

ASEXUAL PLANT PROPAGATION: SPECIAL TECHNIQUES AND CONSIDERATIONS FOR SUCCESSFUL HIGH ALTITUDE REVEGETATION.

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ABSTRACT

High altitude restoration with woody plants poses unique propagation challenges for natural resource managers including unpredictable wildland seed crops, short growing seasons, limited access to wildland plants, genetic considerations, seasonal staffing, uncertain and changing construction schedules, and short revegetation intervals. Although sexual propagation (seed) is generally less labor and equipment intensive, limited seed and long or unknown dormancy requirements can result in lengthy production schedules. Asexual propagation (cuttings) of woody trees and shrubs provides a viable alternative for high altitude settings. Dormant hardwood cuttings provide ease of handling and storage, however, access to donor plants, winter browsing, seasonal staffing, and reduced winter greenhouse operations often limit their use. Summer cuttings facilitate access to donor plants, reduced browse competition, improved percentage rooting, shorter production intervals, adequate labor, and efficient greenhouse operation. The selection of a propagation technique depends on genetic considerations, the propagation characteristics of the species, site and environmental factors, economic and procurement considerations, and construction schedules and goals. Favorable propagation conditions include proper and limited storage, fungicide dip, wounding, recut base, treatment with growth regulators, intermittent mist, sterile well drained media, bottom heat, shade, and strict environmental control.

INTRODUCTION

Natural resource managers responsible for the revegetation of high altitude disturbances are faced with numerous and complex biological, environmental, and economic challenges as a result of the severe climate and limited growing season characteristic of these environs. The revegetation of high altitude disturbances requires land managers to make decisions based on site- and project-specific considerations. The method of plant propagation employed depends on many interrelated variables including genetic considerations, the propagation characteristics of a given species, site and environmental factors, economic and procurement considerations, and construction schedules and goals. The asexual propagation of woody plants from stem cuttings taken during the growing season is an often overlooked approach that may lend itself well to certain high altitude situations.

PROPAGATION DEFINITIONS

Plant propagation is defined as the science and art of multiplication of plants by either sexual or asexual means. *Sexual* reproduction involves meiotic cell division that ultimately produce progeny (seedlings) with new or differing genotypes relative to their male and female parents. Most woody plants are highly *heterozygous*, that is, a relatively high number of genes on one chromosome of a Mendelian pair differ from those on the other chromosome. As a result, the progeny of woody plants grown from seed tend to exhibit a relatively high amount of genetic variation (Hartmann and Kester 1983). For the purposes of this discussion, sexual reproduction is synonymous with propagation by seed, although not all embryos develop from sexual processes.

Asexual propagation by cuttings involves removing sections of stem or root tissue from the parent or donor plant, treating this tissue with plant growth regulators, and then inducing adventitious root or shoot formation under controlled environmental conditions. Asexual propagation is reproduction from the vegetative parts of the donor plant and involves mitotic cell division in which the chromosomes duplicate and divide to produce two nuclei that are genetically identical to the original nucleus. This can occur through the formation of adventitious roots and shoots or through the combining of vegetative tissues, such as in grafting. This clonal process, in which the genotype of the parent plant is exactly duplicated, is made possible because of two unique plant characteristics. *Totipotency* is the property of vegetative plant cells to carry all of the genetic information necessary to regenerate the original plant. *Dedifferentiation* is the ability of mature (differentiated) cells to return to a meristematic condition and produce a new growing point (Hartmann and Kester 1983). In the context of this discussion, asexual reproduction refers to the induction of adventitious roots from stem cuttings and is synonymous with vegetative and clonal propagation.

Although there are numerous variations of the stem cutting, they can be broadly classified into two groups based on the time of year that they are taken. *Dormant hardwood* cuttings are taken in mid to late winter of mature tissue from fully dormant

plants, i.e., plants that have dehiscid their leaves but have not yet initiated spring growth. Usually the previous season's growth is used, although two year and older wood can be used in some cases. Dormant hardwood cuttings are easy to take, handle, and store; which allows for flexibility in the preparation of the cutting and, in general, require less care than cuttings that include actively growing tissue. The cuttings are taken at a time of year when field responsibilities are limited and there is little need for special care, other than cold storage, prior to treatment in the greenhouse. More dormant deciduous cuttings can be stored per unit area than summer cuttings because of the absence of foliage. There is less chance of cutting desiccation or mechanical damage during all stages of handling, storage, preparation, and placement in the greenhouse.

The second type of stem cutting is the *summer cutting* and includes *softwood* and *semihardwood* (greenwood or ripe-wood) cuttings. These types consist, at least in part, of actively growing tissue, and are taken from early to late summer. Softwood cuttings consist of succulent, new growth only, although any cutting that includes actively growing tissue is sometimes erroneously referred to as 'softwood'. True softwood cuttings generally produce adventitious roots faster and have a higher percentage rooting than their hardwood counterparts. Semihardwood cuttings are also new growth but are partially-matured wood taken in mid to late summer. Softwood and semihardwood cuttings, because of their succulent nature, are more susceptible to mechanical injury and tissue desiccation than hardwood cuttings (Hartmann and Kester 1983; Macdonald 1986).

It is also possible to take summer cuttings that include stem tissue that is two or more years old. These are sometimes referred to as *summer hardwood* or *active hardwood* cuttings. Using summer hardwood tissue is practiced infrequently because mature tissue generally does not produce adventitious roots as readily as actively growing, current season's tissue. Also, there is less physiological stress on a dormant hardwood cutting than on a summer hardwood cutting because of the absence of foliage (with deciduous species). This method may prove useful, however, in high elevation situations when softwood and semihardwood tissue is too small or weak to produce roots or when access to dormant hardwood cuttings is limited by inclement weather.

GENETIC CONSIDERATIONS

The degree to which genetic factors will influence plant propagation depends on the goal of the construction project. More latitude may be possible if the goal is simply to establish plant cover on the disturbance, i.e., 'revegetation'. If the goal is to recreate the genetic variation of the site to some predisturbance level through 'restoration', fairly systematic and comprehensive measures will be needed (Majerus 1997). Research suggests that the long term success of high altitude revegetation and restoration efforts depend largely on the genetic makeup of the revegetation material as it reflects the plants ability to tolerate and adapt to a harsh and variable environment. Plant propagation techniques and the manner in which they are implemented can have a dramatic effect on the genetic composition of the target site. The preservation of genetic variation may be particularly important in high elevation settings where biological diversity is

characteristically low (Harris 1984). Poor planning and decision making during plant propagation could ultimately result in alterations in the genetic composition and structure of the revegetated ecosystem causing reduced genetic variation, inbreeding depression, and genetic drift. This in turn may lead to poor plant adaptability and decreased ecosystem stability over time.

Specific, practical methodologies for the preservation of genetic variation within and between woody plant populations during plant production are often lacking or are inappropriate for restoration purposes. Inadvertent selection may occur during propagule collection, processing, propagation, harvesting, and reintroduction (Meyer and Monsen 1992). The technology needed to identify the extent and distribution of genetic variability within or between populations exists, although little of this research has been applied to commercially unimportant woody species. Substantial research has been conducted on the genetics of native and cultivated grasses, agronomic crops, rangeland species, and the commercially important timber species (conifers). The applicability of this information to restoration is questionable, however, in that genetic variation was often evaluated in terms of some short term economic criteria and not long term ecological significance. In fact, natural adaptations that enhance long term survival often reduce the economic value of timber species (Smith 1962).

The development of guidelines for the collection and production of woody plant material for restoration also depends on an understanding of the manner in which they naturally reproduce. The genetic structure of plant populations is largely determined by their mating system, i.e., the degree to which they are self or cross pollinated (Brown 1990). Cross-pollinated species generally exhibit significant genetic variation both among individuals within a population as well as between populations (Millar and Libby 1989). Most trees and shrubs are cross-pollinated and highly heterozygous, having a high potentiality for genetic variability and subsequently, the opportunity for evolutionary change should environments change (Hartmann and Kester 1983). In conifer species, however, much of the genetic variation is distributed within instead of among populations (Loveless and Hamrick 1984). Conifers tend to have greater seed and pollen dispersal than herbaceous species (Levin 1981) as well as a relatively continuous spatial distribution. These traits increase the likelihood of uninterrupted gene flow and consequently decrease the likelihood for frequent genetic differentiation between populations, relative to a life form such as a grass or even a shrub (Knapp and Rice 1996). Tree seed zones have been established for several of the commercially important conifer species based on known patterns of genetic similarity or, on climatic contours when genetic information is lacking. Planting of seed outside the zone in which it was collected is avoided. In addition, within each seed zone, seed is not planted on sites differing more than 1,000 feet (305 meters) vertically or 100 miles (161 kilometers) horizontally from the collection site (Smith 1962; Buck 1970; Kitzmiller 1990).

There is substantial evidence and support for selecting populations native to or in close proximity to the revegetation site (Vallentine 1989; Meyer and Monsen 1992; Guinon 1993). For some species and habitats, however, this may not necessarily be true (McArthur and others 1983; Namkoong 1969). Recommendations for the systematic

sampling from both representative populations and from extreme or unusual populations represents one approach (Ledig 1988). For large scale revegetation, the sampling of all genotypes representative of all of the typical native environments of the species has been recommended. For cross-pollinated species, it may even be possible to produce new genotypes adapted to various intermediate environments (Munda and Smith 1995). Perhaps a cautious and reasonable approach to population selection is the recommendation to use collection sites as closely matched as possible to the restoration site in terms of geographic location, climate, soil, and matrix vegetation (Meyer and Monsen 1992). The issue is complicated by the fact that disturbed sites are environmentally changed, and the ecotypes that are best suited for long term survival may not necessarily be those found growing in close proximity on undisturbed sites.

The method of initial propagule collection and increase is thought to have a significant impact on the survival, establishment, and long term persistence of revegetated sites (Munda and Smith 1995). Adequate sampling of the genetic variation within a population is necessary to assure relatively predictable performance and the ability to adapt to changing environmental conditions over time. Most cross-pollinated species are sensitive to inbreeding depression, and care must be taken to secure an adequate sampling of genotypes, perhaps 100 or more, that are widely dispersed within the reference population. Research indicates that for cross-pollinated species, the loss of genetic variation over time correlates closely with the original number of plants sampled. (Frankel and Soule 1981). Improper sampling and the subsequent loss of genetic variation is especially serious when it occurs during initial propagule collection because little can be subsequently done to reverse this condition (Munda and Smith 1995).

It is possible for genetic change to occur when seed orchards or vegetative cutting blocks are established for the large scale increase of plant propagules. The risks are likely to be greater when these orchards are located at distant sites and/or in different environments relative to the collection site. It is unclear to what degree this may occur for any given species. The risk of change would seem great in seed orchards of open, cross pollinated trees when they are inadequately isolated from undesirable populations of the same species or a close relation. Loss of orchard trees, inbreeding depression, the unequal production of seed by the individual orchard trees, and other factors may be involved. In addition, some hybridization may occur between orchard plants leading to the creation of entirely new genotypes adapted to intermediate environments (Munda and Smith 1995).

In contrast to sexual reproduction, asexual propagation is clonal and assures preservation of the original genotype. Asexual propagation effectively eliminates the need to isolate cutting blocks, for future vegetative propagation, unless volunteer plants contaminate the orchard or random genetic mutations occur. On the other hand, the potential for restricting the genetic variation of a reference population is great with propagation by cutting if exceptional care is not taken during initial population sampling. It is also possible that some of the cutting block trees will die or that there will be differential rooting of the cuttings leading to a bias in the distribution of population genetics.

PROPAGATION CHALLENGES

The selection of a propagation method often depends on the reproductive characteristics of the species involved. Some species propagate readily by seed, producing frequent, abundant, and viable seed crops. Most woody plants indigenous to northern temperate climates possess one or more dormancy mechanisms that prevent seed germination until environmental conditions are favorable for germination, survival, establishment, and ultimately, species perpetuation. Cold chilling, warm stratification, mechanical or chemical seed coat scarification, or some combination of these or other treatments are usually needed before germination will occur. These conditions are fulfilled naturally by sowing seeds outdoors and allowing seasonal climatic conditions and soil processes to break dormancy. The same dormancy breaking requirements can be met with artificial techniques such as acid scarification, warm stratification in a greenhouse, prechilling in a cooler, and others. These requirements vary by individual plant, seed lot, species, population, time of year, method of seed handling and storage, climatic conditions, and other factors. Lengthy dormancy requirements may result in a more costly product or a longer construction schedule. Unknown dormancy requirements may prevent propagation by seed entirely.

As with propagation by seed, some species are easily propagated by cuttings while others are not. In some cases, propagation by stem cuttings is not possible because the species is incapable of producing adventitious roots or there is a lack of technology to do so. Adventitious roots may be produced, but at a rate so low as to not be practical. Some species can be propagated by stem cuttings but only at certain times of the year, i.e., only from hardwood or softwood cuttings and this may conflict with site access, greenhouse operations, etc., as previously described.

SITE AND ENVIRONMENTAL FACTORS

There are numerous site and environmental conditions that can influence the type of plant propagation system selected for producing plants. These conditions can directly impact propagation by effecting the production of wildland seed or by reducing plant and cutting vigor. Factors inhibiting the use of seed include poor weather as it impacts production and timely collection, consumption by animals, attack by insects and disease, and other factors.

Site conditions may also favor one type of asexual propagation technique over another. Despite the inherent ease of hardwood propagation, high altitude revegetation may impose limits on this type of reproduction. Dormant hardwood cuttings are usually taken from December through February when unpredictable weather conditions make travel difficult or impossible. Low stature trees and shrubs may be buried under snow and ice and not accessible. As forage decreases with the onset of winter, many herbivores turn to succulent woody stems and buds for sustenance. In most cases, severe and repeated browsing ultimately reduces the vigor of the plant and subsequently diminishes the ability of its cuttings to produce adventitious roots. Seasonal labor pools are usually low at this

time of year and greenhouse operations curtailed or suspended because of the high cost of heating. Certain combinations of these factors may favor the use of summer cuttings or propagation by seed over dormant cuttings.

ECONOMIC AND PROCUREMENT CONSIDERATIONS

In most cases, propagation by seed is the most labor and cost effective method of reproducing plants, given that genetic variability, such as germination requirements, can be managed within acceptable limits (Hartmann and Kester 1983). Seed may be gathered by commercial collectors on a contractual basis or by the restoration staff directly. Raw fruit requires proper storage prior to cleaning, processing to some clean product level, inventorying, and proper storage prior to sowing. Large amounts of seed can be planted outdoors in woody production beds with relatively simple machinery. For a given species in which seed is readily available, viability high, dormancy requirements known and minimal, and cultural techniques established; sexual propagation represents a low cost, labor and facility efficient method of multiplying plants.

As the conditions for the collecting, sowing, and culturing of seed become less than ideal, asexual propagation by cutting becomes an increasingly viable production option. As noted earlier, seed availability may be low, available seed expensive, or a lengthy or difficult dormancy mechanism involved. Although seed may be in abundant supply, its viability may be low--a condition that may be a regular or periodic phenomena.

Once acceptable collection sites have been identified and the propagules collected, verification of origin and the maintenance of the sampled genetics is necessary throughout all stages of production. It may even be necessary to isolate seed or cuttings by individual parent plant, depending on the restoration strategy. For this reason, the purchase of bulked lots or propagules of questionable origin may not be an acceptable option for National Park and Forest projects. Managers need to recognize the additional expense associated with site specific propagule collection and the cost of verification and maintenance of these sources.

Other factors may increase the cost of production as well. A lack of commercial incentive has resulted in less propagation research being conducted on the native woody species in comparison to ornamental selections that have been through breeding or selection programs. Project-specific wildland collections are often small and irregular in amount and viability, preventing economies of scale from being reached. These factors in combination will increase the cost of production. Procurement specifications need to reflect these needs and resources allocated accordingly. One option may be to reimburse commercial growers in two stages, one for attempting to produce a difficult-to-grow species and the second on a "per plant" basis for the actual product grown. Given some level of success, the sum of the two contracts might approximately equal the per plant cost of producing some relatively easy-to-grow species.

CONSTRUCTION SCHEDULES

Of the aforementioned limitations, seed dormancy often presents the greatest challenge because of its impact on construction schedules. Seed may not germinate until the second or third spring after field sowing and usually requires one to three additional growing seasons prior to lifting. Adequate planning should provide enough lead time in the construction process for propagation by seed. Propagation by cuttings becomes increasingly attractive as construction schedules advance and completion deadlines approach. Although there are wide differences among species, a summer cutting is normally rooted in 6 to 10 weeks, transplanted to a container in 3 to 4 months, and ready for planting sometime the following year.

THE BASICS OF ASEXUAL PROPAGATION BY SUMMER CUTTINGS

Collecting Cuttings

As noted earlier, the pool of plants that can be used as cutting sources (stock plants) will depend largely on the genetic constraints imposed by management, as well as the physiological condition of the stock plant. Given the clonal nature of this technique, attempts should be made to secure cuttings from as many different populations and individual plants as is practical. The broadest range of phenotypes possible should be secured. Genetic concerns notwithstanding, variation in the ability of populations and individual plants to produce adventitious roots warrants the use of multiple sources of germplasm. All donor plants should be relatively vigorous and free of insects and disease. One year prior to taking cuttings, potential donors should be scouted during the growing season, their location marked and recorded, and the plants revisited just prior to taking cuttings to assure the availability of an adequate quantity and quality of tissue. Avoid plants showing signs of severe environmental stress or isolated groups of few individual plants.

Field Equipment and Supplies

The amount of equipment required depends on the number of cuttings needed, the amount of labor available, the mode of transportation, distance to the source of the cuttings, and how the cuttings will be handled and temporarily stored. In most situations, the following supplies will be needed: large zip-lock bags, spray bottles, clean water, permanent markers, plastic labels, metal tags (for labeling stock plants should future reference be required), large white trash bags and ties, high quality pruners, heavy gloves (if handling thorny species), day pack (if transporting the cuttings on foot any distance), large cooler(s), ice or snow, and a vehicle capable of storing the sacks of cuttings out of the sun and wind.

Harvesting Techniques

Summer cuttings are best taken in the early morning hours when turgidity is high. Use only high quality hand pruners to minimize tissue damage to the stock plant and cutting. Cuttings from rank, leggy, or excessively soft growth should be avoided. Softwood cuttings will snap and break cleanly when bent at a sharp angle when they are adequately mature. Semihardwood cuttings, because they are partially matured, are more difficult to break. Thin, weak cuttings or abnormally thick, vigorous cuttings should be avoided. Material grown in partial sun, of an average rate of growth, taken from lateral (side) branches is generally best. Cuttings should be 3 to 6 inches (7.6 to 15.2 centimeters) in length with stems up to pencil diameter (0.25 inch; 6.4 millimeters) in thickness. One or two nodes per cutting is optimal.

Handling and Storage

Immediately place cuttings in zip-lock bags and then moisten with water and place in a cooler or large sack with ice. Cuttings should not be allowed to dry out, sit in standing water, heat up, or freeze. Optimal storage includes a relative humidity near 100 percent and temperatures between 34^o to 37^oF (1.1^o to 2.8^oC). If refrigeration is impossible, the cuttings should be kept wrapped in moist cloth and stored in a cool place in unsealed plastic bags. Summer cuttings are extremely perishable and transportation and storage should be minimized. Placement in greenhouse propagation beds or containers should occur within 48 hours of the time of cutting for best results. Cuttings to be shipped any distance for propagation should be sent by overnight delivery. Avoid taking and sending cuttings late in the week when there is a chance that they may be held in a post office or that no one will be available on a weekend to receive and prepare them for the greenhouse.

Cutting Preparation

Cuttings are prepared by removing the leaves from the basal end of each stem cutting. If the cutting has large or numerous leaves, prune 25 to 50 percent of each of the remaining leaves to reduce water loss. Carefully remove all flowers and fruit from the cuttings. Basal wounding of semihardwood and hardwood cuttings, i.e., the removal of a thin layer of tissue down to the cambium along the axis of the stem for approximately 1 to 2 inches (25 to 50 mm), often improves rooting. The base of each cutting should be recut at an angle with a sharp knife prior to treatment with a growth regulator.. This is done because pruners, especially the 'anvil' type, tend to crush stem cells during the cut thus restricting water uptake prior to adventitious root formation. In addition, some degradation of the base of the cutting stem occurs during transport and storage and requires removal. Cuttings should be kept cool and moist at all times. Keep cuttings wrapped in papers towels moistened regularly with clean water. Submerge cuttings in a broad spectrum fungicide solution (as per label) prior to treatment with growth regulators. Evidence exists that a preplanting soak of the propagation media with fungicide provides additional benefits.

Growth Regulators

In most cases, treatment with plant growth regulators is necessary to encourage adventitious rooting. There are numerous commercial products containing one or more plant growth regulators at various concentrations. Most formulations contain auxin-type compounds or hormones including indolebutyric acid (IBA) or naphthaleneacetic acid (NAA). Both liquid and powder formulations are available. Some products also contain a fungicide to prevent infection, particularly at the base of the cutting. There is wide variation among species in their response to the various types and concentrations of rooting substances. In general, softwood cuttings require the lowest concentration of growth regulator (1,000 to 3,000 parts per million), semihardwood an intermediate concentration (3,000 to 5,000 ppm), and hardwood the highest concentration (3,000 to 10,000 ppm or more). Easily rooted cuttings may not need treatment.

Environmental Control

Environmental control is extremely important for successful asexual propagation, especially for summer cuttings. Air temperatures should be maintained between 65⁰ to 75⁰F (18⁰ to 27⁰C) during the day and 60⁰ to 65⁰F (16⁰ to 18⁰C) at night. The relative humidity of the greenhouse should be as high as possible, without promoting disease, during the early stages of root formation. Overhead, intermittent mist operated by automatic controls is necessary to minimize transpirational losses. Bottom heating of the propagation media may further assist the rooting process. Partial shade may also be helpful in preventing cutting desiccation, although actively growing tissue will require some light for normal growth and survival.

Rooting Media

There are numerous types of rooting media that can be used propagation beds including soil, sand, peat, vermiculite, pumice, Styrofoam, and others. All media should be sterile, and the reuse of media is not recommended. The frequent application of moisture to the media surface from the mist system requires the use of a formulation that is well-drained in order to provide adequate aeration for tissue survival and growth. If multiple species are being propagated simultaneously in a single bench, it may be necessary to use a single, multi-purpose mix. Difficult-to-root species may require a very specific combination of aeration and water holding capacity to facilitate rooting.

SUMMARY

In review, there are certain combinations of factors that will encourage or discourage the use of one propagation method over another. Conditions that favor the use of summer cuttings include poor seed production, a lack of seed availability, poor seed

viability, long or unknown seed dormancy mechanisms, short revegetation intervals, high visibility sites, small or linear disturbances, lack of dormant cuttings, poor rooting of dormant cuttings, and site or plant access limitations. Favorable propagation techniques include proper and limited storage, fungicide dip, wounding, recut base, treatment with growth regulators, intermittent mist, sterile well-drained media, bottom heat, and strict environmental control.

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