropagation system that produces 300,000 to 500,000 redwood (figure 6.3.18A) and 500,000 eucalyptus seedlings per year (Lehar 1997). Compared to normal seedlings, the micropropagated redwood seedlings had up to a 122% gain in volume (figure 6.3.1813).

Microcuttings also have application for the conservation of threatened or endangered species that, for whatever reason, cannot be propagated by conventional techniques. Showy stickseed is a forb that currently is found in only one population of fewer than 500 individuals. Shoot tips of 10 collections were micropropagated in tissue culture (figure 6.3.17C/D), and the resultant plantlets have been outplanted at four different sites (Edson and others 1994).

6.3.7.2 Somatic embryogenesis (SE)

This second type of micropropagation involves chemically inducing tissue cultures to form somatic embryos. Two propagation protocols have been used: **indirect embryogenesis**, which requires an intermediate step of callus tissue to produce the embryos and **direct embryogenesis**, which does not (Dirr and Heuser 1987). Most SE systems for forestry species, including spruces and larches, use indirect embryogenesis. In some pines, however, direct embryogenesis is used to produce numerous identical plantlets from a single immature seed. The process

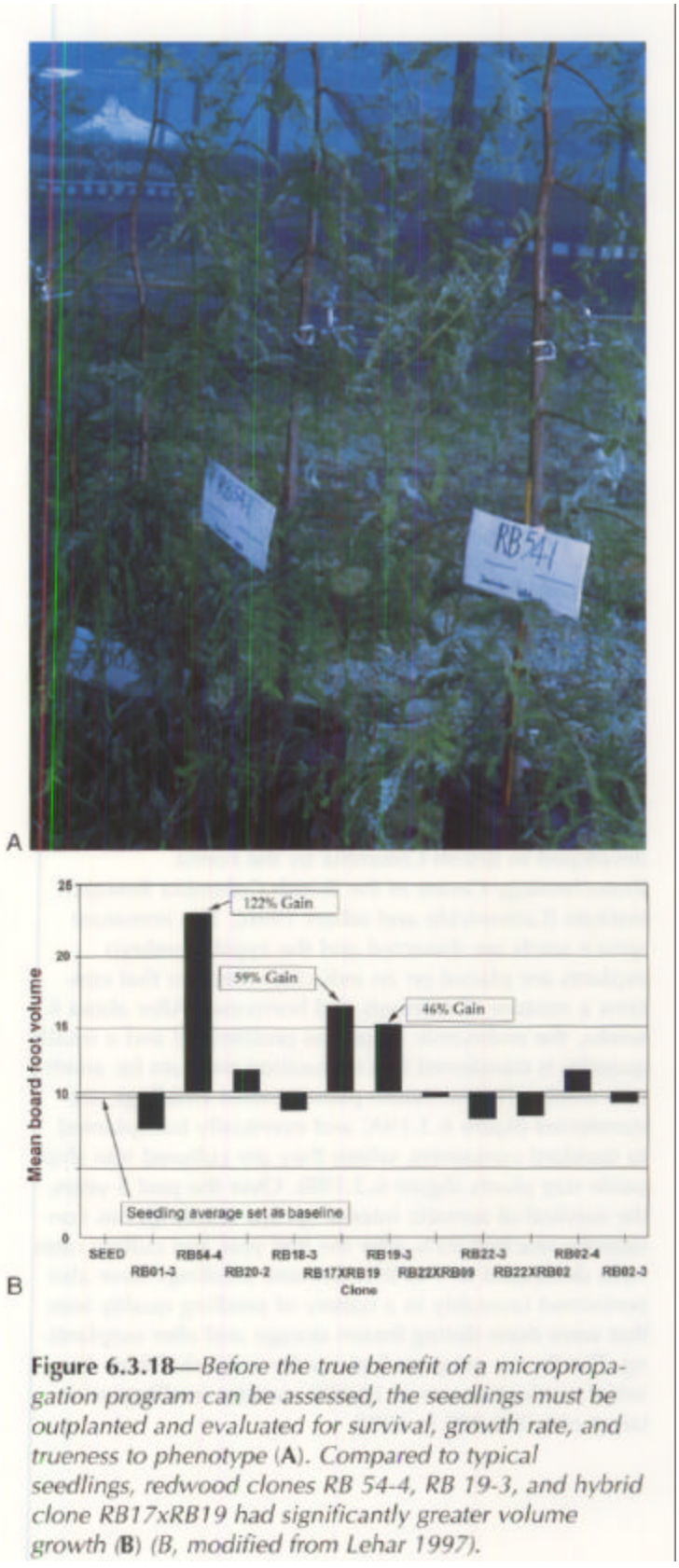

Figure 6.3.18—*Before the true benefit of a micropropagation program can be assessed, the seedlings must be outplanted and evaluated for survival, growth rate, and trueness to phenotype* (**A**). *Compared to typical seedlings, redwood clones RB 54-4, RB 19-3, and hybrid clone RB17xRB19 had significantly greater volume growth* (**B**) *(B, modified from Lehar 1997).*

involves a series of sequential cultural procedures that cause an excised immature seed to produce somatic embryos that are then propagated to produce many plantlets. With radiata pine, at least 10,000 embryo initials can be obtained from 1 g of fresh tissue (Jones 1990)

One of the most critical steps is selecting the immature parent embryo during a relatively short window (as short as 2 weeks) after fertilization of the seed. This step is particularly tricky for pines because the embryo is not fertilized over a year after pollination. In Scots pine, for example, fertilization takes place about 13 months after pollination, but the timing still varies with temperature, so that degree days are used to schedule the excision of the seed (Keinonen-Mettala and others 1996). The SE process has been used for only a few forest species on an operational scale. Spruces are particularly suited for SE because the embryos can be harvested from stored seed, compared to the narrow 1- to 2-week window for Douglas-fir or pines.

In commercial forestry, the most practical application of SE is to bulk-up genetically improved control-pollinated seed. The Canadian Forestry Service is using this new biotechnology to accelerate the tree-breeding cycle of several species of spruces and larches (Charest 1996). One operational SE program for interior spruce has been developed in British Columbia by the Forest Biotechnology Centre of the British Columbia Research Institute (Grossnickle and others 1996). The immature spruce seeds are dissected and the zygotic embryo explants are placed on an induction medium that contains a mixture of nutrients and hormones. After about 6 weeks, the embryonic tissue has proliferated and a small quantity is transferred to a maturation medium for another 6 weeks. The miniature plants, called **emblings**, are transferred (figure 6.3.19A) and eventually transplanted to standard containers, where they are cultured into shippable size plants (figure 6.3.1913). Over the past 5 years, the survival of somatic interior spruce seedlings has consistently reached 95% after the first year and culling rates have decreased to 5 to 8%. Somatic seedlings have also performed favorably in a battery of seedling quality tests that were done during frozen storage and after outplanting. The 5-year program has rapidly expanded from an initial production run of 12,000 somatic seedlings to a target of 1,000,000 in 1998.

Figure 6.3.19—*The somatic embryogenesis (SE) procedure begins when zygotic embryo explants are removed from seeds and cultured on an artificial medium to produce a mass of cotyledonary somatic embryos (**A**). These are then transferred to a different medium to "germinate." The final step is to transfer the emblings to growing media in standard containers where they are cultured into somatic seedlings (**B**). (Courtesy of David Cyr and Steve Grossnickle, Forest Biotechnology Centre, BC Research Inc.)*

6.3.8 Summary

Vegetative propagation is defined as the production of new plants that contain the exact genetic characteristics of the parent plant. If the objective is to generate a large number of plants that have been genetically selected for some characteristic like fast growth, then vegetative propagation is the preferred method. Several different vegetative propagation methods are being used in forest and conservation nurseries. The choice will depend on factors such as species characteristics, type of propagation environment, and the skill of the propagator. Rooted stem cuttings are the most widely used technique and are preferred for easy-to-root species like willow and cottonwood. Rooting cuttings of other species requires experience to determine the best time of collection, treatment with rooting hormones, and other cultural requirements. Some species are completely recalcitrant and cannot be rooted. Others can be propagated from root cuttings, layering, or division depending on the characteristics of the plant species. Grafting is a specialized technique that is only used to establish in tree improvement programs.

Micropropagation, the newest type of vegetative propagation, requires specialized equipment and techniques. Although it is mainly used for bulking-up improved seed of commercial forest tree species, micropropagation can also help preserve valuable species or ecotypes. Therefore, at the present time, vegetative propagation techniques will remain highly specialized and only used for less than 5% of all forest and conservation species.

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