USE OF GROWTH REGULATORS IN ROOTING CUTTINGS OF WOODY PLANTS

M.N. WESTWOOD
Department of Horticulture
Oregon State University
Corvallis, Oregon 97331

Rooting of cuttings is dependent upon interrelated genetic, environmental and chemical factors. Genetics controls such factors as the presence of pre-formed root initials and potential kinds and levels of endogenous rooting substances. Chemical balance is regulated internally by the interaction of genetics and environment but is subject to change by exogenously applied chemicals. The infinite number of possible combinations of genetic, environmental and chemical factors affecting cuttings, both pre- and post-severence, has led to some confusion regarding the importance of specific factors for rooting. This review considers briefly the role of growth regulators in rooting but with the interactive effects of genetic type and environment considered concomitantly. Only sample references are given along with books and review papers which include many additional references (2, 11, 12, 20).

Historical. In 1880 a rhizogenic substance was postulated to be produced in leaves and translocated down the stem to promote root formation (Sachs, 33)). Later Zimmerman and Hitchcock (41) reported that ethylene and other unsaturated gases would stimulate adventitious roots. Thimann and Went (37) established that auxin (indoleacetic acid or IAA) is a principal rooting hormone. Then the synthetic auxins indolbutyric acid (IBA) and napthalene acetic acid (NAA) were found to promote rooting (42). An added factor, rhizocaline, was proposed by Went (39), which is produced in the leaves and moves down the stem, where, with auxin it causes roots to form. During the decade which followed many works reported were empirical ramifications of auxin timing and concentration and post-severence treatments for best rooting (2).

Polarity in Plants. Plants and cuttings exhibit strong polarity, both in the position of roots and other organs (19) and in the strong basipetal transport of auxin and other rooting substances (39). Kawase (19) showed that basipetal centrifugation enhanced the polarity of both leafy and leafless cuttings. However, this increased polarity did not uniformly increase rooting. Rooting was increased in some species, decreased in some and was similar to controls in other species. Such variability possibly explains why inverting cuttings improves rooting in some types and reduces it in others.

Nutrition. Balanced mineral nutrition generally results in better rooting than when a deficiency exists, except that low nitrogen often aids rooting by a resulting carbohydrate buildup or possibly by reducing cytokinin levels. Zinc deficiency seems to reduce IAA levels by reducing tryptophan synthesis (12). Tryptophan is needed for IAA synthesis.

Juvenility. Juvenility in plants was reviewed in detail by Ali (1) He found that pear species resemble others in rooting better as juvenile than as adult cuttings and that juvenile tissues contain the same level of DNA but less RNA than adult tissues. This infers the blockage of "flowering" information transfer in juvenile forms. Not only does the non-flowering state aid rooting (34), but juvenile plants contain more rooting cofactors than adult ones (14) So called "rejuvenation" by repeated shearing of adult mother plants (5) may improve rooting by suppressing flower initiation and by changing the auxin balance rather than causing a return to the true juvenile state.

Seasonal Differences. Seasonal influences can alter the effects of growth regulators on rooting (29) and may explain many of the contradictory results reported. Higdon and Westwood (15) and Ali (1) found that IBA-treated hardwood cuttings of pear rooted well only if the buds were in rest. But Fadl and Hartmann (9), in a different climate, found that active pear buds of easy-to-root 'Old Home' promoted rooting with IBA while disbudding reduced rooting. However, disbudding the hard-to-root 'Bartlett' increased rooting potential. The effect of bud activity in these tests is inconclusive because effects of wounding were not separated from bud removal. Howard (16) reported that enhanced rooting of IBA treated plum cuttings was not related to bud activity but to wounding *per se.* Stimulation occurred either by bud removal or by internodal wounding. Wounding causes ethylene synthesis which is known to stimulate rooting (8). In a seasonal study of Douglas-fir rooting, IBA aided rooting in December and January but equal rooting was obtained in February and March without IBA (31). Little rooting occurred during September and October when buds were in rest. Bud removal reduced rooting but IBA treatment sometimes restored the capacity of disbudded cuttings to root. Lek (24) found that, depending upon the season and stage of growth, active poplar buds stimulated, and dormant ones inhibited, development of pre-formed root initials. Any general statement regarding the role of buds in rooting must account for the separate effects of wounding and disbudding, state of dormancy, the presence or absence of leaves and whether the stimulus is to growth of pre-formed root primordia or to *de novo* root initiation.

Growth Regulant Effects. Besides auxins (IAA, IBA, NAA, 2,4-D), other growth regulators such as gibberellic acid (GA), cytokinins, abscisic acid (ABA), ethylene and possibly traumatic acid may affect rooting. Both GA and cytokinins inhibit root formation (12). In some cases the growth inhibitors ABA (4,7) and succinic acid-2, 2-dimethylhydrazide (SADH) (10,30) improved rooting. ABA may act as an auxin synergist and a GA antagonist, but does not affect kinetin-induced inhibition. The synthetic regulator 2-chloroethyl trimethylammonium chloride (chlormequat) in contrast to SADH, reduced rooting of herbaceous cuttings (30). Skene (35) found that chlormequat-treated grape vines had a higher level of cytokinins in bleeding sap than did controls, which may explain the chlormequat effect. Cytokinin inhibition does not affect amino acid levels in rooting pea stems as does ethionine-induced inhibition (18). Lagerstedt (22) reported that the cytokinin, kinetin, inhibited rooting but was required for budding of begonia leaf disks, and a proper balance between auxin and kinetin was needed for both bud and root regeneration. Some IBA-treated *Prunus* cuttings increased rooting when treated with adenine sulfate while other varieties so treated were unaffected or showed reduced rooting (32). Menhenett (27) concluded that rooting of begonia induced by adenine sulfate was by supplying a nutritional source of N, because it did not produce a cytokinin response. Both 2,3,5-triiodobensoic acid (TIBA) and a morphactin improved rooting of 3 herbaceous species in which the latter failed to antogonize a GA_3-induced inhibition (21). Leshem et. al. (25) reported a gonadotropin-like plant constituent which improved rooting of stems. It may act by depressing GA synthesis or by altering the peroxidase system which affects IAA levels in the plant.

Rooting Cofactors. Rooting cofactors which, with auxin, aid rooting have been reported. Hess (14) found 4 such rooting factors in juvenile *Hedera helix* which were not present in adult plants. Cofactors were reported in evergreen species during late fall to late winter at which time rooting was best (23). Several cofactors of a complex phenolic nature have been reported for fruit species of the rose family (6,9,26). Tukey and Lee (38) found that misting of leafy cuttings not only changed the C/N ratio and delayed dormancy, but also increased rooting cofactors. Rooting cofactors must interact with or accompany auxin to stimulate rooting, hence they work like Went's hypothetical rhizocaline. A possible link between anthocyanins and rooting of cuttings was reported (3), but a comparison in Oregon by the author (unpublished) between cuttings of a red-leafed mutant of 'Bartlett' pear and its green-leafed parent indicated no benefit of the pigment to rooting.

Other Factors and Interactions. The following also affect rooting: method of auxin treatment, etiolation, day-length, host plant treatment and position and type of cuttings used. Howard and Nahlawi (17) found that rooting of hardwood cuttings was inhibited if they were dipped too deeply in high concentration IBA solutions. The application of IBA to the bark somehow inhibited rooting of the callus base. Westwood and Brooks (40) showed that a low concentration IBA soak was superior to high concentration quick dip for pear cuttings. Etiolation of stems improves rooting by increasing the amount of rooting cofactors and by otherwise changing the physiology of the stem (13). Short-day photoperiods aided rooting of *Bryophyllum* (28). Several stock plant treatments, such as girdling, use of a dwarf rootstock and a horizontal position of medium-sized rather than large cuttings improved rooting of pear (15). Also, difficult-to-root varieties rooted well even without IBA if they were inarch grafted to an established tree (15).

From this discussion we see that the role of growth regulators in rooting may change depending upon the genetics of the species and the specific environmental conditions imposed upon it. The balance of biochemical factors required for rooting probably remains constant, but this balance may be achieved by different internal or external means at different seasons of the year.

SUMMARY

The balance between auxin and other constituents in plant tissues controls organ formation and is the basis for rooting of cuttings. This balance may be achieved by various combinations of genetic, environmental and chemical factors. The following facts are important to an understanding of the role of growth regulators in rooting: 1. Budding and rooting are strongly polar, as is the basipetal movement of auxin and rooting cofactors in plants. 2. Nutritional deficiencies usually hinder rooting. 3. Juvenile tissues contain more rooting promoters than adult tissues and lack flower buds which inhibit rooting. 4. Whether bud activity or auxin treatment aids in rooting depends upon 2 and 3 above and the season in which the cuttings are taken. 5. GA and cytokinins tend to inhibit rooting while ethylene, ABA and morphactins may improve rooting. Such synthetics as SADH, chlormequat and TIBA give variable responses. 6. Both environment and genetic makeup affect the kind and amount of rooting cofactors. These cofactors appear to be phenolic compounds which interact with auxin to stimulate rooting. 7. Other factors such as stock plant girdling, photoperiod, etiolation, IBA dipping technique, positional effects and maturity of cuttings may affect rooting.

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AL ROBERTS: Thanks, Mel, for a most informative presentation. Any questions?

HUDSON HARTMANN: Mel, in the experiments you described on rooting pear hardwood cuttings you referred to "callusing" the cuttings. Perhaps it would be useful to describe this in more detail.

MELVIN WESTWOOD: Well, in every instance, when we did the callusing we treated the cutting either with a quick-dip or a slow soak of IBA. This was with freshly cut cuttings; then we placed them in plastic bags in moist conditions and placed them at 60° to 65° F. This permitted the callus to develop following IBA treatment, usually taking about two weeks. We would watch them and as the small root primordia would begin to show a protuberance from the edge of the callus plate then we would either plant them or put them in a cool place (35° F. storage) to keep them from growing. If the roots extend very much beyond a centimeter or so, then they're very difficult to plant and you usually lose them in the transfer.

AL ROBERTS: Any more questions? Any for the other speakers?

MELVIN WESTWOOD: I might mention, since Dr. Black talked about shearing and rejuvenation that certainly there are a number of rooting factors that are benefited by this reinvigoration. At a conference back in St. Paul last week people involved in juvenile physiology from around the world met and tried to plan for working groups in this area — an international working group. It was generally agreed that the only way that we could avoid confusion in the literature on juvenility was to retain one of the original definitions — that a juvenile plant is not capable of flowering. There is a period too, which we called the transition period or stage in which the plant flowers but with difficulty. It doesn't flower as much or with the same kinds of treatments that induce flowering in the fully adult stage. I think much of the confusion may be in dealing with this transitional stage But the true juvenile form we say is not capable of flowering. Then there are all the other characteristics, some of which Dr. Black mentioned this morning.