

Root Formation in Relationship to Auxin Uptake in Cuttings Treated by the Dilute Soak, Quick Dip, and Talc Methods[©]

R.L. Geneve

Department of Horticulture, N-306 Agricultural Science Center North, University of Kentucky, Lexington, Kentucky 40546 U.S.A.

INTRODUCTION

Although many factors can influence the success of adventitious root formation in stem cuttings, auxin remains as a consistent treatment for increasing rooting. The initial observations that auxin promotes root formation date to the 1930s. Since that time, three systems have gained acceptance for delivery of auxin to cuttings. These are talc, quick dip, and dilute soak methods (Blazich, 1988). Quick dip exposes cuttings to auxin in a solvent solution for 1 to 5 sec, while the dilute soak is an aqueous auxin solution with application times up to 48 h. Auxin delivery in talc was developed in the 1930s as an alternative to quick dips and lanolin paste (Loach, 1988). It has generally been suggested that quick dip applications are more effective in promoting root formation than either dilute soak or talc (Bonaminio, 1983). One reason may be the difference in uptake mechanics for these different methods. In general, it appears that auxin in talc or aqueous solutions are only taken up at the cut surface of the cutting and the movement up the stem is by translocation via the transpiration stream. The solvent used for quick dip application seems to facilitate auxin movement through the epidermis as well as the cut surface of the cutting. The objective of this research was to evaluate auxin movement in a woody and herbaceous cutting in relationship to rooting.

MATERIALS AND METHODS

Softwood cuttings were prepared from *Euonymus* (*Euonymus kiautschovicus* 'Manhattan') and mum (*Dendranthema morifolium* 'Bright Golden Princess Anne'). Cuttings were placed under intermittent mist (5 sec every 10 min) with bottom heat 75°F (25°C). Thirty cuttings per treatment were evaluated for root formation after 2 weeks. Auxin uptake was evaluated using radiolabeled NAA (α -naphthaleneacetic acid-carboxyl-¹⁴C) with a specific activity of 2.5 m Ci mmol⁻¹ (Pathfinder Lab, St. Louis). Quick dip and talc formulations contained 2000 ppm, while the dilute soak contained only 200 ppm. In both cases, 0.15% of the NAA was labeled. The quick dip was prepared by dissolving NAA crystals in 95% ethanol and diluting to a final volume of 50% ethanol. The talc was prepared by dissolving NAA in 10 ml of 95% ethanol and mixing with an appropriate volume of talc to form a slurry. The slurry was thoroughly mixed and dried overnight in a fume hood. The dilute soak was an aqueous solution.

Bundles of five cuttings had the basal 2-cm dipped into the 50% ethanol quick dip solution for 0, 1, 5, or 10 sec. The dilute soak had 10 cuttings in 250 ml beakers containing 50 ml of NAA solution for 0, 8, 16, or 24 h. During uptake treatment, the cuttings were kept in a growth chamber at 75°F (25°C), 16-h photoperiod and

illumination by fluorescent lamps ($\sim 70 \mu\text{mol sec}^{-1} \text{m}^{-2}$). In addition, mum cuttings were either directly treated with NAA in talc or pre-treated by dipping in 50% ethanol prior to talc treatment. In both cases, excess talc was tapped free of the cutting. To determine NAA uptake from the talc, the treated cuttings were dipped several times into a scintillation vial containing 10 ml of a toluene based scintillation cocktail. The talc was removed 5 min after treatment or after 24 h in the mist bench. In addition, cuttings that had been in the mist bench for 24 h were directly evaluated for NAA uptake without talc removal. After treatment, the stem below the first leaf was removed from the cutting, dried for 5 days at 50°C (122°F) and combusted in a Packard Tri-carb 306 sample oxidizer and the collected $^{14}\text{CO}_2$ was counted in a Beckman LS 9000 liquid scintillation counter. Thirty cuttings were evaluated for each treatment.

RESULTS AND DISCUSSION

In general, the number of roots produced per cutting for both species was correlated to the uptake of auxin regardless of application method (Fig. 1). For both the quick dip and dilute soak methods, mum cuttings took up more auxin compared to euonymus cuttings. This is probably due to a less waxy stem cuticle on the mum cuttings that allowed auxin entry through the epidermis during the quick dip application and the greater transpiration expected in mum cuttings.

Mum cuttings treated with talc took up only 6% of the auxin absorbed by cuttings that were quick dipped for 5 sec and only about 3% of that taken up by a 16 to 24 h dilute soak. Treating the cutting with a 5-sec ethanol dip prior to talc application

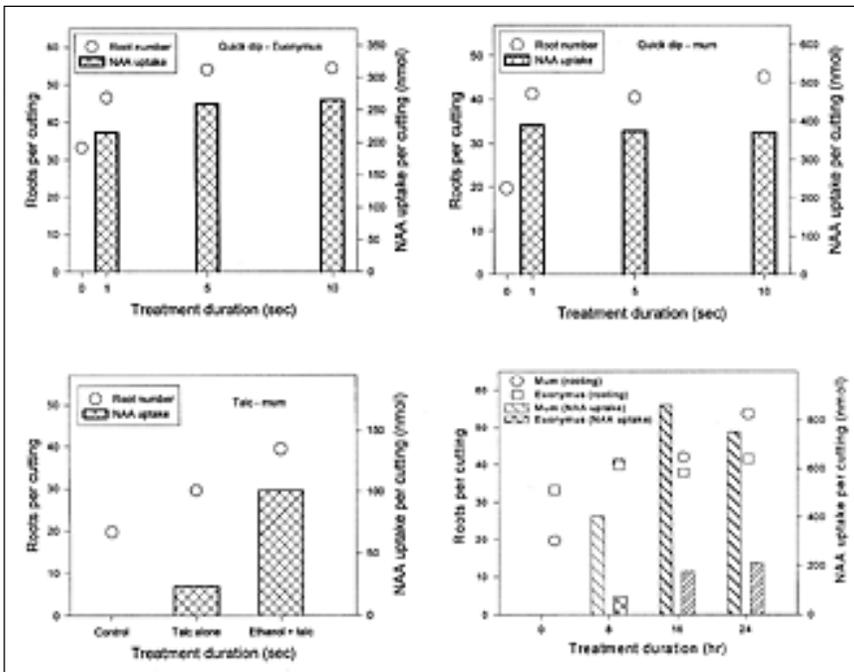


Figure 1. Root formation and auxin uptake in mum and *Euonymus* cuttings.



Figure 2. Location of roots on cuttings treated with auxin by either quick dip, dilute soak, or talc methods.

increased auxin uptake 5-fold with a concomitant increase in approximately 10 roots per cutting. Interestingly, only 3% of the initial talc treatment was still adhering to the surface of the cutting after 24 h in the mist bench.

Mum cuttings in the dilute soak took up the greatest amount of auxin and produced the greatest number of roots. However, these roots did not elongate well and were distributed along the entire stem. It would appear that an auxin concentration (in addition to endogenous auxin) between 100 and 400 nmol per cutting was optimal for increased root formation.

Rooting location along the stem was related to the type of auxin application. Untreated cuttings and talc treated cuttings rooted only at the bottom of the cutting. Cuttings from the quick dip, dilute soak, and ethanol dip prior to talc treatments rooted both at the base of the cutting as well as along the stem (Fig. 2). Cuttings or stem segments treated with an aqueous solution of auxin showed uptake predominantly at the cut surface. This has been shown for stem segments of pea, oats, and cotton (Kenney et al., 1969); seedling cuttings of loblolly pine (Diaz-sala et al., 1996); and apple microcuttings in agar (Guan and de Klerk, 2000). Turetskaya (1957) showed that in order for aqueous solutions of auxin to be taken up from the cut surface and move up the stem it must enter the vascular system and follow the transpirational stream. In black currant cuttings treated with NAA, auxin had moved 6 cm in the first 15 h and was penetrating the leaves. In contrast, the same study showed that auxin was unable to move up the stem of cherry cuttings. This was attributed to limited entry of the auxin into vascular tissue and minimal movement up the stem due to polar auxin transport limitations. However, when auxin was introduced to cuttings as a quick dip in 50% ethanol, auxin was detected in the stem of cherry cuttings (Strydom and Hartmann, 1960). Using autoradiography, they showed that regardless of whether the cutting had leaves to support transpirational transport, auxin moved into and up the stem.

In conclusion, this study supports the hypothesis that talc or aqueous solutions auxin move into the stem and are moved up the stem in the vascular system. In contrast, auxin in ethanol can enter the stem both from the cut surface as well as the epidermis throughout the area of the stem dipped in the solution.

LITERATURE CITED

- Blazich, F.A.** 1988. Chemicals and formulations used to promote adventitious rooting, p. 132-149. In: T.D. Davis, B.E. Haissig, and N. Sankhla (eds.), *Adventitious root formation in cuttings*. Dioscorides Press, Portland, Oregon.

- Bonaminio, V.P.** 1983. Comparison of IBA quick-dips with talc for rooting cuttings. *Comb. Proc. Intl. Plant Prop. Soc* 33:565-568.
- Diaz-Sala, C., K.W. Hutchison, B. Goldfarb, and M.S. Greenwood.** 1996. Maturation-related loss in rooting competence by loblolly pine stem cuttings: The role of auxin transport, metabolism and tissue sensitivity. *Physiol. Plant.* 97:481-490.
- Guan, H. and G.J. De Klerk.** 2000. Stem segments of apple microcuttings take auxin predominantly via the cut surface and not via the epidermis. *Sci. Hort.* 86:23-32.
- Howard, B.H.** 1973. Factors affecting the rooting response of plants to growth regulator application. *Acta Hort.* 34:93-99.
- Kenny, G., J. Sudi and G.E. Blackman.** 1969. The uptake of growth substances XIII. Differential uptake of indole-3yl-acetic acid through the epidermis and cut surfaces of etiolated stem segments. *J. Expt. Bot.* 20:820-840.
- Loach, K.** 1988. Hormone applications and adventitious root formation in cuttings - a critical review. *Acta Hort.* 227: 126-133.
- Strydom, D.K. and H.T. Hartmann.** 1960. Absorption, distribution, and destruction of indoleacetic acid in plum stem cuttings. *Plant Phys.* 35:435-442.
- Turetskaya, R.K.** 1957. Radioactive carbon investigation of the uptake and distribution of growth regulators in plant cuttings. *Plant Physiol. (Fiziologiya Rastenay)* 4:42-51.