Methods of Auxin Application in Cutting Propagation: A Review of 70 Years of Scientific Discovery and Commercial Practice[1]

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Abstract

The discovery of auxins as plant growth regulating chemicals in the 1930s and their ability to stimulate adventitious rooting in stem cuttings marked a major milestone in the modern history of plant propagation. Basic and applied research conducted during the past 70 years has provided information regarding absorption and translocation of auxin in cuttings, as well as the effects of timing of auxin application, methods of auxin application, type of auxin, and concentration of auxin on the rooting response of cuttings. The basal quick-dip method, the powder application method, and the dilute soak method have been the most commonly used methods for applying auxin to cuttings in commercial horticulture over the past 7 decades, with the first two methods still in common use today. In addition, a wide variety of other auxin application methods have been reported beginning in the 1930s and continuing to the present. Some of these methods remain primarily of historical interest, several find limited use in commercial horticulture, and others show potential for greater use in the future. Opportunities exist for further development of auxin application techniques that can enhance plant quality, efficiency and productivity of the cutting propagation process, and worker safety. This review provides horticulturists, from the academic researcher to the commercial propagator, with an exploration of auxin application methods over the past 70 years, along with an examination of the physiological response of cuttings to applied auxin and an overview of issues leading to future opportunities.

Index words: basal quick-dip method, powder application method, extended basal soak method, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 1-naphthaleneacetic acid (NAA), plant growth regulators, rooting hormones, adventitious rooting.

Significance to the Nursery Industry

Following the discovery of auxins as plant growth regulating chemicals in the 1930s, their ability to stimulate root formation on cuttings was soon established and research into efficient and effective methods of applying auxins to cuttings began in earnest. Commercial propagators quickly recognized the advantages of applying auxin for rooting stem cuttings of many nursery crops, including some of the more challenging-to-root taxa, and incorporated methods of auxin application into their standard nursery propagation practices.

Evaluation of the merits of various auxin application methods continued through the 20th century, with the basal quick-dip and powder application methods becoming established as the most commonly used techniques in modern nurseries. Some less common methods of auxin application still find limited use with specific crops or show potential for greater use in the future, while others remain of historical interest and an inspiration for continuing research. Although common methods (such as the basal quick-dip) tend to be easy and simple in daily practice, the development and use of these methods has had a profound impact on clonal plant propagation over the past 70 years. Such an impact is evident in the production and distribution of millions of cutting-grown ornamental landscape plants (both established and newly introduced cultivars) by nurseries throughout the world every year.

The nursery industry continues to face challenges and seek means of improving production efficiency, enhancing product quality, reducing chemical inputs, and promoting worker safety. Improved propagation practices have a part to play in fulfilling these objectives, providing further opportunities for academic researchers, commercial propagators, and professional organizations (such as the International Plant Propagators' Society) to build upon past efforts and enhance current knowledge in moving the nursery industry forward through the 21st century.

Introduction

Use of auxins in cutting propagation. The discovery of auxins as plant growth regulating chemicals and early research into their physiological effects and practical applications form a fascinating story of horticultural research (173). From these studies, the finding that auxins could stimulate adventitious rooting in cuttings was a major breakthrough for commercial plant propagation (5). In the 1930s, Thimann and Koepfli (170) reported the synthetic preparation of indole-3-acetic acid (IAA), a naturally occurring auxin that had recently been found to have root-forming properties, and demonstrated its practical use in stimulating root formation on cuttings. In the same year, the synthetic auxins indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) were shown to be more effective than IAA for rooting (193). Indole-3-butyric acid was later found to occur naturally in plants as a conversion product of IAA, but occurs at lower concentrations than IAA (111, 112). At present, IBA and NAA are the most widely

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used auxins for promoting root formation on stem cuttings. Auxin treatments are commonly used in commercial plant propagation to increase overall rooting percentages, hasten root initiation, increase the number and quality of roots, and encourage uniformity of rooting (72, 113).

Publication of results of rooting trials with stem cuttings of ornamental species conducted at Ohio State University by Chadwick and Kiplinger in 1938 signaled a major advance in the application of technology to the field of commercial plant propagation (75). Chadwick and Kiplinger (34) demonstrated, depending upon species, auxin treatment could enhance rooting percentage and quality of the root system. Plants normally propagated by softwood cuttings could be rooted in less time than nontreated cuttings. However, they also noted auxin treatments did not benefit species that were normally difficult to propagate, thus providing opportunity for further research. The transfer of this technology from scientists to nursery growers was rapid (140). Commercial rooting products containing auxin were being manufactured and made available to commercial propagators within only a few years of the discovery of auxin.

Auxin formulations for commercial propagation. Commercial root-promoting products containing auxins as their primary active ingredients are often labeled or referred to in popular usage as 'rooting hormones'. However, in the scientific literature, the term hormone is typically limited to naturally occurring auxins (IAA and IBA) and other endogenous compounds that are active in small concentrations, synthesized at several sites in the plant, and elicit a variety of growth and morphogenetic responses (156). This definition is less restrictive than that pertaining to animal hormones. The term plant growth regulator (PGR) is used in reference to naturally occurring auxins and other plant hormones, as well as synthetic auxins and other compounds with hormonal properties.

Commercial products containing IBA, NAA, or a combination of IBA and NAA are available to commercial propagators in liquid, powder (talc), and water-soluble tablet formulations (72). Powder (talc) formulations of auxin at low concentration are reported to remain effective for 1 to 2 years and higher concentrations for up to 5 years (108). Although concentrated solutions of IBA in 50% isopropyl alcohol tend to change color during storage, no significant change in biological activity or breakdown was detected when solutions were stored in clear glass bottles under refrigeration and at room temperature for up to 6 months (143). The potassium (K) salt (K-salt) formulations of IBA and NAA (e.g., K-IBA and K-NAA) enable these auxins to be dissolved in water without the use of alcohol or other organic solvents. Potassium salt formulations have been reported to be more, less, or equally affective as acid formulations for rooting cuttings depending on species, type and concentration of auxin, type of cutting, time of year, and other factors (2, 7, 48, 81, 96, 117, 129, 137, 139, 161, 191). Gel formulations of auxin are also available and have been used successfully (15).

Isopropyl alcohol and ethyl alcohol are common solvents in liquid formulations (50). Other solvents or carriers that have been used in commercial and noncommercial auxin formulations include acetone, methanol, dimethylsulfoxide (DMSO), dimethylformamide (DMF), polyethylene glycol, propylene glycol, and ethylene glycol (3, 38, 39, 47, 89). Commercial auxin formulations containing fungicides, boron, and chlorophyll extract have also been used in cutting propagation (47).

Physiological Response of Cuttings to Applied Auxin

Timing, concentration, and type of auxin. Optimal rooting response by cuttings typically occurs when auxin is supplied soon after cutting preparation. In examining the response of stem cuttings of mung bean [*Vigna radiata* (L.) R. Wilcz. (syn. *Phaseolus aureus* Roxb.)] to IBA, Jarvis et al. (93) reported high concentrations of auxin should be supplied immediately after the cutting is prepared to obtain the maximum effect of auxin. Shibaoka (150) reported IAA applied at a high concentration during the first 24 hr increased the number of roots more than IAA applied during the second 24 hr on cuttings of adzuki bean [*Vigna angularis* (Willd.) Ohwi & H. Ohashi var. *angularis* (syn. *Azukia angularis* (Willd.) Ohwi)] seedlings. IAA applied at low concentrations during the first 24 hr did not increase the number of roots on cuttings, but enhanced the effect of IAA applied during the second 24 hr, indicating more than one auxin-promoted action leading to root formation. A sufficiently high dose of IAA applied on the first day was able to fulfill the requirement for auxin on the second day.

In contrast to the standard practice of applying auxin to cuttings soon after their preparation, Luckman and Menary (110) determined root initiation in difficult-to-root cuttings of shining gum [*Eucalyptus nitens* (H. Deane & Maiden) Maiden] occurred in response to a 48-hr basal soak in a solution of IBA at 20 mg/liter (ppm), with the greatest response obtained when auxin was applied 4 or 5 weeks after the cuttings were inserted into the rooting substrate. Auxin treatment immediately after preparation of the cuttings had no significant effect in comparison with nontreated cuttings.

The proven effectiveness of auxin applications in promoting adventitious root formation in cuttings has attracted more interest than any other topic in propagation research (107). However, results from these trials are not readily reproduced. Nevertheless, research findings tend to agree in two respects: auxins are promotive of adventitious rooting when compared with nontreated controls, and high concentrations of applied auxin are often damaging (107).

With increasing concentration, auxins can produce a variety of growth abnormalities within 24 hr after treatment, including leaf epinasty (downward curvature), stem curvature, intensified green leaf pigmentation, and growth inhibition (64). Reductions in stomatal aperture, transpiration, and carbon assimilation can also occur. These changes are followed by accelerated foliar senescence, chloroplast damage, destruction of membrane and vascular system integrity, necrosis, and plant death. A high level of auxin stimulates biosynthesis of ethylene, which in turn triggers abscisic acid (ABA) production (64). The ABA is translocated through the plant, triggering stomatal closure and, together with ethylene, promotes leaf senescence and, ultimately, death (64).

Depending upon type and concentration, auxins can promote rooting, inhibit shoot growth, or produce herbicidal effects. NAA, often used in root-promoting products for cutting propagation, can also be used on landscape and container-grown plants to inhibit unwanted basal sprouting (28, 51, 97). NAA can also be used as a herbicide for weed control (83). The auxin 2,4-dichlorophenoxyacetic acid (2,4-D), commonly used as a herbicide, can also be used at low concentrations to stimulate rooting of stem cuttings (14, 36, 151).

Absorption of auxin. Effectiveness of exogenous applications of auxin in promoting rooting on stem cuttings is dependant on adequate absorption by plant tissue. Absorption of auxin solutions at the base of stem cuttings can be influenced by auxin concentration and treatment duration, with increasing concentration and duration providing greater uptake (86). Amount of time between cutting collection/preparation and treatment can also have an influence on absorption, with greater water loss from the cutting base prior to treatment increasing the suction that develops at the base of the cutting. Depth of treatment can also affect absorption and subsequent rooting response, with increased depth providing additional solution that can run down the epidermis and be absorbed through the cut base. Gouws et al. (61) reported most of the auxin applied basally in liquid form to cuttings of 'Ivy' protea (*Protea* L. 'Ivy') was absorbed during the first second of immersion, and the rate of auxin uptake decreased rapidly with increase in time of treatment.

Absorption of auxin applied in liquid solutions is also influenced by the presence of natural and man-made openings on cuttings. McGuire et al. (126) reported 7.6 to 10.2 cm (3 to 4 in) long stem cuttings of convex-leaf Japanese holly [*Ilex crenata* Thunb. 'Convexa'] absorbed IAA-2-^{14}C when the cut basal ends and freshly exposed leaf scars on the lowest 2.5 cm (1 in) of the cuttings received a 10-sec basal dip in a solution of IAA. Little IAA was absorbed when the base and leaf scars of the cutting were sealed with paraffin prior to receiving the basal dip. Preparation of cuttings with a wound consisting of a 2.5 cm (1 in) long, 0.16 to 0.32 cm (0.063 to 0.125 in) wide incision extending to the xylem prior to a basal dip in IAA did not significantly increase uptake of the auxin.

Absorption of auxin from powder formulations can be influenced by factors operating at the interface of the powder and the cutting stem (87). Prewetting the base of the cutting with water or another solvent increases the loading of the powder and subsequent absorption and rooting response. Extended retention time of the powder can also result in more effective uptake of auxin and improved rooting response, provided auxin levels are not supraoptimal. As with liquid formulations, the entry of auxin from the cut end of the cutting tends to elicit the most efficient rooting response, although epidermal applications of powder can be more effective if preceded by a solvent dip. Hitchcock and Zimmerman (81) reported powder remaining on rose of Sharon (*Hibiscus syriacus* L.) cuttings for a period as short as 2 hr allowed sufficient uptake of auxin to induce better rooting than on nontreated cuttings. Powder remaining on the cuttings for 24 hr appeared to provide an optimal rooting response.

Uptake of auxin by softwood cuttings of Manhattan euonymus (*Euonymus kiautschovicus* Loes. 'Manhattan') and herbaceous cuttings of 'Bright Golden Princess Anne' chrysanthemum [*Chrysanthemum* ×*morifolium* Ramat. (syn. *Dendranthema morifolium* (Ramat.) Tzvelev) 'Bright Golden Princess Anne'] was studied by Geneve (58) using the quick-dip [NAA at 2000 mg/liter (ppm)], powder application [NAA at 2000 ppm (0.2%)], and dilute soak [NAA at 200 mg/liter (ppm)] methods using ^{14}C-labeled NAA. Number of roots per cutting was correlated with auxin uptake regardless of application method. Cuttings of chrysanthemum absorbed more auxin than cuttings of euonymus, likely due to a less waxy cuticle that permitted greater entry through the epidermis with the quick-dip, as well as a greater transpiration rate. Cuttings of chrysanthemum treated with talc absorbed only 6% as much NAA as that absorbed by cuttings receiving a 5-sec quick-dip and about 3% of that absorbed by cuttings soaked for 16 to 24 hr. Treating cuttings in ethanol for 5 sec prior to applying talc increased auxin uptake 5-fold. When cuttings were treated with talc, only 3% of the initial amount of talc was still adhering to the cuttings after 24 hr under mist. The dilute soak method provided the greatest uptake of NAA and the greatest number of roots; however, these roots did not elongate well. Nontreated and talc-treated cuttings rooted only at the bottom of the cutting, while the quick-dip, dilute soak, and ethanol pre-dip treatments resulted in rooting both at the bottom and along the stems of the cuttings. On cuttings treated with auxin solutions, the greatest uptake of auxin occurred at the cut surface.

Absorption of auxin solutions by plant foliage can be strongly affected by the physical structure of the foliage itself. Leopold (106) noted waxy leaf surfaces [as with garden pea (*Pisum sativum* L.)] are more difficult to wet, causing applications of auxin at a given concentration to be less effective than on plants with leaves that wet easily [e.g., tomato (*Solanum lycopersicum* L.)]. In addition, the physical form of the foliage can influence absorption, as noted by the greater run-off of spray droplets from leaves with smaller surface-to-volume ratios. However, stomata do not appear to have much bearing on the absorption of foliar-applied auxin (181).

Environmental conditions, including temperature and light, can also affect absorption of foliar-applied auxin. Rice (141) showed total absorption of ammonium 2,4-dichlorophenoxyacetic acid (2,4-D) was considerably more rapid at 32C (90F) than at 10C (50F), although the duration of absorption was apparently less due to more rapid drying of the spray droplets at the higher temperatures. Rice (141) also noted absorption of 2,4-D was greater in the dark than in the light. This effect was likely due to an extended period of wetting in the dark and more rapid evaporation of droplets in the light. The relative rapidity of foliar absorption of auxin was apparent in a study by Overholser et al. (134) in which simulated rain applied 8 hr after application of NAA did not perceptibly reduce the effect of auxin in preventing preharvest drop of apple (*Malus domestica* Borkh.), while simulated rain applied after 2 hr had an adverse effect. Weaver et al. (182) reported an immediate heavy rain caused no lessening in the response to 2,4-D applied in an oil solution to soybean [*Glycine max* (L.) Merr.] and red kidney bean (*Phaseolus vulgaris* L.), but rainfall often lessened the response to 2,4-D applied as an aqueous solution. Response of greenhouse-grown plants to an aqueous solution of 2,4-D was not diminished by artificial rainfall 6 hr or more after chemical application, but response of field-grown plants was diminished if rainfall occurred within 24 hr of chemical application, indicating developmental stage of the plants or dissimilar environmental conditions could affect absorption.

The pH of the auxin solution or powder can also influence the degree of absorption. Crafts (43) noted polar solutions are generally more effective on young foliage, while nonpolar solutions, such as esters, are more effective on the more heavily cuticled (more lipoidal) older foliage. Albaum et al. (1) demonstrated auxin entered stonewort (*Nitella* Agardh.) cells in solution as the undissociated acid through a simple diffusion process, and occurred most rapidly at pH values below the pKa value of 4.75. In a study with tissue cultures of 'Gala' and 'Triple Red Delicious' apple, Harbage et al. (65) found root formation and uptake of ^{3}H-IBA were inversely related to root initiation substrate pH, with maximum root count and IBA uptake occurring at pH 4.0. However, pH of the root initiation substrate did not affect IBA metabolism. Chadwick and Swartley (35) mixed malic acid with commercial rooting powders to reduce the pH to 3.0 and found the acid mixtures to be more effective than the unaltered powder formulations in rooting cuttings of Pfitzer juniper [*Juniperus ×pfitzeriana* (Späth) P. A. Schmidt] and Japanese yew (*Taxus cuspidata* Siebold & Zucc.); however, results varied according to the time of year.

Based on a study examining uptake of 2,4-D by oat (*Avena* L.) coleoptiles, Johnson and Bonner (94) described the uptake of auxin by plant tissue as consisting of three separable processes. The first, completed in 20 to 30 min, resembles a diffusion into the tissue in the sense that auxin taken up by this process is readily leached to the outside solution. The second, also a rapid process, resembles a binding, since the bound auxin can be removed by exchange for unlabeled auxin. The third is a continuing absorption, maintained at a steady rate for several hours. They also determined auxin could be accumulated by the tissue to a concentration higher than the external concentration and the rate of continuing uptake was inhibited by low temperature and metabolic inhibitors, while initial diffusion was little inhibited by these same inhibitors. In addition, the rapid initial amount of uptake of auxin was increased (primarily with the exchangeably bound component) in the presence of salts such as KCl, K malate, or KH_2PO_4, while both the exchangeable binding and the continuing uptake of auxin were inhibited by the presence of a high concentration of a second auxin, IAA.

Surfactants can also affect the uptake of auxin by plant tissues. Lownds et al. (109) demonstrated surfactants can enhance foliar penetration of NAA and the response is related to the chemical nature of the surfactant. The three nonionic surfactants tested decreased surface tension of NAA solutions, resulting in an increase in droplet to leaf interface area, but differed in degree of increase in NAA penetration. Enhancement of NAA penetration was greatest during the droplet-drying phase, but penetration continued to take place from the deposit after drying. There was little change in penetration associated with increased drying time. In addition, surfactant-enhanced NAA penetration caused an increase in NAA-induced ethylene synthesis. Shafer et al. (149) noted surfactant concentration could affect sorption by plant cuticles. In one study, NAA sorption was not significantly affected below the critical micelle concentration (CMC), but decreased with increasing surfactant concentration above the CMC. In a study using NAA, 2,4-D, and several nonionic surfactants, Tan and Crabtree (168) showed nonionic surfactants could have effects on foliar penetration of plant growth regulators in addition to changing wetting, drying time, and stomatal entry of the spray solution. The effects were related to the chemical classification and hydrophilic-lipophilic balance of the surfactants and depended on the type of growth regulators.

Translocation of auxin. Effectiveness of exogenous applications of auxin in promoting rooting on stem cuttings also depends on adequate translocation from the site of application to the site of adventitious root initiation. Auxin translocation can occur in three primary manners: 1) basipetally by polar transport; 2) passively through the phloem; and 3) acropetally in the xylem (with exogenous application).

Auxin is unique amongst plant hormones in exhibiting a polarity (unidirectional nature) in its movement within plants (13). As summarized by Taiz and Zeiger (167), the polar transport of auxin occurs primarily in a basipetal direction, with the shoot apices serving as the primary sources of endogenous auxin for the entire plant. Polar transport contributes to the creation of an auxin gradient from the shoot to the root, with the auxin gradient affecting various physiological processes. According to the commonly accepted chemiosmotic model for polar auxin transport, uptake of auxin is driven by the proton motive force (ΔE + ΔpH) across the plasma membrane, while auxin efflux (transport from the cell) is driven only by the membrane potential (ΔE). Auxin enters cells either by passive diffusion across the phospholipid bilayer or secondary active transport via a proton symporter. Auxin exits cells via auxin efflux carriers that are located mainly at the basal end of each cell. The repeated uptake of auxin at the apical end of the cell and release from the basal end of the cell results in the overall polar transport effect. Polar transport is thought to occur primarily in parenchyma cells associated with the vascular tissue.

The importance of polar auxin transport for adventitious rooting of cuttings was demonstrated by Ford et al. (57) comparing rooting responses of difficult-to-root stem cuttings of lilac (*Syringa vulgaris* L.) and easy-to-root stem cuttings of forsythia (*Forsythia ×intermedia* Zabel). Rooting ability of lilac cuttings declined over the growing season (May to June). Initial free IAA concentration at the cutting base declined, but the relationship between basal IAA concentration and rooting ability was not strong, indicating other factors were also involved. The ability to transport IAA declined in lilac over the period of maximum growth, and declined to a lesser extent in forsythia; this difference was also maintained in winter hardwood cuttings. Use of the polar transport inhibitor 2,3,5-triiodobenzoic acid reduced rooting ability in both species. A basal treatment with IBA did not maintain the rooting ability of lilac cuttings during the growing season. It was suggested a basal auxin dip might not lead to sufficient levels of auxin in the specific tissues for root initiation in lilac.

Among the naturally occurring auxins, only IAA and IBA are translocated via polar transport (156). Biologically active synthetic auxins, such as 1-NAA, also show polar transport; however, 2,4-D shows little polar transport. Biologically inactive analogs, such as 2-NAA and 2,6-dichlorophenoxyacetic acid, do not exhibit polar transport. Went and White (187), using an oat coleoptile curvature test, demonstrated differences in the transport rate among various auxins, with IAA being greater than IBA, and IBA greater than NAA.

Most of the auxin synthesized in mature leaves appears to be transported to the rest of the plant in a nonpolar direction through the phloem along with carbohydrates and other components of the phloem (167). Phloem transport of auxin is primarily passive and occurs at greater velocities than with polar transport in other plant tissues. Research by Cambridge and Morris (32) with ^{14}C-labeled IAA suggested auxin could be transferred from the nonpolar phloem pathway to the polar transport pathway in garden pea, with the transfer apparently taking place in the immature tissues of the shoot apex.

Weaver and DeRose (181) found 2,4-D was translocated more readily from well-lighted leaves than from shaded leaves, indicating translocation of foliar-applied auxin had some relationship to carbohydrates. While translocation of 2,4-D did not appear to take place when applied to plants in darkness, Weintraub and Brown (183) found translocation occurred readily when plants were moved into the light. Rohrbaugh and Rice (144) determined application of sugar to darkened leaves treated with 2,4-D would substitute for light in causing translocation of 2,4-D from the treated leaf. Weintraub and Brown (183) confirmed these findings by showing a wide variety of sugars would serve in bringing about translocation of 2,4-D.

Early research by Hitchcock and Zimmerman (78) on the translocation of exogenous auxin in plants showed auxins applied to the soil could move acropetally in plants, suggesting movement within the transpiration stream. Cooper (42) found when the cut ends of the base of leafy lemon (*Citrus ×limon* (L.) Burm.) or 'Lady Perkins' rose (*Rosa* L. 'Lady Perkins') cuttings were placed in an auxin solution, there was some upward movement of the auxin depending on the rate of transpiration, with stronger solutions effective for inducing root formation.

Skoog (155) found auxin moves upward in the xylem with the transpiration stream, then moves laterally into the surrounding tissues, and then is reexported by normal polar basipetal transport. The downward movement appeared to consist of cell-to-cell movement in nonvascular tissues and (at a faster rate) in the phloem. Under special conditions, in the presence of very high concentrations, auxin introduced into the plant appeared to also move downward in the xylem.

After killing a portion of a stem with a flame, Weaver and DeRose (181) found upward movement of 2,4-D from the soil to the apex was unimpeded, but downward movement was impeded, supporting movement of auxin in the xylem tissue. Leopold (106) indicated such translocation in the xylem was unlikely to occur to any great extent when auxins are applied as foliar sprays, but could occur when auxin was applied to the soil or to a cut plant part, providing direct access to the transpiration stream.

In trials using ^{14}C-labeled IAA for rooting leafy stem cuttings of plum (*Prunus* L. 'Mariana 2624'), apically and basally applied auxin was absorbed and distributed throughout the cuttings in 24 hr (163). However, most of the radioactivity remained in the basal portion of the cutting when the auxin was applied basally. Cuttings without leaves absorbed the same amount of auxin as leafy cuttings, indicating transpiration pull was not a primary factor in the absorption and translocation of auxin.

In a study examining transport and metabolism of IBA, Epstein and Ackerman (54) incubated the bases of stem cuttings of two cultivars of flametip (*Leucadendron discolor* E. Phillips & Hutch.) in a solution containing ^{3}H-IBA. IBA was rapidly metabolized in cuttings of both cultivars, although some free IBA was present in the cuttings throughout the 4-week rooting period. More of the radiolabeled IBA was transported to the leaves of the easy-to-root cultivar than the difficult-to-root cultivar, while more free IBA accumulated in the cutting base of the easy-to-root cultivar than the difficult-to-root cultivar.

Using ^{14}C-labeled NAA, Geneve (58) concluded auxin applied to cuttings of 'Bright Golden Princess Anne' chrysanthemum in talc or an aqueous solution moves into the stem primarily at the cut surface and is moved up the stem through the vascular system. In addition, NAA applied to cuttings in an ethanol solution could enter the stem both from the cut surface and through the epidermis along the entire treated length of the stem.

Common Methods of Auxin Application

Three methods of applying auxin to stem cuttings have been most common in commercial horticulture over the past 70 years: 1) the basal quick-dip (concentrated-solution dip or, simply, quick-dip) method; 2) the powder (talc or dust) application method; and 3) the dilute soak (dilute-solution soaking) method. The basal quick-dip and powder application methods tend to be most common, with the quick-dip generally considered to be the superior method of the two (50). The dilute soak method, an older technique, is sometimes practiced with cuttings of certain species (113).

The basal quick-dip method involves dipping the basal portion [0.5 to 2 cm (0.2 to 0.8 in)] of a stem cutting into a concentrated [500 to 10,000 mg/liter (ppm) or greater] solution of one or more auxins for 1 to 5 sec (or longer) prior to inserting the cutting into the rooting substrate. Advantages of quick-dips include: 1) speed and simplicity of application; 2) uniformity of application (with individual or bundled cuttings); 3) greater economy (particularly if a large quantity of cuttings is to be treated); 4) ability to prepare a variety of final concentrations; and 5) general uniformity of results. Disadvantages of quick-dips include: 1) preparation requires experience and equipment; and 2) auxin concentration can change during extended use due to evaporation of alcohol or dilution with water (from wet cuttings or other sources) (16, 72, 113, 190).

The powder application method involves dipping the basal portion [0.5 to 2 cm (0.2 to 0.8 in)] of a stem cutting (often pre-moistened to enhance adhesion) into a blend of auxin and talcum powder, followed by a light tap to remove excess powder prior to inserting the cutting into the rooting substrate. Advantages of powders include: 1) ease of application; 2) preparation is not required with commercial formulations; 3) easy storage; and 4) visible evidence of application for workers. Disadvantages of powders include: 1) less uniformity of application; 2) loss of powder when inserting the cutting into the rooting substrate; 3) limited selection of concentrations; and 4) higher cost compared to a quick-dip when a large quantity of cuttings must be treated (16, 72, 113).

The dilute soak method involves placing the basal portion [1 to 3 cm (0.4 to 1.2 in)] of a stem cutting into a dilute solution [20 to 250 mg/liter (ppm)] of auxin for an extended period (2 to 48 hr) in a warm [20C (68F)] location with indirect illumination. An advantage of a dilute soak is that it allows greater uptake of auxin by the cuttings, which may enhance rooting of some difficult-to-root species. A dilute soak tends to be unpopular due to several disadvantages: 1) the process is cumbersome and requires more time; 2) additional space and equipment are required for treating cuttings; 3) additional planning is required to ensure removal of cuttings from the solution; and 4) results can be variable because the environmental conditions affect the amount of solution absorbed (16, 72, 113).

Early use of the extended basal soak method. Early research with auxin solutions for rooting stem cuttings focused on their use as an extended basal soak. In the 1930s, Cooper (42) found soaking the bases of lemon or 'Lady Perkins' rose cuttings in auxin solution for 8 hr before insertion in sand induced a much greater number of roots than on nontreated cuttings soaked in water, provided the auxin concentration was sufficiently high. He also found number of roots produced were from two to five times greater when auxin was applied at the base than when applied at the tip. However, it was also noted that basal application at higher concentrations could cause injury to cuttings.

When Hitchcock and Zimmerman (80) summarized results of research with IAA, IBA, and NAA at concentrations of 1 to 400 mg/liter (ppm) applied using an extended basal soak to cuttings of approximately 100 varieties, treatment times were also measured in hours. They found effectiveness of the treatment was primarily a function of concentration and not the total amount of solution absorbed. Hitchcock and Zimmerman (79) further reported preparations of IAA, indolepropionic acid, IBA, and NAA resulted in earlier rooting, increased number of roots, and promoted root emergence from a greater area of stem tissue in comparison with nontreated cuttings. On a concentration basis, aqueous solutions of auxin were 100 to 500 times more effective than lanolin preparations of auxin.

Skinner (154) utilized a basal soak in dilute auxin solutions for 8 to 48 hr in trials examining the rooting of 45 different ericaceous plants in the genera *Enkianthus* Lour. (enkianthus), *Erica* L. (heath), *Kalmia* L. (laurel), *Leucothoe* D. Don (fetterbush), *Oxydendrum* DC (sourwood), *Pieris* D. Don (pieris), and *Rhododendron* L. (azalea, rhododendron). While some plants rooted satisfactorily without auxin treatment, most exhibited an increase in average rooting over nontreated cuttings. Auxin treatment also reduced rooting time by an average of 2 weeks and produced larger root systems in comparison with nontreated cuttings.

Chadwick and Kiplinger (34) used a 24-hr basal soak in their studies with auxin application for rooting cuttings of selected woody and herbaceous ornamentals. DeFrance (44) found cuttings of kinnikinnick [*Arctostaphylos uva-ursi* (L.) Spreng.] could be rooted successfully by immersing the basal 2.5 cm (1 in) of multiple terminal, single terminal, and heel cuttings in IBA solutions for 24 and 43 hr; nontreated cuttings failed to root.

Preliminary examination of the extended basal soak method by Tincker (172) focused on determining an appropriate range of auxin concentrations and time of exposure, with high concentrations and extended exposure periods determined to be injurious to cuttings. In further studies with a broad selection of woody and herbaceous species, Tincker (174) reported positive rooting results using various auxins applied as a basal soak to cuttings for 24 and 48 hr. Auxin treatments were advantageous with most of the readily propagated species tested. None of the nontreated cuttings showed an advantage over the treated cuttings when an appropriate dilution was used, but strong solutions caused tissue discoloration, tissue necrosis, and defoliation. Treatments were also advantageous to 70% of 47 less readily propagated species with acceleration of rooting and more copious development of roots compared to nontreated cuttings. Using 30 difficult-to-root species, only five showed positive results with auxin treatment.

Warner and Went (180) reported hard-to-root cuttings of camellia (*Camellia japonica* L.) treated with an extended basal soak in a dilute solution of IAA showed improved rooting when the auxin treatment was made 2 weeks after preparation of the cuttings. In addition, when cuttings of daphne (*Daphne* L.), glorybower (*Clerodendrum* L.), citrus (*Citrus* L.), and camellia (*Camellia* L.) failed to root in response to an initial application of IAA, a second treatment with auxin after several weeks or months resulted in additional rooted cuttings.

Development of the powder application and basal quick-dip methods. Although an extended basal soak was an effective means of supplying auxin to cuttings to stimulate rooting, research in the 1930s soon turned to developing more simplified procedures for practical use in which treatment times could be reduced to a few seconds. Grace (62) reported, depending on the species, effectiveness of the powder (dust) application method was frequently superior to the extended basal soak method for rooting cuttings. Even when similar results were obtained with the two methods, the powder application method was recommended for its simplicity and convenience.

Stoutemyer (157) generally found the powder application method to be as effective as a 24-hr soak in dilute auxin solutions. Application of auxin in talc form was also easier and allowed greater latitude in the concentration of auxin that could be used on cuttings without being toxic. Application of the powder to the basal 1 cm (0.4 in) of the cutting was also shown to be preferable to application to the basal cut surface only. The importance of preventing loss of the powder by inserting treated cuttings into a sufficiently wide opening in the substrate was also noted, as insertion into narrow openings caused powder to be rubbed off the cutting base.

Hitchcock and Zimmerman (81) compared an extended basal soak, brief powder dip, and concentrated solution dip using selected concentrations of IBA for rooting stem cuttings of 70 woody ornamentals. Their studies showed optimum rooting varied by species, age and relative developmental stage of the shoot, time of year, auxin concentration, and method of application. Treatments with concentrated solutions [1000 to 20,000 mg/liter (ppm)] and powder preparations [500 to 12,000 ppm (0.05% to 1.2%)] were about equally effective for a given species, but represented values 10 to 1000 times higher than equivalent concentrations used for the extended basal soak method [1 to 80 mg/liter (ppm)].

In examining the rooting of stem cuttings of Pfitzer juniper and Japanese yew (*Taxus cuspidata* Siebold & Zucc.), Chadwick and Swartley (35) retreated cuttings with auxin powders after the cuttings had produced callus. Although the retreatment tended to produce heavier root systems, the effects of retreatment were inconsistent and appeared to not warrant the extra labor.

As commercial nurseries incorporated use of auxins for rooting cuttings into their propagation practices, the convenience and availability of powder (talc) formulations made their use common. By the 1950s, propagators were using commercial powders and preparing their own talc formulations (184). The dilute soak and concentrated solution dip were also used to some extent.

Additional attention turned to use of a concentrated solution dip during the 1950s, with the term ‘quick-dip’ commonly used to refer to the technique. Commercial propagators generally prepared their own formulations with alcohol as a solvent. Hess (74) reported, while rooting percentages showed little difference among cuttings of yew (*Taxus* L.), Pfitzer juniper, and Manetti rose [*Rosa* ×*noisetteana* Thory ‘Manettii’ (syn. *Rosa manetti* Criv. ex Rivers)] treated with no auxin, talc, and a concentrated dip, a concentrated dip tended to promote more roots per cutting. This was likely due to improved uniformity of application and greater retention using the solution. Gray (63) examined a quick-dip for difficult-to-root species, obtaining both positive and negative results. Negative results were excessive rooting, bud dormancy, or phytotoxicity of the treated tissue. Pinney (138) described the quick-dip method as economical, resulting in labor savings due to more rapid development of a quality root system that allowed rooted cuttings to be transplanted sooner. Roller (145) reported use of quick-dips gave inconsistent results from year to year. However, Stroombeek (160) reported erratic rooting results were not noticed when the quick-dip solutions were prepared with alcohol and water, rather than alcohol alone, thus reducing evaporation of the alcohol from the solutions and a resulting increase in auxin concentration as the solutions were used during the workday.

The comparative merits of a basal dip in talc formulations of auxin versus a basal quick-dip in concentrated auxin solutions continued to be examined in the 1960s and beyond. Meahl and Lanphear (128) tested both methods with species and cultivars of juniper (*Juniperus* L.), apple (*Malus* P. Mill.), sweetgale (*Myrica* L.), plum (*Prunus* L.), and lilac (*Syringa* L.). They determined, in general, there was equal or greater rooting with the same concentration of IBA in solution as compared to IBA in talc, possibly due to more uniform application of the solution to the base of the cutting. However they also suggested some species might respond better to talc preparations due a greater amount being held by the stem and thus more IBA available to the cutting over time. They also examined different holding times for cuttings treated with solutions and found little difference between 5 sec and 160 sec, but a decrease in rooting response at 320 sec, and thus concluded a quick-dip appeared sufficient for optimum response.

Heung and McGuire (76) compared basal dips with two talc formulation of IAA at 4000 mg/liter (ppm) for effects on rooting of cuttings of coleus [*Solenostemon* Thonn. (syn. *Coleus* Lour.)]. One formulation was prepared by grinding IAA crystals with talc in a mortar and pestle, and the other by dissolving IAA crystals in 95% ethanol, pouring the solution over talc and mixing the two components, and drying the mixture at 25C (77F). After 10 days of rooting, the number of roots on cuttings treated with the auxin and talc mixture formulations was greater than with no treatment or talc alone. Number of roots on cuttings treated with talc prepared by dissolving IAA in ethanol was twice that of cuttings treated with the mixture prepared by grinding due to more thorough mixing of the former.

Heung and McGuire (76) also compared liquid and talc formulations of IAA for amount of auxin absorbed into cuttings or adsorbed to the outer surface and for rate of IAA uptake. In one experiment, cuttings of convex-leaf Japanese holly were basally dipped in water followed by talc or dipped for 10 sec in a 40% ethanol solution to simulate auxin treatment. Assuming the talc and alcohol solution had both contained IAA at 6000 mg/liter (ppm), a far greater amount of auxin would be taken up using the alcohol solution than would be adsorbed using the talc formulation. In a second experiment, ^{14}C-labeled IAA was used along with unlabeled IAA to a prepare talc formulation of 6000 ppm (0.6%) IAA and a 50% ethanol formulation of 6000 mg/liter (ppm) IAA. Cuttings of convex-leaf Japanese holly exhibited the greatest uptake of IAA in ethanol solution within 24 hr after application, while cuttings treated with IAA in talc reached a maximum after 72 hr. Uptake of IAA and subsequent rooting of the cuttings were greater from the liquid formulation than from the talc. Although uptake of auxin from the talc formulation was slower, it was noted, for some species, a slower rate of uptake might provide a suitable level of auxin at a time when endogenous materials are at an optimum level for root initiation.

Ticknor (171) examined rooting of cuttings of 10 woody ornamentals in response to one commercial talc formulation [Hormex 8 (8000 ppm (0.8%) IBA)] and two commercial liquid formulations [Dip ’N Grow (IBA + NAA) and Wood’s Rooting Compound (IBA + NAA)]. Treatment with the liquid formulations produced similar or better results than with the powder. Bonamino (27) evaluated rooting response to treatment with talc and quick-dip applications of auxin on cuttings of wax myrtle [*Morella cerifera* (L.) Small (syn. *Myrica cerifera* L.)], Leyland cypress [x *Cupressocyparis leylandii* (Dallimore & Jackson) Dallimore], and convex-leaf Japanese holly using commercial formulations. Quick-dip application resulted in similar or better rooting than with talc. It was also noted liquid formulations were easier to apply than talc since cuttings could be treated in bundles at one time rather than individually.

A combination of a basal quick-dip in a concentrated auxin solution followed by a dip in an auxin powder has also been used for treating cuttings (72). In trials conducted by Beck and Sink (11) with poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) cuttings prepared in late winter, basal treatment with a combination of Jiffy Grow (liquid) at 1000 mg/liter (ppm) auxin (IBA + NAA) and Hormodin 2 powder [3000 ppm (0.3%) IBA] significantly increased the number of roots on all cultivars tested compared with nontreated cuttings and cuttings treated with either product alone. Number of roots obtained using two dual liquid/powder treatments in late summer was greater than with nontreated cuttings, but similar to cuttings treated with the single products alone. Sink and Knowlton (153) tested the same combination of auxin treatments on cuttings of carnation (*Dianthus caryophyllus* L.). Root fresh weight and dry weight were greater on treated than nontreated cuttings, but similar to most other auxin formulations applied singly.

During the 1990s, both powder and liquid formulations continued to be used in cutting propagation. Dirr (49) listed nine talc products (some available with multiple concentrations of auxin), one water-soluble tablet product, and five liquid products available in the United States and Europe. Liquid formulations no longer included dimethylsulfoxide and dimethylformamide as solvents or carriers. Due to governmental regulations in the U.S., propagators were limited to purchasing registered products from end-use formulators and could no longer purchase chemicals to prepare their own formulations.

As a modification of the basal quick-dip technique, Beeson (12) compared water and aqueous solutions of Cell-U-Wett (sodium cellulose glycolate or sodium carboxymethylcellulose) as dilutants for solutions of Dip ’N Grow (IBA + NAA). Auxin prepared at three concentrations with Cell-U-Wett and water was applied as a basal quick-dip to stem cuttings of five woody ornamental species. The type of dilutant did not affect rooting percentage and root mass. A similar study showed no difference in rooting on cuttings of 'Little Gem' southern magnolia (*Magnolia grandiflora* L. 'Little Gem') (37). However, auxin application was faster with Cell-U-Wett-diluted solutions (quick in-and-out dip) than with water-diluted solutions (2 to 4 sec). It was also noted Cell-U-Wett solutions had a longer shelf life and greater stability than water-based solutions. Blythe and Sibley (19) found inclusion of sodium cellulose glycolate in liquid auxin solutions improved rooting response of 'Collie Mullens' rose of Sharon (*Hibiscus syriacus* L. 'Collie Mullens') and 'Red Cascade' miniature rose (*Rosa* L. 'Red Cascade').

While basally applied auxin is capable of stimulating adventitious rooting, at elevated levels it can also inhibit subsequent budbreak. Le Fanu (105) reported axillary buds of garden pea on single-node stem cuttings were inhibited when the cuttings were placed with their ends in an auxin solution, as well as when auxin in gelatin was applied to the cut ends of the stems above or below the buds (but more strongly if applied from above). DeVries and Dubois (45) treated single-node softwood cuttings of 'Amanda' rose (*Rosa* L. 'Amanda') with a 10-sec dip in IBA at concentrations ranging from 0 to 5000 mg/liter (ppm). Although IBA promoted greater root weight, budbreak of all IBA-treated cuttings occurred significantly later and less frequently with increasing auxin concentration; however, IBA increased length of new shoots, particularly at low concentrations.

Sun and Bassuk (165) treated cuttings of 'MM.106' apple rootstock (*Malus domestica* Borkh. 'MM.106') with a 10-sec basal dip in IBA at concentrations of 0 to 2000 mg/liter (ppm) and found subsequent budbreak on rooted cuttings was reduced with increasing auxin concentration. Many of the cuttings treated with IBA at 2000 mg/liter (ppm) failed to grow even after several months in a greenhouse. In the same experiment, basal application of IBA at 1000 and 2000 mg/liter (ppm) to cuttings of Franklin tree (*Franklinia alatamaha* Bartr. ex Marsh.) almost completely inhibited budbreak. Sun and Bassuk (166) also found a 10-sec basal dip in IBA at a concentration of 600 mg/liter (ppm) or higher greatly inhibited budbreak on single-node cuttings of 'Royalty' rose (*Rosa hybrida* L. 'Royalty') during 4 weeks of rooting, and determined inhibition of budbreak resulted primarily from elevated ethylene levels. Root initiation and root elongation of cuttings initially inhibited budbreak, but later promoted budbreak. Geneve and Heuser (59) had previously measured ethylene evolution while examining rooting response of mung bean cuttings treated with four auxins. Ethylene production was greatest with cuttings treated with 2,4-D and least on cuttings treated with IBA. Cuttings treated with IAA and NAA produced similar quantities of ethylene, intermediate between cuttings treated with IBA and 2,4-D.

Uncommon Methods of Auxin Application

A variety of other auxin application methods have been reported beginning in the 1930s and continuing to the present. Some of these methods continue to find limited application in commercial horticulture, generally for rooting cuttings of certain types of plants or specific species, including difficult-to-root species. Other methods find no current application in commercial horticulture, having served a useful purpose in early research studies or being less effective or efficient than more common techniques. However, such methods remain of historical interest and a source of inspiration for continuing research.

Lanolin paste methods. In the 1930s, Cooper (41) obtained improved rooting results in comparison to nontreated cuttings by applying lanolin paste containing auxin to the upper stem tissue of cuttings of 'Eureka' lemon [*Citrus ×limon* (L.) Burm. 'Eureka'], fig (*Ficus carica* L.), lantana (*Lantana* L.), copperleaf (*Acalypha* L.), and spiderwort (*Tradescantia* L.). One part IBA was mixed with 2000 parts of pure lanolin and approximately 10 mg (0.00035 oz) of the resulting paste was spread on a small area on one side near the top of each cutting (which had previously been scraped to remove the epidermis and outer cortical layers). Auxin treatment in a lanolin paste resulted in root formation on leafless cuttings of lemon, while nontreated cuttings did not root.

In initial studies using a lanolin paste containing auxin for rooting cuttings, Tincker (172) described encouraging results with cuttings of some herbaceous species; however, the technique was ineffective on cuttings of woody plants. In further studies with a broader range of woody and herbaceous plants, Tincker (174) described rooting results as disappointing. He also noted lanolin was not easy to apply in small quantities of uniform weight and the technique could not be considered highly efficient.

Hitchcock and Zimmerman (79) reported lanolin preparations of auxin were highly effective for rooting cuttings of many types of plants, especially herbaceous species, but were relatively ineffective in promoting rooting of many woody plants, including species and cultivars of pear (*Pyrus* L.), plum (*Prunus* L.), and hawthorn (*Crataegus* L.). In their studies, auxin in a lanolin paste was applied to the basal portion of attached shoots of numerous species growing indoors and outdoors. Several days to several weeks after application, shoots were severed just below the treated region and inserted in a mixture of peat and sand. Lanolin pastes failed to induce rooting on cuttings of most taxa. Japanese maple (*Acer palmatum* Thunb.) was one of the few species of woody plants responding consistently to the lanolin treatments, with an increase in rooting percentage and number of roots per cutting. However, high concentrations of auxin also caused an increase in the amount of callus produced and retarded elongation of young shoots. Application of lanolin paste to intact shoots (with subsequent removal of the shoots from the plant for cuttings) was noted to be more effective than application directly to prepared cuttings. Overall, Hitchcock and Zimmerman (79) determined an extended basal soak in auxin solutions was preferable to the use of lanolin.

Vial, vacuum, and vapor methods. In the 1930s, Hitchcock and Zimmerman (79) supplied auxin solutions to shoots of woody and herbaceous plants through a cut surface prior to collection of the cuttings. Small vials containing auxin solution were tied to the shoot such that the overhanging portion of a slit cut into the shoot was positioned in the solution. After 3 to 10 days, shoots were severed at the slit and inserted into a rooting substrate. Cuttings of chrysanthemum (*Chrysanthemum ×morifolium* Ramat.), American holly (*Ilex opaca* Ait.), and gardenia (*Gardenia jasminoides* Ellis) showed more root growth, earlier rooting, and root growth from a greater area of stem tissue than nontreated cuttings. Although this method was limited in its practical application, it was noted solutions of higher concentration could be used compared to solutions used for soaking cuttings.

In trials aimed at improving rooting of stem cuttings of difficult-to-root species, Butterfield and McClintock (31) conducted tests in which cuttings were placed in 6.4 cm (2.5 in) of auxin solution in 2-liter (2.1-qt) bottles and subjected to a vacuum of 73.7 cm (29 in) of mercury for 4 hr. Results were compared to nontreated cuttings and cuttings soaked in auxin solution for 24 hr. Hardwood cuttings receiving the vacuum treatment absorbed twice as much solution as cuttings that were soaked, with indications the vacuum treatment caused the solution to be distributed through a greater amount of tissue. Vacuum-treated cuttings of pussy willow (*Salix discolor* Muhl.) and grape (*Vitis* L.) had more extensive distribution of roots along the stem, exhibited greater root proliferation, and rooted in less time than nontreated and soaked cuttings. The vacuum and soaking treatments produced similar rooting results with softwood cuttings, while both treatments produced better results than nontreated cuttings.

When leafless cuttings of 'Mrs. Louis Wathen' bougainvillea (*Bougainvillea* ×*buttiana* Holttum & Standl. 'Mrs. Louis Wathen') failed to root in response to immersion of the basal ends in a dilute solution of auxin for 18 hr, Jackson (92) made fresh cuts on the cutting bases and submerged the basal 2.5 cm (1 in) of the cuttings in an auxin solution contained in a vacuum desiccator. The desiccator was exhausted until bubbles emerged from the base of the cuttings, at which point the vacuum was broken, forcing the solution into the cutting bases. After 2 to 3 months, most of the cuttings had rooted.

Zimmerman and Hitchcock (192) examined response of cuttings to vapor application of auxins. Cuttings of euonymus (*Euonymus* L.) and privet (*Ligustrum* L.) were exposed to vapors of the methyl esters of IAA, IBA, and NAA for 30 min under bell jars. Cuttings of euonymus responded readily to methyl indolebutyrate and privet cuttings responded to methyl naphthaleneacetate. Rooting responses were qualitatively similar to responses to auxin applied using liquid solutions.

Injection, insertion, and basal spray methods. Tincker (174) briefly mentions injection of cuttings with hormones in the 1930s, but without providing methods or data. Difficulties when injecting liquids into herbaceous tissues and loss of material by exudation were noted as problems with this technique.

Leakey (103) treated stock plants of obeche (*Triplochiton scleroxylon* K. Schum.), a commercially important West African hardwood, using injections of auxin prior to taking cuttings in the 1990s. Solutions of IBA or NAA (250 µg each) were injected into the xylem either 10 cm (4 in) from the plant base or 10 cm (4 in) below the uppermost lateral shoot of the stock plants at 1 to 8 weeks before the cuttings were severed from the plants. Cuttings taken from lateral shoots treated with auxin at the shoot base rooted better than nontreated control cuttings. Injection at 4 and 8 weeks before severance provided greater rooting percentages than injection at 1 or 2 weeks before severance, although there were more roots per cutting on cuttings taken from plants injected 1 week prior to collection.

In examining use of etiolation, girdling (or 'ring barking'), and auxin on the rooting of cuttings of avocado (*Persea americana* P. Mill.) cultivars 'Fuerte' and 'Colin V-33' in the 1980s, Barrientos-Priego et al. (8) applied auxin to cuttings using thin pieces of wood treated with IBA at 10,000 mg/liter (ppm) and NAA at 300 mg/liter (ppm). The pieces of auxin-treated wood were inserted into a lengthwise cut in each cutting [following a technique described by Salazar-Garcia and Borys (146) for air-layering avocados] prior to planting in a propagation bed. A combination of etiolation, girdling, and auxin produced the best rooting percentages, root number, and root length, followed by a combination of etiolation and girdling (with no auxin). Nonetiolated cuttings failed to root, even with the use of girdling and IBA (separately or in combination).

In an experiment conducted by Struve and Blazich (162), stem cuttings of eastern white pine (*Pinus strobus* L.) treated with toothpicks soaked in solutions of IBA at 4500 or 8000 mg/liter (ppm), dried and inserted into the basal end of the cuttings, initiated more roots per cutting than cuttings treated with a rooting powder containing 8000 ppm (0.8%) IBA or toothpicks soaked in a solution of IBA at 1000 mg/liter (ppm). There were also differences in total root length and weighted root score (an index of root number and spatial symmetry among initiated roots), but not in rooting percentage. In a second experiment, cuttings treated with a solution of IBA at 10,000 mg/liter (ppm) for 30 sec or inserted toothpicks soaked in an IBA solution at 1000 mg/liter (ppm) showed no differences in rooting percentage. However, cuttings receiving the toothpick treatment initiated more roots during the period between 3 and 13 months after the first roots were observed than cuttings receiving a basal dip, suggesting slow release of auxin from the toothpicks.

Auxin solutions can be applied to the base of cuttings using a spray application. Cuttings are typically bundled prior to treatment so that multiple cuttings can be sprayed at one time. In the 1970s, Hartley et al. (71) reported rooting of cuttings of carnation receiving a basal spray of Jiffy Grow (IBA + NAA) was comparable to, or higher than, cuttings receiving a 20-sec basal dip using equivalent concentrations. Nisio (132) evaluated rooting of 'Seiun' chrysanthemum (*Chrysanthemum* ×*morifolium* Ramat. 'Seiun') cuttings with IBA applied as a powder, dip, and spray. Rooting occurred on almost all nontreated cuttings and cuttings treated with IBA powder, while approximately two-thirds of cuttings rooted when the cutting bases were sprayed with IBA or dipped in a solution of IBA.

Wounding, prewetting, and acid/base pretreatment methods. Powder and basal quick-dip applications of auxin are sometimes carried out in conjunction with a wound treatment, particularly on harder-to-root cuttings of woody species. Techniques of wounding include stripping the leaves or branches at the cutting base, making one or more vertical incisions down the side of the cutting base, splitting the stem base (leaving both sides, or removing one side, of the split stem base), or removing a shallow or deep slice of tissue from one side (or slices from two sides) of the proximal end of a woody stem cutting so as to expose more of the cambium tissue to the auxin (72, 89). Wounding appears to increase the amount of auxin absorbed by cuttings, based on results of research by Scalabrelli and Couvillon (147) examining ethylene release from hardwood cuttings of peach [*Prunus persica* (L.) Batsch]. Howard et al. (89) found a significant wounding response of hardwood cuttings of M.26 apple rootstock (*Malus pumila* P. Mill.) occurred only with application of IBA, both when wounding preceded and followed IBA treatment. Mackenzie et al. (114) concluded the critical factor in improving rooting performance of hardwood cuttings of M.26 apple wounded by splitting the cutting base prior to treatment with an IBA solution was exposure of a greater quantity of tissue susceptible to the root-promoting influence of applied auxin. Research conducted by Edwards and Thomas (52) demonstrated the influence of a wounding treatment on rooting can vary depending on factors such as plant species, type of wound, and auxin concentration.

Stuart and Marth (164) prepared cuttings of American holly with a 0.64 cm (0.25 in) long, upward splitting of the cuttings bases with two right-angle cuts prior to soaking cuttings in IBA at 100 mg/liter (ppm) for 18 hr. These cuttings rooted at 100% and produced root systems that were somewhat larger and better distributed than those on nonwounded, IBA-treated cuttings. Cuttings prepared without a wound and not treated with IBA failed to root, while there was almost no rooting on cuttings wounded but not treated with IBA. Another group of cuttings was prepared and inserted in a rooting bench to produce callus, but the cuttings were not treated with IBA until 76 days later. Cuttings wounded by removing two thin slices of bark from opposite sides of the stem just above the basal callus prior to the delayed IBA treatment showed a better rooting response than wounded cuttings receiving no IBA treatment.

Chadwick and Swartley (35) wounded cuttings of Pfitzer juniper by making two opposite slits on the lower 2.5 cm (1 in) of each cutting, slightly twisting the knife as the slits were made so as to create a slight groove. Wounded cuttings treated with IBA powder rooted at 52% while nonwounded cuttings treated with IBA powder rooted at 24%. Also, wounded cuttings tended to produce more roots than nonwounded cuttings. Wells (184) reported a higher percentage of Pfitzer juniper cuttings rooted in response to a 3.8-cm (1.5-in) incision wound followed by treatment with 4000 ppm (0.4%) or 8000 ppm (0.8%) IBA powder compared with no treatment, auxin treatment alone, or an incision wound followed by a 24-hr soak in a solution of IBA at 40, 60, or 80 mg/liter (ppm). Wells (185) also found a combination of wounding and IBA to be effective for cuttings of several other conifer cultivars; however, rooting of Brown's yew (*Taxus* x *media* Rehder 'Brownii') cuttings treated in this manner was described as excessive and unnatural in comparison with nonwounded cuttings treated with IBA. Nursery trials conducted in Great Britain (85) indicated a trend toward improved rooting when cuttings of several woody ornamentals were treated with a slice wound on the proximal ends of the cuttings prior to a powder application of IBA and reduced rooting when wounded cuttings received a basal quick-dip in an alcohol-based solution of IBA. In some cases, wounding was beneficial whether IBA was applied before or after the wound was made.

Howard et al. (88) found use of one or two incision wounds, followed by a dip in K-IBA at 4000 mg/liter (ppm), enhanced rooting of jojoba [*Simmondsia chinensis* (Link) Schneid.] cuttings, particularly cuttings prepared without a proximal node. When Palzkill and Feldman (135) prepared jojoba cuttings with two shallow slice wounds followed by a dip in K-IBA at 1000 mg/liter (ppm), roots emerged along a greater length of the stem on wounded cuttings than on nonwounded cuttings. Teklehaimanot et al. (169) tested three wounding treatments [each with and without NAA at 100 mg/liter (ppm)] for rooting cuttings of the undomesticated African locust bean [*Parkia biglobosa* (Jacq.) R. Br. ex G. Don]: a diagonal cut made to expose additional tissue, a bruising performed by squeezing to loosen the bark from the woody tissue, and four equidistant horizontal cuts. In general, only the diagonal cut enhanced rooting in comparison with nonwounded cuttings; however, application of NAA negated the positive effect. Al-Salem and Karam (2) found wounding by making two longitudinal slits at the cutting base prior to a 5-sec dip in an IBA solution greatly increased rooting of Grecian strawberry tree (*Arbutus andrachne* L.) cuttings.

A technique described by Howard (86) involved application of auxin in talc formulation following treatment of cuttings with solvents as prewetting agents. Fifty percent aqueous solutions of acetone, ethanol, and methylated spirits were more effective than water in stimulating rooting response on winter cuttings of 'MM.111' apple rootstock (*Malus domestica* Borkh. 'MM.111') and 'Myrobalan B' plum rootstock (*Prunus cerasifera* Ehrh. 'Myrobalan B'), but less effective than dimethylsulfoxide. However, this method produced rooting responses less than those obtained by dipping cuttings directly into solutions of IBA in the same solvents.

Auxin has also been applied to stem cuttings of ornamental crops in association with a pretreatment with acid or base. In a study by Lee et al. (104), stem cuttings were basally dipped in 2N sulfuric acid for 15 sec or soaked in sodium hydroxide at pH 10.5 for 10 min, washed with distilled water, and then dipped in an aqueous solution of K-IBA at 3000 mg/liter (ppm) for 10 sec for herbaceous cuttings and 20 sec for hardwood cuttings, inserted into rooting substrate, and placed under intermittent mist. Acid treatment promoted rooting of 'San Diego Red' bougainvillea (*Bougainvillea* Comm. ex Juss. 'San Diego Red'), carob (*Ceratonia siliqua* L.), chrysanthemum, Japanese euonymus (*Euonymus japonicus* Thunb.), poinsettia, English ivy (*Hedera helix* L.), star jasmine [*Trachelospermum jasminoides* (Lindl.) Lem.], northern California black walnut (*Juglans hindsii* Jeps. ex R. E. Sm.), Chinese pistache (*Pistacia chinensis* Bunge), and red willow (*Salix laevigata* Bebb). Pretreatment with base increased rooting of 'Sweetheart Supreme' rhododendron (*Rhododendron* L. 'Sweetheart Supreme'), bougainvillea (*Bougainvillea* Comm. ex Juss.), American sweetgum (*Liquidambar styraciflua* L.), holly osmanthus [*Osmanthus heterophyllus* (G. Don) P. S. Green], and Monterey pine (*Pinus radiata* D. Don). It was suggested short exposure to acid may break acid-labile linkages in the cell walls of calciphilous plants, while base pretreatment may break base-labile linkages in the cell walls of acid-loving or acid-tolerating plants, thus increasing water permeability, facilitating absorption of applied auxin, and/or facilitating emergence of root initials.

Khosh-Khui and Tafazoli (98) also tested the acid and base pretreatments of Lee et al. (104) in association with IBA and NAA for rooting hardwood cuttings of damask rose (*Rosa* ×*damascena* Mill.). Acid pretreatment increased root number, root length, and root dry weight, but did not affect rooting percentage. Ingram et al. (91) found sulfuric acid negated the beneficial effect of IBA, while sodium hydroxide had no effect on rooting cuttings of Indian hawthorn [*Rhaphiolepis indica* (L.) Lindl. ex Ker Gawl.].

Stock plant spray treatment methods. Some researchers have investigated auxin application to cuttings by spraying stock plants with auxin prior to cutting collection. Stoutemyer and O'Rourke (158) sprayed stock plants of several woody ornamentals with selected concentrations of the auxin, 2,4,5-trichlorophenoxyacetic acid (TCPA) and its sodium salt, and prepared cuttings from the treated plants after several days or weeks. The results, which the researchers considered to be preliminary in nature, suggested cuttings taken from plants treated with TCPA at a suitable concentration after a certain interval would show essentially the same rooting responses as cuttings treated with generally accepted methods of application.

Whatley et al. (188) sprayed stock plants of rose of Sharon with a solution containing NAA, dimethylsulfoxide (DMSO), acetone, glycerin, and Triton X100 approximately 10 days before collecting cuttings for one treatment. Cuttings from nontreated stock plants received no auxin treatment, a 15-min soak in IBA at 1000 mg/liter (ppm) with 0.5% DMSO, or a 30-min soak in IBA at 1000 mg/liter (ppm) with 0.5% DMSO. Cuttings were rooted in both a continuously aerated Hoagland solution (82) and distilled water. Cuttings receiving the 15-min soak in IBA rooted better than cuttings receiving the foliar spray, while cuttings from both of the treatments rooted better than nontreated cuttings. Cuttings receiving the foliar spray rooted better in water than in Hoagland solution.

Girdling and banding pretreatment methods. In research examining cutting propagation of difficult-to-root species, a technique of girdling (also known as 'ringing' or 'ring barking') and pretreating shoots with auxin several weeks before removal of the shoots from the plant and preparation as cuttings has shown to be useful with a number of species. Working with $^{14}C0_2$ and seedling and mature plants of Monterey pine, Cameron (33) found girdling the shoot increased the proportion of radioactive photosynthates accumulating at the site of root initiation (the base of a subsequently prepared air layer or stem cutting) compared with nongirdled shoots, while treatment of the base of the shoot with IBA at the time of girdling enhanced this effect.

Using girdling (or 'ringing') by removal of a 2-cm (0.8 in) ring of bark at the base of seedling shoots of mango (*Mangifera indica* L.) and pretreatment with lanolin paste containing IBA at 2000 ppm (0.2%) at the time of girdling, Basu et al. (10) reported improved rooting (88 and 12% rooting for cuttings from girdled and non-girdled shoots, respectively). Use of NAA in the lanolin paste was less effective than IBA. Supplementary treatment with a 24-hr basal soak in solutions of IBA or NAA at 100 mg/liter (ppm) following cutting preparation had an adverse affect.

A protocol described by Hare (66) for propagation of slash pine (*Pinus elliottii* Engelm.) involved girdling <1-year-old branches of 12-year-old trees by removing a 2-cm (0.8 in) ring of bark 15 cm (6 in) below the terminal bud, covering the upper portion of the girdled area with paste (powder and water) containing IBA at 1000 ppm (1%) and other compounds, and covering the girdled area with saran and aluminum foil. After 2 months, these shoots were severed at the upper end of the girdled area and the resulting cuttings were inserted into a perlite/vermiculite substrate for rooting under intermittent mist. Survival and rooting of pretreated cuttings were notably better than with conventional, nongirdled cuttings receiving a basal treatment in the same rooting powder prior to placement into the rooting substrate; however, success of the technique was dependant on the time of year. Further experiments by Hare (70) confirmed slash pine cuttings receiving the girdling and auxin pretreatment rooted faster, produced larger root systems, and survived better both before and after rooting than nongirdled cuttings.

The technique of girdling and pretreatment with an auxin-containing paste has also shown to be useful in improving the rooting of cuttings from older (nonjuvenile) trees of American sycamore (*Platanus occidentalis* L.), Formosan sweetgum (*Liquidambar formosana* Hance), and water oak (*Quercus nigra* L.) (67, 68, 69). No improvement in rooting was noted using this technique with cuttings of American sweetgum, probably due to being performed too late in the season (68).

Another method of pretreating cuttings with auxin while the shoots are still attached to the stock plant involves application of auxin in association with a banding treatment. Banding involves wrapping a short length of shoot (eventually to be the cutting base) with an opaque material to exclude light, and may be performed on etiolated (dark- or shade-grown) shoots upon their shift to lighted conditions or on developing light-grown shoots to blanch the tissue. Maynard and Bassuk (119) examined the use of 2 cm (0.8 in) wide black plastic electrical tape and Velcro (a reusable adhesive material) as banding materials, along with the application of IBA powder at 8000 ppm (0.8%) to the bands prior to banding etiolated and light-grown shoots of a range of difficult-to-root woody species. Bands were removed after 4 weeks and cuttings were prepared and treated with IBA powder at 4000 ppm (0.4%) before planting in the rooting substrate. The banding technique and initial application of auxin was facilitated by use of the Velcro strips in comparison with the black tape and sometimes resulted in stem wounding, swelling, and root formation on the treated shoots. Rooting response to inclusion of IBA in the Velcro bands varied by species. In similar experiments (118), cuttings of Japanese stewartia (*Stewartia pseudocamellia* Maxim.) obtained from shoots treated with auxin-treated Velcro bands benefited from an additional basal quick-dip in IBA at 4000 mg/liter (ppm) prior to planting in the rooting substrate, but cuttings of Japanese tree lilac [*Syringa reticulata* (Blume) H. Hara] did not. In other trials (120, 121, 122, 148), rooting results obtained using auxin-treated Velcro bands have varied depending on species and other treatment factors.

Upper stem dip, foliar dip, and total immersion methods. Auxin application has been performed by dipping the upper (distal) cut end, foliage, or terminal portion of cuttings into an auxin solution prior to insertion into the rooting substrate. Auxin has also been applied by completely immersing cuttings in an auxin solution prior to insertion into the rooting substrate.

Krisantini et al. (100) treated two-node cuttings of two grevillea (*Grevillea* R. Br. ex Knight) cultivars with a 5-sec upper [1 cm (0.4 in)] stem dip in a solution of IBA at 1000 mg/liter (ppm), basal dip in a solution of IBA at 1000 mg/liter (ppm), basal dip in an IBA powder at 16,000 ppm (1.6%) (standard treatment), or no auxin treatment. Rooting percentages with cuttings of 'Royal Mantle' grevillea were higher using the upper stem dip and the basal powder application than the basal solution treatment or no auxin treatment. Some cuttings of 'Coastal Dawn' grevillea rooted in response to the upper stem dip and the standard powder treatment, while no cuttings rooted in the other two treatments.

McGuire and Sorensen (127) conducted studies using cuttings of 12 ornamentals including cultivars of juniper, rhododendron, and viburnum (*Viburnum* L.). All cuttings were prepared with a terminal bud and four nodes. Treatment 1 was a terminal dip to a depth of two nodes with leaves in a mixture of 10,000 mg/liter (ppm) IBA and 500 mg/liter (ppm) NAA in 40% polyethylene glycol, with cuttings blotted to remove all excess. Treatment 2 was a basal dip to a depth of 2.5 cm (1 in) of defoliated stem in a mixture of 2000 mg/liter (ppm) IBA and 500 mg/liter (ppm) NAA in 40% polyethylene glycol. Treatment 3 was a basal application to a depth of 2.5 cm (1 in) of defoliated stem in a commercially prepared talc containing IBA at 3000 ppm (0.3%). Terminal application resulted in significantly greater root development than both forms of basal application for two species, similar to one form of basal application with five species, and similar to both forms of basal application with three species; results were inconclusive with two species due to limited rooting. McGuire and Sorensen (127) concluded terminal treatments were feasible and could be used on a practical basis provided optimum concentrations were determined for each species.

In another study, McGuire et al. (125) applied 10,000 mg/liter (ppm) IBA in a 50% solution of ethanol to cuttings of convex-leaf Japanese holly as a terminal dip, as a spray applied to the entire leaf area, or as a basal dip of the cutting and reported similar results among all three treatments. Four applications of 2500 mg/liter (ppm) IBA in 50% ethanol resulted in significantly fewer roots per cutting than the previous three treatments, but similar to nontreated cuttings. Nontreated cuttings produced roots at the lowest part of their basal stem section, cuttings treated with a basal dip produced roots higher up on the basal part of the stem and none on the lowest part, and cuttings treated with a terminal dip produced roots all along the lower and upper parts of the basal stem section.

Working with ^{14}C-labeled IAA applied to 12.7 cm (5 in) long cuttings of convex-leaf Japanese holly through a terminal dip, McGuire (124) concluded sufficient auxin entered the foliage and terminal bud of cuttings to result in effective increases in rooting compared to nontreated cuttings. He also determined more auxin was absorbed using a basal dip than with a terminal dip, with the auxin carried to the uppermost 2.5 cm (1 in) of the cuttings within 24 hr. The largest amount remained in the lowest 2.5 cm (1 in) with progressively lesser amounts in each succeeding 2.5 (1 in) cm up the cutting. With a terminal dip, the greatest amount of auxin remained in the 2.5 (1 in) cm that was dipped, with progressively less in each lower 2.5 cm (1 in), with a slight exception in the lowest 2.5 cm (1 in). In addition, removal of the shoot apex resulted in increased auxin uptake of terminally dipped cuttings, but did not result in increased transport.

A foliar dip in solutions of IBA was reported by Anuradha and Sreenivasan (4) to be beneficial for rooting single-node cuttings of 'Cauvery' coffee (*Coffea arabica* L. 'Cauvery'). Rooting at 90 days after planting cuttings in trenches increased from 35% in the nontreated controls to 53% with a basal dip in 5000 mg/liter (ppm) IBA, 90% with a foliar dip in 7000 mg/liter (ppm) IBA, and 90% with a foliar dip in 10,000 mg/liter (ppm) IBA. Among these same treatments, the foliar dip in 7000 mg/liter (ppm) IBA was rated as superior as it provided the highest values for number and length of primary roots and number of leaves per cutting. Notable shoot retardation was observed on cuttings receiving a foliar dip in 10,000 mg/liter (ppm) IBA.

Tincker (174) mentions an early study using the total immersion technique. Cuttings of myrtle (*Myrtus communis* L.) (an easily rooted species) were placed with their leaves in a dilute solution of IAA and NAA for 12 hr, with results described as unsatisfactory compared with treatment of the stem.

Complete immersion of cuttings in auxin solutions was also studied by Van Bragt et al. (179). In a preliminary experiment, cuttings of several woody ornamentals were totally immersed for 2 sec to 2 min in K-IAA or K-IBA solutions at concentrations of 1, 10, 100, or 1000 mg/liter (ppm) (plus 1.5 mL/liter Tween 20 as a wetting agent), and results were compared to cuttings receiving a basal application of 10,000 ppm (1.0%) powder preparations of IAA or IBA. Immersion treatments for 2 min in 1000 mg/liter (ppm) auxin provided the best results. Cuttings of three cultivars treated with IAA rooted similarly with both treatment methods, while two cultivars treated with IAA and two cultivars treated with IBA rooted better with the immersion treatment. In a second experiment using IBA to treat cuttings of three cultivars of barberry (*Berberis* L.) and two cultivars of firethorn (*Pyracantha* M. Roemer), cuttings receiving the immersion treatment rooted similarly or better than cuttings treated with powder. It was also noted that shoot growth was retarded for the first 3 months after immersion; however, initial loss of growth was compensated at a later stage of growth so that both treatments yielded marketable plants on the same date.

Treatment of cuttings with auxin by total immersion has been used as a commercial practice for perennials. Strasko (159) described use of the technique in which tip cuttings of herbaceous perennials were dipped into tubs containing a solution of K-IBA and a fungicide for 2 min before insertion into rooting substrate. Singletary and Martin (152) utilized the same procedure to examine rooting of three perennials using K-IBA at 0, 500, 1000, and 2000 mg/liter (ppm). Rooting percentage for cuttings of 'Noah Williams' speedwell (*Veronica* L. 'Noah Williams') was lowest and root number was greatest at the highest concentration, while responses with the other three treatments were mostly similar. Rooting response for fern leaf yarrow (*Achillea* L. 'Moonshine') appeared highest using 1000 and 2000 mg/liter (ppm) K-IBA. Rooting percentage for white wild indigo [*Baptisia alba* (L.) Vent. var. *alba* (syn. *Baptista pendula* Larisey] appeared to increase up to 1000 mg/liter (ppm) K-IBA, while root number appeared highest with K-IBA at 2000 mg/liter (ppm).

Foliar spray methods. Cuttings may also be treated with auxin by spraying cuttings with an auxin solution after insertion into the rooting substrate. Although other agricultural chemicals (including some plant growth regulators) are applied as sprays in commercial practice, auxins as root-promoting chemicals are not commonly applied in this manner. Spray applications of auxin are useful in agriculture for a variety of purposes, including their use as herbicides, thinning agents on fruit trees, and agents able to control regrowth of tree sprouts after pruning, induce flowering of bromeliads, and promote fruit set (56).

In the 1930s, Chadwick and Kiplinger (34) tested a foliar spray of IAA on chrysanthemum cuttings in an effort to avoid the inconvenience of soaking the basal ends of cuttings in auxin solutions. Although data on the trials were not presented in their report, they noted spraying cuttings once with IBA at a concentration of 100 mg/liter (ppm) and three times with a concentration of 20 mg/liter (ppm) was not as effective as soaking the basal ends of cuttings in IBA at a concentration of 2.5 mg/liter (ppm) for 24 hr. The spray technique also required a greater amount of auxin.

Tincker (174) sprayed cuttings of strawberry tree (*Arbutus unedo* L.) after insertion in a rooting frame with 100 mg/

liter (ppm) IAA three times over 2 days, resulting in some injury, defoliation, and no successful rooting. A similar test with Tasmanian snow gum (*Eucalyptus coccifera* Hook. f.) cuttings resulted in leaf injury, but no rooting.

Physiologists at the U.S. Department of Agriculture (77) recommended using a fine foliar spray of auxin as an aqueous solution or an emulsion to the tops of cuttings of herbaceous perennials after they had been inserted into the rooting substrate. A concentration of 300 mg/liter (ppm) IBA was suggested for cuttings of bean (*Phaseolus* L.), marigold (*Tagetes* L.), coleus, marguerite [*Argyranthemum frutescens* (L.) Schultz-Bip.], and carnation. Spray treatment with an emulsion of IBA resulted in a greater number of roots on cuttings in comparison to nontreated cuttings. A similar concentration of IAA or NAA was also effective on cuttings of carnation and coleus. Compared to a dilute basal soak, the spraying technique was reported to be easier to apply, less likely to cause injury to cuttings, and permitted repeat applications without disturbing cuttings. However, the spray technique was noted to be somewhat less economical and no more effective than some of the soaking treatments.

Mitchell and Marth (130) briefly discussed spray application of auxin to the leaves and upper ends of stems as an aid in rooting stem cuttings of flowering, fruiting, and vegetable plants. A solution of IBA dissolved in alcohol and diluted with water was noted to provide good results with certain herbaceous species, while lanolin-emulsion sprays were suggested for species requiring longer periods for rooting. The lanolin was used to retain the auxin on the foliage and make it available to the cutting over an extended period of time.

Fish (55) reported using a spray application of auxin in association with a powder dip for rooting cuttings of camellia. Cuttings were treated with Hormodin 3 [8000 ppm (0.8%)] powder prior to insertion into the rooting substrate and, after an additional 6 weeks, were sprayed with a light application of Jiffy Grow (IBA + NAA) liquid formulation (diluted 1:5). Excessive application of the liquid was reported to retard root growth.

In reviewing potential uses of a water-soluble IBA product using a foliar spray application, Kroin (101) noted the 'spray drip down' method is cost effective since it uses minimum labor and low IBA concentrations. After cuttings have been inserted in trays, a rooting solution is sprayed onto the leaves until beads of liquid drip down into the substrate. Aqueous solutions of IBA at 50 to 250 mg/liter (ppm) were recommended for cuttings of chrysanthemum, begonia (*Begonia* L.), dieffenbachia (*Dieffenbachia* Schott), heath, and hibiscus (*Hibiscus* L.). Chrysanthemum cuttings treated using the spray drip down method with IBA at 5 to 150 mg/liter (ppm) are noted to produce high quality, symmetrical root systems based on production tests in Holland. In addition, rose cuttings were noted to produce quality roots using the spray drip down method with 50 to 100 mg/liter (ppm) IBA based on tests at a commercial research center.

Bartolini and Fiorino (9) tested biweekly foliar spray applications of 0, 50, 100, or 200 mg/liter (ppm) IBA or the amide form of NAA (NAD) on cuttings of 'Maurino' olive (*Olea europaea* L. 'Maurino'), the auxin sprays being used alone and in combination with a 4- to 5-sec basal dip in a solution of 4000 mg/liter (ppm) IBA. Cuttings treated with the IBA sprays alone showed increased rooting response with increasing IBA concentration. Rooting of cuttings sprayed with 200 mg/liter (ppm) IBA was similar to that of cuttings receiving only the basal dip. Rooting was similar or less using a foliar spray of NAD. Root number per cutting was greater when cuttings received both a foliar and basal application of auxin compared with a foliar spray alone. With increasing auxin concentration in the foliar spray treatments, there were indications of decreasing axillary budbreak of rooted cuttings at the end of the rooting period.

Trials were conducted by Blythe et al. (21) to determine whether a foliar spray application of the auxins IBA and NAA as a dilution of the commercial root-promoting formulation Dip 'N Grow (IBA + NAA) would be as effective as a basal quick-dip for rooting cuttings. A foliar spray application of 50 mg/liter (ppm) IBA + 25 mg/liter (ppm) NAA after insertion into the rooting substrate was as effective as the basal quick-dip for terminal cuttings of silver and gold chrysanthemum [*Chrysanthemum pacificum* Nakai (syn. *Ajania pacifica* (Nakai) K. Bremer & Humphries)], while other spray treatments (at lower concentrations) were less effective. Cuttings of 'Lynwood Gold' border forsythia (*Forsythia* ×*intermedia* Zab. 'Lynwood Gold') rooted well using a basal dip; however, subsequent shoot development from the rooted cuttings was better with the foliar spray treatments.

Further trials by Blythe et al. (23) evaluated use of a foliar spray application of Dip 'N Grow, as well as K-IBA, for rooting stem cuttings of ornamentals. Cuttings of silver and gold chrysanthemum sprayed with 10 mg/liter (ppm) K-IBA produced greater total root length than cuttings receiving the basal quick-dip; otherwise, root development was similar between spray treatments and the basal quick-dip. Root and shoot development measures were similar or lower for cuttings of glossy abelia [*Abelia* × *grandiflora* (André) Rehd.], panicled hydrangea (*Hydrangea paniculata* Sieb.), and 'Natchez' crapemyrtle [*Lagerstroemia* (*indica* x *fauriei*) 'Natchez'] sprayed with auxin compared to a basal quick-dip.

In testing foliar sprays of auxin on stem cuttings of four tropical ornamentals, Blythe et al. (20) found terminal cuttings of Chinese evergreen (*Aglaonema modestum* Schott ex Engl.) receiving a basal quick-dip produced the greatest number of roots and total root length per cutting, nontreated cuttings produced the fewest roots and lowest total root length, while auxin spray treatments gave intermediate results. Nontreated and sprayed two-node cuttings of dwarf gardenia [*Gardenia jasminoides* Ellis 'Radicans' (syn. *Gardenia augusta* (L.) Merrill 'Radicans')] and terminal cuttings of weeping fig (*Ficus benjamina* L.) produced similar or lesser results in comparison to a basal quick-dip. Results with subterminal cuttings of 'Ivalace' English ivy (*Hedera helix* L. 'Ivalace') were similar among all treatments. Subsequent shoot or foliage development on cuttings of all species receiving the spray treatments was similar in most cases to cuttings receiving no auxin treatment or a basal quick-dip treatment.

In five experiments conducted by Blythe et al. (25), single-node cuttings of 'Red Cascade' miniature rose were treated with a basal quick-dip (prior to insertion into the rooting substrate) or sprayed to the drip point with a single foliar application (after insertion) of Dip 'N Grow, K-IBA, or K-NAA; a single foliar spray application of Dip 'N Grow with and without Kinetic surfactant; or multiple foliar spray applications of Dip 'N Grow. Spray treatments were compared with their respective basal quick-dip controls. Sprayed cuttings exhibited rooting percentages, total root length, percent of rooted cuttings with shoots, and shoot length similar to or less than control cuttings. Exceptions were cuttings sprayed with 0 to 0.5 mg/liter (ppm) K-NAA which exhibited shoot length greater than the control cuttings. Addition of 1.0 mg/liter (ppm) Kinetic organosilicone surfactant to spray treatments resulted in greater total root length and shoot length. Repeated sprays (daily up to 7 consecutive days) had no or negative effects on root and shoot development.

Substrate application methods. Until recent years, scientific literature has reported little regarding auxin application to conventional stem cuttings by way of the rooting substrate. However, auxin-containing substrates are commonly utilized in micropropagation (tissue culture) to promote adventitious rooting, suggesting such a technique has potential for propagation by stem cuttings.

In the 1930s, Tincker (174) briefly mentioned obtaining successful root formation on cuttings stimulated by pouring an auxin solution onto the rooting substrate (sand), but did not provide information on species tested or quantitative data. Tincker (174) suggested use of an organic substrate, although of practical value, would not permit determination of the auxin concentration to which the cuttings were exposed since the breakdown of organic material (e.g., peat) in the substrate might provide active (root-promoting) products.

Stem cuttings rooted in a hydroponic system can be treated with auxin via a recirculating hydroponic solution. Using a hydroponic system based on the nutrient film technique, Boland and Hanger (26) inserted terminal cuttings of winter daphne (*Daphne odora* Thunb.) into a layer of black polypropylene beads within plastic-lined, galvanized channels with recirculating solutions containing tap water only or tap water containing 10 mg/liter (ppm) K-IBA, with groups of cuttings in the K-IBA solution transferred to tap water only channels at selected intervals. Cuttings exposed to K-IBA exhibited accelerated root formation compared to cuttings with no K-IBA exposure. Root number, root length, and root dry weight were maximized with around 10 days of exposure to auxin. Heating the solution to maintain a minimum temperature improved rooting percentage.

Wilkinson (189) used a hydroponic system similar to that of Boland and Hanger (26) to root stem cuttings of several woody ornamentals. Cuttings of Sturt's desert pea [*Swainsona formosa* (G. Don) Joy Thomps.] exposed to 10 mg/liter (ppm) IBA produced a similar rooting percentage, but did produce a larger root mass, than cuttings in water only. Cuttings of Lady Banks rose (*Rosa banksiae* W. T. Aiton var. *banksiae*) rooted successfully in water only, while exposure to 5 mg/liter (ppm) IBA was toxic to all cuttings. Rooting of slender honeymyrtle (*Melaleuca gibbosa* Labill.) and winter daphne was stimulated with exposure to IBA. Rooting of heath-leaf banksia (*Banksia ericifolia* L. f.) was improved when peat moss was added to the beads in the substrate and the solution was recycled intermittently, rather than continuously. The technique provided only limited success with cuttings of species and hybrids of grevillea and failed to produce roots on cuttings of mature redflower gum [*Corymbia ficifolia* (F. Muell.) K.D. Hill & L.A. Johnson (syn. *Eucalyptus ficifolia* F. Muell.)].

In micropropagation, microcuttings of some species are rooted in a Stage III (root induction) substrate containing specific types and concentrations of organic and inorganic chemicals, including nutrients, sucrose, a solidifying agent, and often at least one type of auxin (102). Microcuttings to be rooted in tissue culture can receive either a short (acute) or prolonged (chronic) application of auxin, depending upon the requirements of the particular species (60). Acute applications of auxin can be accomplished either by culturing shoots in a substrate containing concentrated auxin for a short period or by briefly dipping shoots into a concentrated auxin solution or powder, with treated shoots then cultured in an auxin-free substrate. Chronic applications of auxin are accomplished by culturing shoots in a substrate containing a low concentration of auxin for an extended period of time.

Stabilized organic substrate treated with auxin has been used to successfully root microcuttings in vivo. In a series of experiments, Malavasi and Ranieri (116) transferred microcuttings of the peach clonal rootstock 'GF 677' [*Prunus dulcis* (P. Mill.) D.A. Webb (syn. *Prunus amygdalus* Batsch) x *Prunus persica* (L.) Batsch] into small plugs composed of peat and a proprietary binder, having pretreated the plugs with 0.1 mL (0.0034 fl oz) of IAA, IBA, or NAA solutions at concentrations from 10 to 500 mg/liter (ppm). Although auxin was not essential for rooting, root formation was increased using 25 to 100 mg/liter (ppm) auxin in comparison to nontreated controls. Treatment of microcuttings with an auxin dip was also examined, but was not investigated further because it seemed to demand more labor as compared to pretreating the plugs with auxin.

Malavasi and Predieri (115) tested this same auxin-treated plug technique with microcuttings of 'Hayward' kiwi (*Actinidia chinensis* Planchon 'Hayward') and European plum [*Prunus domestica* L. var. *insititia* (L.) Fiori & Paoletti (syn. *Prunus insititia* L.)] clonal rootstock. Microcuttings of kiwi rooted in vivo in both treated and nontreated plugs; however, microcuttings treated with 25 mg/liter (ppm) NAA produced a greater rooting percentage than the control, while both 25 mg/liter (ppm) NAA and 25 mg/liter (ppm) IBA produced more roots (but shorter root length) than the nontreated control. Microcuttings of European plum rootstock exhibited high rooting ability in all treatments; however IAA at 25 mg/liter (ppm) increased the rooting percentage over the control.

McComb and Newton (123) reported successfully rooting microcuttings of kangaroo paw (*Anigozanthos* Labill.) in cubes of flexible polyurethane in vitro. Auxin was supplied to the microcuttings by inserting microcuttings into the foam cubes and placing the cubes into culture tubes containing liquid rooting substrate with IBA. Roots grew through the foam and emerged from the base and sides. Rooted shoots were transferred directly to soil without removing the foam.

Auxin-treated substrate has also been used for layering. Wells (186) described the air-layering process utilized at a commercial nursery for propagating compact Oregon grape holly (*Mahonia aquifolium* (Pursh) Nutt. 'Compacta') in which sphagnum moss is soaked in a solution of 60 mg/liter (ppm) IBA and inserted into a cut flap of stem tissue prior to completion of the air layer.

In a study conducted by Blythe et al. (22) to evaluate auxin application to stem cuttings via the rooting substrate, single-node cuttings of English ivy and 'Red Cascade' miniature rose were inserted into plugs of a stabilized organic rooting substrate (plugs comprised of peat and a polymer binder) that had been soaked in water or aqueous solutions of K-IBA + K-NAA at concentrations ranging from 50 + 25 mg/liter (ppm) to 2000 + 1000 mg/liter (ppm). With increasing auxin concentration in the plugs, treatments first provided a root-promoting response of the lower stem tissue of the cuttings, then a phytotoxic response of the lower stem tissue and some root-promoting response of the upper stem tissue, and finally a phytotoxic response by all stem tissue. Results suggested cuttings inserted in plugs treated with a low concentration of auxin [below 100 mg/liter (ppm)] could potentially provide results similar to cuttings receiving a conventional basal quick-dip. In a second experiment conducted in a similar manner, cuttings were inserted into plugs that had been soaked in water, aqueous solutions of K-IBA at concentrations ranging from 15 to 75 mg/liter (ppm), or aqueous solutions of K-IBA + K-NAA at concentrations ranging from 15 + 7.5 mg/liter (ppm) to 60 + 30 mg/liter (ppm), with results compared to cuttings receiving a conventional basal quick-dip in K-IBA at1000 mg/liter (ppm) or K-IBA + K-NAA at 1000 + 500 mg/liter (ppm). Although auxin was not essential for rooting, number of roots and total root length on cuttings of English ivy were greater in plugs treated with K-IBA at 45 or 60 mg/liter (ppm) and similar with other treatments compared to the basal quick-dip. Shoot length was similar on cuttings in nontreated plugs and all K-IBA treatments compared to the basal quick-dip, and lower with all K-IBA + K-NAA treatments. Auxin was not essential for rooting cuttings of 'Red Cascade' miniature rose; however, cutting response trends indicated a low level of auxin [K-IBA at 30 or 45 mg/liter (ppm)] in the plugs could be beneficial.

In a subsequent study by Blythe et al. (24), results indicated improvements in rooting of both easy- and less-easy-to-root woody ornamental species over a conventional basal quick-dip are possible by applying auxin to stem cuttings via a stabilized organic substrate provided an appropriate formulation and concentration of auxin is selected. In this study, cuttings of 'Wintergreen' boxwood [*Buxus sinica* (Rehd. & Wils.) M. Cheng var. *insularis* (Nakai) M. Cheng 'Wintergreen'], Ebbinge's silverberry (*Elaeagnus* ×*ebbingei* Boom ex Door.), weeping fig, dwarf gardenia, 'Nigra' inkberry [*Ilex glabra* (L.) A. Gray 'Nigra'], dwarf yaupon holly (*Ilex vomitoria* Ait. 'Nana'), 'Blue Pacific' shore juniper (*Juniperus conferta* Parl. 'Blue Pacific'), Japanese ternstroemia [*Ternstroemia gymnanthera* (Wight & Arn.) Sprague], and Asian star jasmine [*Trachelospermum asiaticum* (Siebold & Zucc.) Nakai] were inserted into plugs that had been soaked in water or aqueous solutions of K-IBA [15 to 75 mg/liter (ppm)] or K-IBA + K-NAA (15 + 7.5 mg/liter (ppm) to 60 + 30 mg/liter (ppm)]. Rooting and initial shoot growth responses were compared with cuttings receiving a basal quick-dip in K-IBA (1000 mg/liter (ppm)) or K-IBA + K-NAA [1000 + 500 mg/liter (ppm)]. Rooting percentage, number of roots per rooted cutting, and total root length per rooted cutting for cuttings rooted in auxin-treated plugs were similar to or greater than cuttings receiving a basal quick-dip; lesser results were obtained in a few cases with K-IBA + K-NAA. Percent of rooted cuttings with new shoots and shoot length per rooted cutting for cuttings rooted in plugs treated with K-IBA were mostly similar to cuttings receiving a basal quick-dip in K-IBA, while cuttings rooted in plugs treated with K-IBA + K-NAA exhibited similar or lesser results compared to cuttings receiving a basal quick-dip in K-IBA + K-NAA.

Future Research Opportunities

Opportunities continue to exist for research in the use of auxins for rooting cuttings. Blazich (16) commented that, in the years subsequent to their discovery, auxins remain the only applied compounds that consistently enhance adventitious rooting, and there has been little improvement in the effectiveness of auxins beyond that achieved with IBA, NAA, and related compounds. The need for research to develop new, more effective auxins is noted, the need being justified by the economic importance of adventitious rooting in horticulture and forestry worldwide. Precise identification and characterization of possible rooting co-factors (auxin synergists) is also proposed as a critical area of research. However, until new auxins and rooting co-factors are clearly identified, Blazich (16) points to the need for research in the efficient use of current rooting formulations, along with facilitating auxin uptake, minimizing auxin destruction, and encouraging the health and vigor of cuttings during the critical period preceding rooting.

The Horticultural Research Institute in Washington, DC, the research division of the American Nursery and Landscape Association, lists among its research priorities the topics of propagation-related management issues, as well as mechanization and applied technology (84). Included within the latter topic are labor reduction and efficiency issues, use of new machinery and technologies, and ergonomic issues. Development of alternative methods of auxin application can play a part in each of these focus areas.

For alternative methods of auxin application in cutting propagation to be successfully integrated into commercial nursery production, such methods must meet the needs of the changing horticultural industry. In discussing quality management systems in ornamental horticultural production, Briercliffe (29) reported, in addition to reducing costs and enhancing efficiency, modern production systems call for an approach that also emphasizes quality, safety, and environmental responsibility. In reviewing key issues in the management of a commercial propagation program, Blythe and Sibley (18) noted effective propagators must continually strive to improve their methods of production by evaluating processes, eliminating unneeded steps, streamlining production, and investing in laborsaving systems.

Jones (95) reported engineering in horticulture has the potential to reduce chemical inputs and reduce repetitive, labor-intensive practices by automation and robotics. Examples of engineering solutions that have been applied successfully to nursery production systems include airblast transplanters in seedling production, repotting machines for nursery stock production, and gantry cranes for handling and transporting container-grown plants. As another example, the National Institute for Occupational Safety and Health funded a series of projects by the University of California Agricultural Ergonomics Research Center, one of which involved development of a power cutter for woody plant cuttings (131). The machine reduces repetitive and forceful gripping, with reduced need for handling cuttings and improved productivity by reducing worker fatigue.

Alternative methods of auxin application should be applicable to both well-established, manual propagation processes, as well as newer, automated cutting propagation processes. Although cutting propagation has traditionally been a largely manual process, recent innovations point to the increasing incorporation of automated systems in commercial propagation. Howard (90) described use of the Jansen Plant Cut machine that mechanizes production of cuttings of some woody ornamentals. An operator feeds stems of plant material into sets of rubber belts, which in turn move the material to blades that prepare cuttings by severing at both ends, and then deposits the cuttings into a collection bin. The machine can produce 1500 to 2000 cuttings per hour, depending on the uniformity of the material. Kondo et al. (99) described an automated system that individually separates and, using a robotic manipulator, picks up chrysanthemum cuttings based on size, shape, and orientation and sends them to a cutting sticking robot that carries out the cutting insertion operation. A planting machine described by Hayashi et al. (73) is able to remove the lower leaves from chrysanthemum cuttings and insert them in plugs trays at a rate faster than that of manual planting. An automated cutting and placement device has also been developed for micropropagation (40).

Where manual procedures are employed in cutting propagation, alternative methods of auxin application should play a part in reducing labor requirements and thus reducing production costs. In a study examining the costs of cutting propagation for Fraser's photinia (*Photinia ×fraseri* Dress) and tamarix juniper (*Juniperus sabina* L. 'Tamariscifolia') in nurseries in the Willamette Valley of Oregon, labor accounted for >50% of the total cost of propagation (17). The process of preparing and inserting cuttings into the rooting substrate comprised 22 and 27% of the total cost of propagation for Fraser photinia and tam juniper, respectively. In examining work flow and costing in cutting propagation, Baldwin and Stanley (6) noted the importance of concentrating on three value operations: 1) cuts made on the cutting in the correct position; 2) treating with auxin; and 3) inserting the cutting in the growing substrate. Bunker (30) also observed one of the steps in the propagation process with potential for increased efficiency involves rooting hormones and their application. Richey (142) emphasized that cutting crops with lower rooting percentages are not necessarily more costly to produce than cutting crops with higher rooting percentages; rather, he noted the labor of handling cuttings is a more expensive factor than rooting percentage.

Finally, alternative methods of auxin application must take into account the issues of chemical use and chemical safety. Elliott (53) noted employee health and safety issues, including chemical safety, merit the same attention as other issues in nursery production.

Plant growth regulators, including auxin formulations intended for use in rooting cuttings, are classified as pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act of 1988 (FIFRA), which governs regulation of pesticides in the United States (178). Under FIFRA, all pesticides must be registered (licensed) by the Environmental Protection Agency (EPA) before they may be sold or distributed in commerce. The EPA classifies IBA and products containing IBA as their only active ingredient as biopesticides, and considers such biopesticides to be nontoxic to humans and animals and posing no risk to the environment (176, 177).

Precautionary labeling on pesticides includes information on health hazards, restricted entry interval (REI), and personal protective equipment (PPE) required under the Worker Protection Standards (WPS) of FIFRA (178). PPE requirements are based on acute toxicity tests performed on the end-use product and the solvents (e.g., alcohol) used in the end-use product (136). As an example, precautionary statements on the product label for Dip 'N Grow (IBA + NAA) liquid rooting concentrate instruct users to avoid eye contact, avoid breathing vapors, and wear long-sleeved shirts, pants, and chemical-resistant gloves to avoid skin contact (46). Use of PPE is not required during the REI for products such as Dip 'N Grow provided there is no contact with the treated area (136). However PPE is required during dilution, mixing, and use of such liquid formulations. Requirements under WPS also include pesticide safety training for nursery workers (175).

Although the volume of root-promoting chemicals used in horticulture is small in comparison to other agricultural chemicals, changes in the method of applying these chemicals to cuttings can, at least in small part, help to enhance employees' well-being and overall job satisfaction in a number of ways by: 1) lowering the chemical concentration being used; 2) reducing the time spent handling chemicals; and 3) reducing the number of employees who handle chemicals. The Occupational Safety and Health Administration (133) suggests engineering controls, work practices, and administrative controls be employed to avoid situations where worker safety will require the use of PPE.

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