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Stock Plant Nutrition and Stem Cutting Water Relations During Propagation of Four Woody Nursery Crops

by

William Henry Rein

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APPROVED:

Robert D. Wright, Chairman

John A. Barden

John R. Seiler

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(ABSTRACT)

To evaluate the effects of stock plant nutrition and propagation medium moisture content on stem cutting propagation, two separate studies were conducted. In one study, stem cuttings of *Juniperus horizontalis* Moench 'Wiltonii', *Rhododendron* (Lindl.) Planch 'Hino-Crimson', and *Ilex crenata* 'Helleri', were propagated in 1 peat : 1 perlite (v/v) at 125 %, 250 %, 375 %, 500 %, and 625 % moisture. Stem cutting survival and rooting, midday xylem water potential, and basal water uptake all generally increased with increasing medium moisture level. Incidence of cutting basal rot was not directly related to medium moisture level, but was related to species and growth stage of the stock plant. Basal water uptake by cuttings was highest during the first few days after insertion and thereafter decreased until root emergence. Propagation was most successful in the wettest medium (625 %).

In a second study, containerized stock plants of *Ilex crenata* Thunb. 'Rotundifolia' were liquid-fertilized with 25, 50, 100, 200, and 300 mg N • liter^{-1} in two forms (100 % NH_4NO_3 or 50 % Urea + 50 % NH_4NO_3) in a factorial treatment design. Percent rooting of stem cuttings decreased linearly with fertilizer rate. Leaf and stem percent N increased from suboptimal to excessive levels with fertilizer rate. Total nonstructural carbohydrates (TNC) levels increased in leaves and remained constant in stems with in-

creasing fertilizer rate. Stem cutting percent moisture was highly correlated with fertilizer rate. The form of N applied made no statistical difference in these trends. The decrease in percent rooting with increasing fertilizer rate was attributed to increases in shoot growth activity.

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Introduction

The nursery industry relies on the adventitious rooting of stem cuttings as a major method of woody plant propagation. It is a tradition which began centuries ago with easily-rooted food crops such as grape and fig and has continued to provide growers with the easiest and most reliable method of clonal propagation of landscape plants.

Unfortunately, variability in stem cutting survival and rooting remains an industry-wide problem. It is not uncommon for the same plant material to yield high percentages of rooted cuttings one year and to fail the next. Such experiences can disrupt the grower's production schedule and reduce profits.

Probably the most serious challenge to survival of a stem cutting is a consequence of its separation from the stock plant -- the loss of its water- and nutrient-supplying support system. Cultural manipulation of the stock plant prior to cutting severence, and the maintenance of turgidity in the cutting prior to root formation, have been the obvious solutions to this challenge. Enhancing shoot growth on stock plants and the placement of cuttings under intermittent mist systems are now standard procedures in propagation. Nonetheless, balancing increased growth with a carbon-nitrogen ratio favorable for rooting, and maintaining turgid cuttings without suffocating them with an overwet medium, are principles based more on grower experiences than on a clear understanding of their underlying physiology.

The unpredictability of survival and rooting percentages of commercial woody plant cultivars may be due to the lack of data quantifying both the ideal nutritional balance of the stock plant and the factors determining stem cutting water status during propagation. Both stock plant nutrition and the moisture content of the propagation medium may be related to the frequently unexplained basal rotting of stem cuttings.

The research presented in this thesis is an attempt to reduce such causes of variability in propagation by defining the levels of both stock plant fertility and propagation medium moisture that improve survival and rooting of leafy stem cuttings of woody nursery crops. The specific objectives include: (1) determining whether excessive fertilization rates are detrimental to survival and rooting of woody stem cuttings; (2) determining whether the levels of N and carbohydrate in the cutting can be correlated with rooting as well as with fertilization rate; (3) determining the effect of propagation medium moisture level on survival and rooting of stem cuttings; and (4) determining whether medium moisture levels and/or excessive fertilization are related to basal rot of cuttings.

Chapter One: Literature Review

I. *The Water Relations of Stem Cuttings*

Introduction

Moisture stress prevention may be the greatest challenge during propagation (Dirr and Heuser, 1987; Elstrodt and Milbocker, 1983). This has been understood for many years, since stems cut from the parent plant will wilt and die unless water loss is suppressed.

The prevention of water stress has been the subject of several papers dealing with the propagation environment, but quantification of the water relations of stem cuttings has received limited attention. Over thirty years ago, Evans (1952) published an early report on water stress during stem cutting propagation. He cited three physiological conditions which must be overcome for successful rooting of cacao: rapid transpiration, the blocking of the vascular tissue by "mucilaginous secretions or biochemical oxidations at the cut surface," and extreme water tensions in the stem which cannot be overcome by excising it under water. He observed that even a slight water deficit, with no visible wilting, substantially delayed or reduced adventitious root formation. Subsequent reports by several other authors (Hess and Snyder, 1955; Slezinski and Davidson, 1973) have emphasized methods of maintaining an "atmosphere saturated with water vapor" to reduce the loss of cuttings to water stress.

Physiology and Quantification of Stem Cutting Water Relations.

Cell expansion, which is a function of cell turgor, is one of the plant processes most sensitive to water stress (Hsaio, 1973). Since adventitious root formation is dependent upon the expansion of dedifferentiated root initial cells (Hartmann and Kester, 1983), any reduction in stem cutting turgidity would likely reduce the number and length of roots (Snyder and Hess, 1955). The development of practical methods for measuring plant tissue water potential has enabled the evaluation of these assumed reductions in cutting water status. Plant tissue water potential is the sum of both cell turgor (positive pressure of cell contents, mainly vacuolar solutions, against the cell wall) and osmotic potential (the reduction in water potential of pure water, due to the presence of solutes, organic or inorganic) (Kramer, 1983). Reduction in cell size has been highly correlated with a reduction in the water potential of the medium in which growing tissue has been immersed (Hsaio, 1973). Rajagopal and Andersen (1980) subjected stock plants of 'Alaska' pea to periods of artificial moisture stress; the mean number of roots of cuttings taken from these stock plants decreased linearly with increased duration of moisture stress.

Loach (1977) published a numerical description of the severity of water stress endured by cuttings under typical propagation microclimates. In stem cuttings of rhododendron, ceanothus, and hebe, he found that leaf water potentials well below -1.0 MPa (-10 bars) frequently occurred. Rooting was negatively linearly related to afternoon water potentials within the -1.3 to -1.8 MPa range. Grange and Loach (1985) later found that the turgor pressure in the basal portion of *Forsythia* cuttings was maintained even if leaf turgor decreased under water stress. They concluded that basal stem tissue solute levels, not leaf water potential, better determined turgor in the rooting region of a cutting.

Causes of Water Stress During Propagation.

Transpiration is the predominant cause of water loss from a leafy stem cutting (Grange and Loach, 1983a). Transpirational water loss increases with an increase in the difference between leaf and atmospheric water vapor pressure. Stomatal opening, controlled directly by guard cell turgidity, is controlled by basal water supply as well as the atmospheric vapor pressure, light, carbon dioxide concentration, and possibly abcissic acid and cytokinins (Kramer, 1983). If the basal water supply is limited to the point where uptake does not keep pace with water loss, leaf turgidity will decrease until stomata close and turgidity can be restored. However, if the water-supplying medium dries to the point where plant turgidity cannot be restored under ideal conditions (i.e. low vapor pressure deficit and darkness), the plant will become wilted permanently and will die. The same concepts can be applied to plant cuttings. Leaf conductance, a relative measure of stomatal aperture, decreased to a very low level within sixteen hours after rhododendron and dogwood stem cuttings were excised and inserted into the propagation medium, and did not increase until root emergence (Gay and Loach, 1977). Stomata of *Populus robusta* cuttings closed rapidly after excision (Sivakamuran and Hall, 1979). In herbaceous stem cuttings of chrysanthemum, stomata were "fully" closed within 30 minutes after excision and preparation for sticking (Orton, 1979). Stomatal closure reduces transpiration but does not eliminate the loss of water from stem cuttings. Recognition of this phenomenon has formed the basis for environmental controls, such as intermittent mist systems, used in propagation to prevent wilting and to enhance rooting of cuttings (Hess and Snyder, 1955; Slezinski and Davidson, 1973).

Water Uptake by Stem Cuttings.

Although limited by internal resistances and the lack of roots, cut stems absorb water from the propagation medium (Grange and Loach, 1983). The tensions created in a cutting by transpirational and cuticular water loss, as noted by Evans (1952), are multiplied as the water supply becomes limited. Propagators have recognized this; thus, maintenance of a moist but not overwet medium is a standard propagation procedure (Hartmann and Kester, 1983). Using gravimetric methods, Grange and Loach (1983) found that transpiration exceeded uptake when the leaf-to-air vapor pressure deficit exceeded 0.1 kPa. Under high irradiance (greater than 100 $W \bullet m^{-2}$) water deficits developed even in cuttings inserted into water alone. Uptake rates were increased by removing a 5 mm section of stem from the cutting base, substantiating Evans' (1952) observation that uptake is hindered by the formation of stem blockages at or near the severed end. Grange and Loach (1983) found that despite these blockages, all cuttings rehydrated overnight, when transpirational demand was minimal. However, if the propagation medium is insufficiently moist, full hydration may not be reached because stem resistances cannot be overcome.

Water uptake by stem cuttings may be directly proportional to the volumetric water content of the propagation medium (Loach, 1985). Incomplete contact of the cutting base with the film of water surrounding medium particles may be a major source of uptake resistance. The practice of wounding the stem cutting base may decrease this contact resistance, but has no effect on the internal stem resistance (Grange and Loach, 1983).

Water vs. Oxygen in the Propagation Medium.

Although the stem base must be provided ample moisture, the availability of oxygen must also be maintained or cuttings will produce few or no roots (Evans, 1952; Gislerød, 1983; Soffer and Burger, 1988). Such a balance is difficult to achieve (Loach, 1985; Tilt and Bilderback, 1987). Because oxygen is much less soluble in water than in air (Guttormsen, 1974), the availability of oxygen to the base of a stem cutting is primarily determined by the ratio of air to water in the propagation medium (Gislerød, 1983). The quality of roots produced increases with the availability of water if the oxygen supply is above a threshold level in the propagation medium (Long, 1933). The minimum concentration varies with species, from one part per million (ppm, in water) for *Salix* to four ppm for *Hedera helix* (Zimmerman, 1930). Air concentrations less than 15 % (by volume) in the propagation medium were detrimental to rooting of carnation and chrysanthemum (Gislerød, 1983).

Maintaining turgid stem cuttings without overwetting the rooting medium creates a dilemma in intermittent mist propagation. Considering the resistances to water uptake, the wetter the medium the better; but as water fills medium pore spaces, less oxygen is available for adequate adventitious rooting (Loach, 1985). In his early work, Evans (1952) noted that a propagation medium cannot be defined without reference to its porosity properties as well as the frequency of water application. Porosity and other physical characteristics of propagation media influence the adventitious rooting process only through their direct effects on air and water availability (Long, 1933; Tilt and Bilderback, 1987).

Moisture levels in the rooting medium have been recognized as important to stem cutting survival and rooting but few quantitative data exist defining their optimum range (Tilt and Bilderback, 1987).

II. The Carbohydrate/Nitrogen Balance in Stem Cuttings

Introduction

Having been removed from a more complete source of water, mineral nutrients, photosynthate, and other growth substances, some metabolic adjustments should occur in a stem cutting in order to survive. In the previous section on stem cutting water relations, it was shown that stomatal conductance may decline as a response to water stress. Stomatal closure not only reduces transpirational water loss but may reduce gas exchange and net photosynthetic rates in cuttings, as shown in *Populus* (Eliasson and

Brunes, 1980). Photosynthetic carbon fixation has been found to decrease to a level just above the compensation point until root emergence in *Pinus radiata* cuttings (Cameron and Rook, 1974). Respiration rates may increase over 40 percent over the same period. The internal balance of carbohydrates and rooting promoters or inhibitors in the cutting may be altered in response to excision from the parent plant.

For many years, propagators were encouraged to maintain high carbohydrate levels in cuttings by limiting fertility, especially nitrogen (N) nutrition, of the stock plants which supply those cuttings (Dirr and Heuser, 1987; Haissig, 1986; Hartmann and Kester, 1983). There are practical as well as physiological problems with this approach. All nutrients essential to plants are important to adventitious rooting, so emphasizing carbohydrate and nitrogen alone may be misleading (Dirr and Heuser, 1987). Carbohydrate levels may not be as important as the availability of carbohydrates to the site of root primordia development (Breen and Muraoka, 1974; Haissig, 1984; Veierskov et al.,1982). Because net photosynthetic (Pn) rates are often lower in cuttings than in the parent (stock) plant, carbohydrate levels must be adequate for rooting (Haissig, 1986). Nitrogen must be readily available in the stock plant for optimal shoot growth for efficient stem cutting production (Grange and Loach, 1985). Not only N but light levels and the internal balance of other growth substances (such as auxin) may interact in determining carbohydrate metabolism after the cutting has been excised from the parent plant (Altman and Wareing, 1975; Davis and Potter, 1981; Grange and Loach, 1985; Haissig, 1982). Finally, the simplest route to controlling the ideal C : N balance -- through appropriate N fertilization regimes (Hartmann and Kester, 1983) -- has yet to be defined for the propagator (Dirr and Heuser, 1987; Andersen, 1986).

Carbohydrates and their Role in Adventitious Rooting.

A number of studies have indicated that adventitious rooting ability is positively correlated with the carbohydrate content of the cutting (Ali and Westwood, 1966; Breen and Muraoka, 1974; Reuveni and Adato, 1974). Early investigators of stem cutting propagation found that a high carbohydrate : N ratio in cuttings was correlated with rooting success (Kraus and Kraybill, 1918; Pearse, 1943). If a cutting is completely girdled above its base prior to insertion in the propagation medium, little or no root initiation occurs below the girdle. Stoltz (1968) found that the substances which accumulate above the girdle were primarily carbohydrates, amino acids, and an unidentified root-promoting oxygenated terpenoid. Of all these substances, carbohydrates have been given the most study, probably because they are the most easily identified and quantifiable factor found in the cutting.

Although some studies have positively correlated total extractable (nonstructural) carbohydrate levels in the cutting with rooting success, other studies imply that particular carbohydrates, their location in the stem cutting, and their translocation to the site of root initiation may determine rooting success more than total nonstructural carbohydrate (TNC) levels. In ^{14}C labeling studies with 'Marianna 2624' plum, Breen and Muraoka (1974) found that, over time in the propagation bench, TNC concentrations decreased in the midstem and base of softwood cuttings until only 13 percent of the original carbohydrate concentrations remained. Haissig (1984) compared specific carbohydrate changes in Jack pine (*Pinus banksiana*) seedlings with those of seedling cuttings. Over time, cuttings had a higher TNC concentration than seedlings. The terminal portion of cuttings showed the highest net rate of TNC accumulation; reducing sugars accumulated most in the base of cuttings. Starch levels were highest in cuttings. Veierskov et al. (1982) found similar results with cuttings and intact plants of 'Alaska' pea. They also observed little top growth of cuttings compared to intact plants, and concluded that this may have allowed greater carbohydrate accumulation in cuttings. In all three examples, the terminal portion of cuttings contained the highest TNC concentrations at the end of the experiment. TNC may not only be redistributed within the cutting but may be metabolized to form insoluble molecules such as cellulose, lignins, proteins, and nucleic acids (Haissig, 1982). The basal segment of cuttings, the zone of adventitious rooting, is apparently not a strong sink for carbohydrate until roots actually develop (Breen and Muraoka, 1974; Haissig, 1984; Veierskov et al., 1982).

Although TNC concentration in basal segments of Jack pine stem cuttings were positively related to root development, Haissig (1982) found no obvious cause-effect relationships. Because carbohydrates may act as both metabolites and modifiers of cell osmotic potential, their role in adventitious rooting is still not fully understood. Total nonstructural carbohydrate content of stem cuttings of the leguminous tree *Prosopis alba* was not significantly correlated with rooting success (percent of cuttings rooted) (De Souza and Felker, 1986). The authors suggested that cuttings from legumes may differ from nonlegumes in their carbohydrate : N requirement for rooting.

The Role of Current vs. Stored Photosynthate.

Most cuttings do not require high light levels until after roots have formed. Low Pn rates were found in the cuttings of a number of woody plants, such as aspen (Eliasson and Brunes, 1980), dogwood and rhododendron (Gay and Loach, 1977), and pine

(Cameron and Rook, 1974). If Pn rates remain close to the compensation point, carbohydrates present in the cutting at the time of excision from the stock plant would have to be sufficient for the requirements of adventitious rooting. Breen and Muraoka (1974) found that less than five percent of photosynthetically-fixed ^{14}C was translocated to the rooting zone prior to root emergence. Cuttings of *Pinus radiata* (Cameron and Rook, 1974), and poplar (Okoro and Grace, 1976) which exhibited low Pn rates rooted as well as cuttings with Pn rates that had been increased by supplemental lighting. Twenty-fold increases in photosynthetically-active radiation (PAR), despite significantly decreasing leaf water potential, increasing the net photosynthetic rate, and increasing the sucrose and starch levels in the basal segment of cuttings, failed to affect rooting percentages of a slow-to-root rhododendron cultivar (Davis and Potter, 1987).

There is evidence that some leafy woody and herbaceous cuttings benefit from increased net rates of photosynthesis for the rooting process. Increasing light intensities during propagation has resulted in increased rooting percentages of leafy pea cuttings (Davis and Potter, 1981; Eliasson, 1978). Davis and Potter (1981) found that reduced photosynthetic rates were correlated with a decline in both rooting and carbohydrate levels. The production of "current" photosynthate may be beneficial to the adventitious rooting of some species, especially if shoot growth becomes a competing sink for carbohydrate in the unrooted cutting.

The Role of Nitrogen in Carbohydrate Metabolism in Cuttings.

Assimilation of inorganic N by plant tissue is usually a carbohydrate-utilizing process. Amino acid synthesis requires intermediates ultimately derived from carbohydrate reserves. It has therefore been assumed that the amount of carbohydrate stored in stem tissue can be manipulated by the level of inorganic nitrogen applied to a plant as fertilizer. Many years ago Kraus and Kraybill (1918) concluded that tomato cuttings rooted better if they had a relatively high carbohydrate : N ratio. The basic assumption has since been that sufficient or excessive nitrogen for shoot growth will generally diminish subsequent adventitious rooting of stem cuttings (Haissig, 1986).

Early work with grape (*Vitis vinifera*) cuttings, taken from stock plants grown in sand culture and fed with solutions of different nutrient concentrations, indicated that dormant cuttings taken from "starved" or "semi-starved" vines responded better to IBA (1*H*-Indole-3-butyric acid) treatment and rooted more easily than cuttings taken from adequately- or over-fed stock vines (Pearse, 1943). Later, Haun and Cornell (1951) reported that low and medium N levels (1 and 3 meq. $N \bullet liter^{-1}$) applied to geranium stock plants grown in sand culture produced cuttings which had higher rooting percentages than a higher (9 meq. $N \bullet liter^{-1}$) treatment. Varied levels of phosphorus (as Na_2PO_4) or potassium (as K_2SO_4) had no effect on rooting. In a similar experiment with azalea, Preston et al. (1953) found that cuttings taken from stock plants receiving low N levels (1.5 and 3 mmol $N \bullet liter^{-1}$) rooted better than those from plants receiving a higher N level (6 mmol $N \bullet liter^{-1}$). Again, varied levels of phosphorus or potassium had no effect on the rooting response.

A low N fertilization regime is not invariably associated with ease of rooting (Hartmann and Kester, 1983). Carbohydrate and nitrogen levels vary within a plant according to tissue age and stem region. An actively growing terminal stem cutting would be lower in carbohydrate reserves than a cutting taken from further down the shoot, but both cuttings may root similarly (Dirr and Heuser, 1987). Cuttings taken from young, succulent growth on azalea responded to N treatments differently than cuttings taken from more mature, hardened stem tissue (Preston et al., 1951). Mature cuttings taken from high N-treated stock plants rooted better than the same tissue from low N-treated stock. Succulent cuttings taken from low N-treated stock rooted better than succulent stem tissue taken from high N-treated stock. Overall, succulent cuttings survived less than mature cuttings. With *Prosopis alba* cuttings, there was a significant positive correlation between stem N concentration and the percentage of rooted cuttings (De Souza and Felker, 1986). This indicates that stock plant fertilization regimes may be varied according to the type of plant, the time of year, and growth stage of shoots.

Whether the stem cutting base -- the site of adventitious root formation -- can compete with the leaves and terminal meristem for carbohydrate may be largely determined by a complex interaction of light, nitrogen nutrition, carbohydrate reserves, and auxin levels within the stock plant. The strength of the basal rooting zone "sink" may be diminished by high nitrogen fertility, which tends to strengthen the leaf- and terminal-sink since these are the sites of greatest inorganic nitrogen breakdown. In whole plants, such as 'Helleri' holly, the ratio of shoot dry weight to root dry weight increases with increasing concentrations of applied nitrogen fertilizer (Yeager and Wright, 1981). In cuttings, the basal sink is strengthened after root initiation. Synthetic auxin (as IBA) treatments appear to further increase the strength of this sink in cuttings (Altman and Wareing, 1975; Blazich et al., 1983; Breen and Muraoka, 1974; Haissig, 1982).

An inverse relationship between nitrogen and carbohydrate contents in stem tissue may not always determine successful adventitious rooting of cuttings. Unfertilized plants may retain higher leaf and stem carbohydrate levels than fertilized plants, and this may be controlled by growth, which utilizes carbohydrate reserves. Increasing the level of nitrogen within a plant increases the shoot growth, strengthening it as a sink for carbohydrate and diluting the carbohydrate reserves. Sometimes cuttings taken from the shoots of such plants will root, and sometimes they will not (Ali and Westwood, 1966). There may be wide variations in rooting percentages from cuttings of different yet clonal stock plants given the same treatment and taken the same time of year, implying an additional factor not considered and not controlled (De Souza and Felker, 1986).

Nitrogen is required for the adventitious rooting process, so it must be provided at some baseline concentration in the cutting. Much of the evidence presented here has shown that the excessive shoot growth provided by high nitrogen fertility is generally detrimental to rooting. However, efficient numbers of cuttings must be provided by each stock plant. Considering that stock plants are often the same plants being grown for later sale, they often receive the same heavy fertilization regime required in the efficient production of nursery stock. Furthermore, the variation in response between plants, and the few species tested, warrants further investigation into the lower and upper limits of nitrogen nutrition and their effects on adventitious rooting of a number of commercial woody plants.

Literature Cited

Ali, N. and M.N. Westwood. 1966. Rooting of pear cuttings as related to carbohydrates, nitrogen, and rest period. Proc. Amer. Soc. Hort. Sci. 88:145-150.

Altman, A., and P.F. Wareing. 1975. The effect of IAA on sugar accumulation and basipetal transport of ^{14}C-labeled assimilates in relation to root formation in *Phaseolus vulgaris* cuttings. Physiol. Plantarum 33:32-38.

Andersen, A.S. 1986. Environmental influences on adventitious rooting in cuttings of nonwoody species. pp 223-250. In: M.B. Jackson, ed. *New Root Formation in Plants and Cuttings.* Martinus Nijhoff Pub., Dordrecht.

Blazich, F.A., R.D. Wright, and H.E. Schaffer. 1983. Mineral nutrient status of 'Convexa' holly cuttings during intermittent mist propagation as influenced by exogenous auxin application. J. Amer. Soc. Hort. Sci. 108:425-429.

Breen, P.J. and T. Muraoka. 1974. Effect of leaves on carbohydrate content and movement ^{14}C-assimilate in plum cuttings. J. Amer. Soc. Hort. Sci. 99:326-332.

Cameron, R.J. and D.A. Rook. 1974. Rooting stem cuttings of Radiata pine: Environmental and physiological aspects. N. Z. J. For. Sci. 4:291-298.

Davis, T.D., and J.R. Potter. 1981. Current photosynthate as a limiting factor in adventitious root formation on leafy pea cuttings. J. Amer. Soc. Hort. Sci. 106:278-282.

Davis, T.D. and J.R. Potter. 1987. Physiological response of rhododendron cuttings to different light levels during rooting. J. Amer. Soc. Hort. Sci. 112:256-259.

De Souza, S.M. and P. Felker. 1986. The influence of stock plant fertilization on tissue concentrations of N, P, and carbohydrates and the rooting of *Prosopis alba* cuttings. For. Ecol. Mgt. 16:181-190.

Dirr, M.A. and C.W. Heuser. 1987. *The Reference Manual of Woody Plant Propagation.* Varsity Press, Athens, GA.

Eliasson, L. 1978. Effects of nutrients and light on growth and root formation in *Pisum sativum* cuttings. Physiol. Plantarum 43:13-18.

Eliasson, L. and L. Brunes. 1980. Light effects on root formation in aspen and willow cuttings. Physiol. Plantarum 48:261-265.

Elstrodt, C.J. and D.C. Milbocker. 1983. Improving propagation with vhhp. Nursery Business 8:36-38,55.

Evans, H. 1952. Physiological aspects of the propagation of cacao from cuttings. Rpt. XIII Intl. Hort. Congr. 2:1179-1190.

Gay, A.P. and K. Loach. 1977. Leaf conductance changes on leafy cuttings of *Cornus* and *Rhododendron* during propagation. J. Hort. Sci. 52:509-516.

Gislerød, H.R. 1983. Physical conditions of propagation media and their influence on the rooting of cuttings. Plant and Soil 75:1-14.

Grange, R.I. and K. Loach. 1983. The water economy of unrooted leafy cuttings. J. Hort. Sci. 58:9-17.

Grange, R.I. and K. Loach. 1985. The effect of light on the rooting of leafy cuttings. Scientia Hort. 27:105-111.

Guttormsen, G. 1974. Effects of root medium and watering on transpiration, growth, and development of glasshouse crops I.Effects of compression at varying water levels on physical state of root media and on transpiration and growth of tomatoes. Plant and Soil 40:65-81.

Haissig, B.E. 1974. Metabolism during adventitious root primordium initiation and development. N. Z. J. For. Sci. 4:324-337.

Haissig, B.E. 1982. Carbohydrate and amino acid concentrations during adventitious root primordium development in *Pinus banksiana* Lamb. cuttings. For. Sci. 28:813-821.

Haissig, B.E. 1984. Carbohydrate accumulation and partitioning in *Pinus banksiana* seedlings and seedling cuttings. Physiol. Plantarum 61:13-19.

Haissig, B.E. 1986. Metabolic processes in adventitious rooting of cuttings. pp.154-193. In: M.B. Jackson, ed. *New Root Formation in Plants and Cuttings.* Martinus Nijhoff Pub., Dordrecht.

Hartmann, H.T. and D.E. Kester. 1983. *Plant Propagation, Principles and Practices.* 4th ed. Prentice-Hall, Englewood Cliffs, N.J.

Haun, J.R. and P.W. Cornell. 1951. Rooting response of geranium as influenced by nitrogen, phosphorus, and potassium nutrition of the stock plant. Proc. Amer. Soc. Hort. Sci. 58:317-323.

Hess, C.E. and W.M. Snyder. 1955. A physiological comparison of the use of mist with other propagation procedures used in rooting cuttings. Rpt. XIV Intl. Hort. Congr. 2:1133-1139.

Hsaio, T.C. 1973. Plant responses to water stress. Annu. Rev. Plant Physiol. 24:519-570.

Kramer, P.J. 1983. *Water Relations of Plants.* Academic Press, Orlando, Fl.

Loach, K. 1977. Leaf water potential and the rooting of cuttings under mist and polythene. Physiol. Plantarum 40:191-197.

Loach, K. 1985. Rooting of cuttings in relation to the propagation medium. Proc. Intl. Plant Prop. Soc. 35:472-485.

Long, J.C. 1933. The influence of rooting media on the character of roots produced by cuttings. Proc. Amer. Soc. Hort. Sci. 29:352-355.

Okoro, O.O. and J. Grace. 1976. The physiology of rooting *Populus* cuttings: I. Carbohydrates and photosynthesis. Physiol. Plantarum 36:133-138.

Orton, P.J. 1979. The influence of water stress and abcisic acid on the root development of *Chrysanthemum morifolium* cuttings durring propagation. J. Hort. Sci. 54:171-180.

Pearse, H.L. 1943. Effect of nutrients and phytohormones on rooting of vine cuttings. Amer. J. Bot. n.s. 7:123-132.

Preston, W.H., J.B. Shanks and P.W. Cornell. 1951. Influence of mineral nutrition on production, rooting, and survival of cuttings of azaleas. Proc. Amer. Soc. Hort. Sci. 61:499-507.

Rajagopal, V. and A.S. Andersen. 1980. Water stress and root formation in pea cuttings. Physiol. Plantarum 48:144-149.

Reuveni, O. and I. Adato. 1974. Endogenous carbohydrates, root promoters, and root inhibitors in easy- and difficult-to-root date palm (*Phoenix dactylifera* L.) offshoots. J. Amer. Soc. Hort. Sci. 99:361-363.

Sivakamuran, S. and M.A. Hall. 1979. Hormones in relation to stress recovery in *Populus robusta* cuttings. J. Exp. Bot. 30:53-63.

Slezinski, J. and H. Davidson. 1973. Vapor pressure deficit and polyethylene in plant propagation. Proc. Intl. Plant Prop. Soc. 23:238-241.

Snyder, W.E. and C.E. Hess. 1955. An evaluation of the mist technique for the rooting of cuttings as used experimentally and commercially in America. Rpt. XIV Intl. Hort. Congr., pp.1125-1132.

Soffer, H. and D.W. Burger. 1988. Effects of dissolved oxygen concentrations in aerohydroponics on formation and growth of adventitious roots. J. Amer. Soc. Hort. Sci. 113:218-221.

Stoltz, L.P. 1968. Factors influencing root initiation in an easy- and difficult-to-root chrysanthemum. Proc. Amer. Soc. Hort. Sci. 92:622-626.

Struve, D.K. 1981. The relationship between carbohydrates, nitrogen, and rooting of stem cuttings. The Plant Prop. 27(2):6-7.

Tilt, K.M. and T.E. Bilderback. 1987. Physical properties of propagation media and their effects on rooting of three woody ornamentals. HortScience 22:245-247.

Veierskov, B., A.S. Anderson, and E.N. Eriksen. 1982. Dynamics of extractable carbohydrates in *Pisum sativum* I. Carbohydrate and nitrogen content in pea plants and cuttings grown at two different irradiances. Physiol. Plantarum 55:167-173.

Yeager, T.H. and R.D. Wright. 1981. Influence of nitrogen and phosphorus on shoot:root ratio of *Ilex crenata* Thunb. 'Helleri'. HortScience 16:564-565.

Zimmerman, P.W. 1930. Oxygen requirements for root growth of cuttings in water. Amer. J. Bot. 17:842-861.

Chapter Two: Propagation Medium Moisture Content and the Water Relations and Adventitious Rooting of Stem Cuttings

Abstract

Stem cuttings of Blue Rug juniper (*Juniperus horizontalis* Moench 'Wiltonii'), Hino-Crimson azalea [*Rhododendron* (Lindl.) Planch 'Hino-Crimson'], and Helleri holly (*Ilex crenata* Thunb. 'Helleri') were propagated in 1:1 peat/perlite (v/v) at one of five moisture levels based on medium dry weight (125 %, 250 %, 375 %, 500 %, and 625 %). In all three species, cutting survival and percentage of rooted cuttings increased with increasing medium moisture content. Incidence of cutting basal rot was not directly related to medium moisture content, but was related to species and growth stage of the stock plant. Midday xylem water potential of cuttings usually increased with increasing percent moisture in the propagation medium. During propagation, stem cutting water potential below - 2.0 MPa occurred even in the wettest medium tested, and frequently reached - 4.0 MPa in cuttings in the driest treatment (125 %). Basal water uptake by cuttings was increased with increasing medium moisture level. It was highest during the first few days after insertion, and thereafter decreased until root emergence. Stem cutting propagation of all three species was most successful with the wettest medium tested (625 %).

Introduction

Intermittent mist systems are commonly used in plant propagation to reduce transpirational water loss from cuttings by periodically wetting the leaves. A problem with this system is maintaining a wet leaf surface without overwetting the rooting medium. Water and air compete for pore space in a medium (Loach, 1985), so oxygen availability is reduced as the volume of water is increased. The availability of oxygen in the propagation medium must be maintained or rooting will be limited (Evans, 1952; Gislerød, 1983; Soffer and Burger, 1988). Anaerobic conditions may increase the likelihood of basal rotting of stem cuttings, a common cause of propagation failure.

Cut stems will absorb water from the propagation medium, despite limitations to water movement by internal stem resistances and the lack of roots (Grange and Loach, 1983). Water uptake by stem cuttings may be directly proportional to the water content of the propagation medium (Loach, 1985). If transpiration exceeds water uptake, cutting turgidity will be reduced. Cuttings may rehydrate to full turgidity overnight -- when the vapor pressure deficit (vpd) is normally reduced -- provided the propagation medium is sufficiently moist (Grange and Loach, 1983). Incomplete contact of the stem cutting base with the film of water surrounding the medium particles can be a major source of uptake resistance. If the propagation medium is insufficiently moist, full hydration of cuttings may not occur, limiting propagation success.

Evans (1952) noted that a propagation medium cannot be defined without reference to its porosity properties as well as the frequency of water application. Studies of various media have revealed that physical characteristics such as particle size are less deterministic of rooting performance than are air and water content (Long, 1933; Tilt and Bilderback, 1987). Because few quantitative data exist defining the optimum levels of moisture in a propagation medium (Tilt and Bilderback, 1987), propagators face a dilemma in providing adequate but not excessive moisture to stem cuttings both above and below the medium surface.

The objective of this study was to determine the influence of a range of moisture levels in the propagation medium on the water potential, adventitious rooting, quality, and survival of stem cuttings of Blue Rug juniper, Hino-Crimson azalea, and Helleri holly.

Materials and Methods

Treatment establishment. Five medium moisture levels -- 125%, 250%, 375%, 500%, and 625% (w/w) -- were established for a steam-pasteurized 1:1 (v/v) mix of sphagnum peat moss and coarse perlite that had been sieved to pass through a 5.7 mm mesh screen. Individual 29 mm × 104 mm (50 ml) translucent plastic centrifuge tubes (Nalge

Co., div. Sybron Corp., Rochester, N.Y.) were filled with the medium with uniform compaction. Water was applied gravimetrically to each tube until a specific moisture level was reached. Clear polyvinyl film (Polyvinyl Films, Inc., Wilkinsonville, Mass.) was placed over each tube opening and sealed with a strip of Parafilm® 'M' (American National Can Co., Greenwich, Conn.) tape to eliminate evaporation from the medium. Cuttings were inserted into the tubes as described below for each species.

To maintain the medium moisture levels, each cutting-tube unit was weighed every four days, and water was injected through the Parafilm® into the medium with a syringe to compensate for water loss. To differentiate between water losses due to cutting uptake and evaporative losses from from the medium, 0.5 cm × 12 cm wooden dowels, treated like stem cuttings, were placed in randomized complete blocks among the cuttings on the propagation bench. Therefore, stem cutting water uptake data is the amount of water lost from the cutting-tube unit minus that from the dowel-tube unit at each date.

Comparison of the tested range of medium moisture with typical propagation conditions. Tubes of the peat/perlite medium, if placed under intermittent mist (on-time 8 sec every 8 min) with tops left uncovered and the tube bases perforated for drainage, would equilibrate at 400 % to 500 % moisture by dry weight. Therefore, the highest tested medium moisture level, 625 %, is greater than the level of moisture found in the same medium at an equivalent depth and volume under standard intermittent mist conditions.

Blue Rug juniper. On 27 February 1989, 15 cm long stem tip cuttings were taken from *Juniperus horizontalis* 'Wiltonii' (Blue Rug juniper) growing in a residential landscape in Blacksburg, Va. The cuttings were trimmed acropetally to 13 cm, leaves removed from the basal 6 cm, and their basal 2.5 cm were dipped into a 50 percent ethanol solution of 5000 mg IBA (1*H*-indole-3-butyric acid) •liter^{-1} for 5 sec. A 10 cm square of Parafilm® was wrapped skirtwise around each cutting, approximately 5 cm from its base, before inserting the cutting's basal 4 cm into the tubes of propagation medium. The "skirt" reduced water leakage into and evaporation from the tubes, while allowing air exchange within the medium. The cutting-tube units were weighed and then placed in randomized complete blocks inside a walk-in high-humidity propagation tent constructed within the glasshouse. The tent was covered with 4 mil clear polyethylene film supported by 3 hoops of 1.3 cm o.d. pvc tubing bent to form a quonset. Relative humidity was maintained at or near 100 % during daylight hours by three venturi-type pressurized atomizing nozzles (Delavan model 3060). Nozzle air pressure was maintained at 22 psi and water was provided through 5 mm i.d. tubing inserted into a plastic carboy. There were five treatments per block, with thirty blocks of cuttings and five dowel-tube control blocks.

Midday (1200 to 1400 hr) xylem water potential was measured on two dates, with a pressure chamber (Scholander et al., 1965). Stem cuttings were destructively sampled from five blocks. Prior to insertion in the chamber, one cm of stem tissue was removed from the cutting base to eliminate variation due to internal stem blockages which may develop over time (Grange and Loach, 1983). Stem cutting survival, the percentage of rooted cuttings, the number and length of roots, and the occurrence of basal rot were evaluated 24 April, the termination of this experiment.

'Hino-Crimson' azalea. On 21 June, 12.6 cm long stem tip cuttings of *Rhododendron* 'Hino-Crimson' (Hino-Crimson azalea) were taken from container plants at a Suffolk, Va. nursery, and were placed on ice and transported overnight to Blacksburg. On 22 June the cuttings were unpacked, trimmed acropetally to 10 cm, and stripped of leaves

along the basal 6 cm of stem. They were basally dipped into a 50 percent ethanol solution of 1250 mg IBA • liter^{-1}. The medium moisture treatments were applied as in the juniper experiment. The tubes were weighed every five days to determine water loss, and cuttings were evaluated for survival, basal rot, and rooting eight weeks after insertion (18 August). The cuttings were placed in a randomized complete block design with 28 blocks of cuttings and five dowel-tube control blocks.

'Helleri' holly. On 29 March 1989, 12 cm stem tip cuttings of *Ilex crenata* Thunb. 'Helleri' were taken from container plants growing in a commercial nursery in Suffolk, VA., placed on ice in a cooler, and transported to Blacksburg, VA. On 31 March, the cuttings were trimmed to 11.5 cm, their leaves stripped from the basal 4.5 cm of stem, dipped basally into 5000 mg IBA • liter^{-1} for 5 sec, and treated as in the above experiments. There were 26 blocks of cuttings, and four dowel-tube control blocks. Water potential was measured only once -- 14 April -- because these cuttings initiated roots within two weeks. This experiment was repeated on 24 April, when stem cuttings were taken from a landscape planting in Blacksburg. Water potential was measured on four blocks of cuttings once every six days until rooting was evaluated (25 May). The experiment was repeated again on 24 August, when stem cuttings were taken from 3 gallon container plants at a commercial nursery in Piney River, VA. Water potential was measured once, and the experiment was terminated 21 Sept.

Statistical analyses. Percentage data were transformed via arcsin [square root (percentage)] prior to statistical analysis. To detect trends in tabular data, regression coefficients were tested for significant addition to the full polynomial model via the General Linear Models procedure of SAS® (SAS Institute, Cary, N.C.). Best-fit regression models are presented in the figures.

Results and Discussion

Stem cutting quality and rooting percentages. In most cases, percent survival and adventitious rooting of stem cuttings increased as percent moisture in the propagation medium increased (Tables 2.1 and 2.2). Survival was high in juniper at all medium moisture levels, with 77 percent of cuttings in the lowest medium moisture level (125 %) alive and green eight weeks after insertion (Table 2.1). It is possible that the surviving cuttings may have rooted later; it is not unusual for juniper cuttings to take 12 weeks to root. Generally, few cuttings of the azalea and Japanese holly cultivars tested survived the lowest moisture treatment. One exception is the 24 August 'Helleri' trial, where 79 % of the cuttings in the 125% treatment were alive after three weeks. In this case, survival percentages would have probably been reduced if the cuttings were left under propagation conditions for a longer period before evaluation.

The variability in rooting percentages between species was expected. Stem cuttings of juniper are generally the slowest and least predictable to root; those of 'Helleri' holly the quickest and most dependable. However, the repeated experiments with 'Helleri' holly illustrated that rooting success can vary with time of year or growth stage of the stock plants (Table 2.2). 'Helleri' holly growth occurs in flushes throughout the growing season; it is usually recommended to take these cuttings between the periods of shoot growth (Dirr and Heuser, 1987). Within this cultivar the highest rooting percentages dropped from approximately 90 percent for dormant cuttings taken 31 March and 27 April, to only eight percent for cuttings taken in August, when a shoot flush was beginning. The latter stage of growth is considered limiting to adventitious rooting success (Dirr and Heuser, 1987).

In no case was the incidence of basal rot significantly related to the percentage of moisture in the propagation medium (Tables 2.1 and 2.2). Instead, basal rot seemed related more to species and time of year. For example, basal rot of 'Helleri' holly cuttings taken in August was much greater than on those taken in March or April (Table 2.2). Increasing the percentage of water in the propagation medium seemed to aggravate basal rotting of the August 'Helleri' cuttings, but trends were not significant. Even though 75 to 88 percent of these cuttings appeared green and alive across all treatments, rotting of the stem base had occurred on most of them (Table 2.2).

Water potential. Midday xylem water potential of stem cuttings increased as percent medium moisture increased from 125 % to 625 % (Figs. 2.1, 2.2, and 2.3). The water potential of cuttings which had as few as one or two roots was much higher than the water potential of unrooted cuttings (data not shown). Apparently, water stress was reduced as water uptake resistance was reduced through rooting.

Variation in stem cutting water potential increased with time. In most cases, R-square values became much lower at the later dates for water potential measurement, especially in azalea (Fig. 2.2) and holly (Fig. 2.3). This could be due to increasing entropy as water stress is prolonged in unrooted cuttings, or to the rooting of some but not all cuttings within a treatment, as noted above.

Even at the highest medium moisture percentage, stem cutting water potential became quite negative, often much below what is considered the turgor loss point for a plant (-1.5 to -2.0 MPa). The water potential of juniper cuttings at all treatment levels was quite low relative to the other species. Apparently, juniper maintains a relatively low water potential even as a mature, rooted plant. Pre-insertion stem cutting water potential averaged - 1.52 MPa (mean of five cuttings) for Blue Rug juniper and only - 0.36

MPa and - 0.32 MPa (mean of four cuttings) for 'Helleri' holly and 'Hino-Crimson' azalea, respectively. Within five days after insertion into the medium, xylem water potential of azalea and holly cuttings usually fell to levels as low as those measured at later dates. This occurred most often with cuttings propagated in media at the lowest percentage of moisture. Using thermocouple psychrometry, Loach (1977) found that the mean leaf water potential of rhododendron stem cuttings propagated under intermittent mist decreased from - 1.37 MPa three days after insertion to - 1.97 MPa 44 days after insertion. In the present experiment, water potential of cuttings at all medium moisture levels were more negative than those found by Loach. Differences between our studies may be due to differences in propagation conditions and species tested. Leaf and air temperatures may be much greater under a high humidity propagation system than under intermittent mist (Hess and Snyder, 1955; Slezinski and Davidson, 1973). Evaporative cooling is probably greater under intermittent mist, allowing stem cutting water stress to be reduced and stem cutting water potential to be greater than it would be under a high humidity system.

Water uptake by stem cuttings. Stem cuttings in the wettest medium generally showed the greatest amount of water uptake (Tables 2.3 and 2.4). This is in agreement with the findings of Grange and Loach (1983) that water uptake by stem cuttings is limited by the availability of water to the cut stem base. Our data also show that water uptake by cuttings is greatest during the first four days of propagation, followed by a period of lower water absorption from the propagation medium until adventitious rooting occurred. The latter increase in uptake by 'Helleri' holly cuttings occurred between the third and fourth week after insertion, corresponding to the period in which rooting was noted. The increase in uptake occurred earliest in cuttings in the wettest medium, and was so abrupt in juniper that it seemed possible to detect the occurrence of root emergence by the periodic weighing of the cutting-tube units.

The high initial water uptake may be due to the continued transpirational water loss through stomata that have not yet responded to the stress of propagation. Evans (1952) and later Grange and Loach (1983) noted that water uptake by cut stems may become restricted by gum-like residues which form near the stem base shortly after excision from the stock plant. This event may have contributed to the reduction in uptake measured after the first four days of propagation. This may further contribute to the reduction in stem cutting water potential over time.

Final Discussion

The level of moisture in a propagation medium influences the ability of a stem cutting to absorb water and produce adventitious roots. The availability of moisture in a propagation medium at least partially controls the ability of a stem cutting to absorb enough water to offset transpirational and nonstomatal water losses. A standard 1 : 1 peat/perlite medium under intermittent mist may contain 400 % to 500 % moisture on a dry weight basis. We found that stem cuttings inserted into a relatively wet propagation medium (625 %) exhibit greater water absorption, less negative water potential, and greater adventitious rooting percentages than cuttings in a medium at lower percent moisture levels.

An increase in contact between the cut stem base and the moisture in the medium probably accounted for greater water uptake by stem cuttings in the higher medium moisture treatments (Grange and Loach, 1983). The extent of this contact could control stem cutting water potential and adventitious rooting. Water potential was less negative in cuttings propagated in the highest medium moisture levels, probably because their water demands were more easily met. It is therefore possible to reduce water stress during propagation not only by reducing leaf-air vapor pressure deficits but also by maintaining high medium moisture levels. Limiting water stress in stem cuttings increases the possibility of adventitious rooting.

Within the first four days after insertion, the absorption of water by stem cuttings was highest until rooting occurred. It is not clear whether this is due to the greater transpirational demands of freshly cut stems or to the clogging of basal stem tissue over time. The application of greater quantities of water to stem cuttings during this period could help reduce water stress. This is actually the practice of many propagators.

Within the range of moisture and species tested here, a reduction in stem cutting quality due to basal rotting was not a direct result of high moisture levels in the propagation medium. The basal rotting of stem cuttings appeared to be most closely linked to the species and growth stage of the stock plant. However, the highest medium moisture level tested was probably not saturated to the point of anaerobiosis. Further study with a saturated medium with a known air content may be necessary to clarify the causes of basal rot. It is therefore suggested that successful stem cutting propagation requires a highly moist, but aerated, propagation medium.

Table 2.1. Quality and adventitious rooting percentages of stem cuttings propagated in a peat/perlite medium at five moisture levels.

Medium Moisture (%)	Survival (%)	Basal rot (%)	Rooting (%)
		Blue Rug Juniper	
125	77[z]	43	0
250	95	24	0
375	100	43	10
500	100	43	5
625	100	33	48
Regression[y]			
Linear	0.01	0.99	0.05
Quadratic	0.03	0.99	0.40
		Hino-Crimson Azalea	
125	13	0	0
250	19	0	0
375	81	0	19
500	100	0	38
625	100	0	75
Regression			
Linear	0.04	0.99	0.02
Quadratic	0.70	0.99	0.33

[z] Percentage data transformed via arcsin [square root (proportion)] prior to statistical analysis. For Blue Rug juniper, n = 21; for Hino-Crimson azalea, n = 16.

[y] Regression coefficients tested for significant addition to the polynomial model by backward selection F test; *P*-values presented.

Table 2.2. Quality and adventitious rooting percentages of 'Helleri' holly stem cuttings in a peat/perlite medium at five moisture levels.

Medium Moisture (%)	Survival (%)	Basal rot (%)	Rooting (%)
		31 March to 22 April 1989	
125	0[z]	0	0
250	74	0	26
375	96	0	74
500	91	0	74
625	100	0	92
Regression[y]			
Linear	0.05	0.99	0.02
Quadratic	0.18	0.99	0.18
		27 April to 26 May 1989	
125	30	0	20
250	80	20	60
375	90	0	60
500	100	20	90
625	100	20	70
Regression			
Linear	0.01	0.42	0.10
Quadratic	0.12	0.99	0.23
		24 Aug. to 21 Sept. 1989	
125	79	50	0
250	75	75	0
375	75	71	4
500	83	88	4
625	88	83	8
Regression			
Linear	0.04	0.09	0.05
Quadratic	0.06	0.37	0.91

[z] Percentage data transformed via arcsin [square root (proportion)] prior to statistical analysis. For 31 March, n = 27; 27 April, n = 10; 22 Aug., n = 24.

[y] Regression coefficients tested for significant addition to the polynomial model by backward selection F test; *P*-values presented.

Table 2.3. Water uptake by 27 April 'Helleri' holly stem cuttings from a 1:1 peat/perlite medium at five moisture levels.

Medium Moisture	Days after sticking					
	4	8	12	16	23	27
(%)			*Uptake (g)*			
125	0.16[z]	0.12	0.11	0.06	0.02	0.03
250	0.28	0.17	0.16	0.06	0.32	0.42
375	0.34	0.18	0.12	0.07	0.31	0.42
500	0.20	0.20	0.20	0.05	0.31	0.42
625	0.41	0.35	0.21	0.08	0.17	0.53
Regression[y]						
Linear	0.08	0.02	0.12	0.79	0.18	0.03
Quadratic	0.94	0.37	0.83	0.82	0.01	0.32
Cubic	0.08	0.39	0.90	0.72	0.43	0.26
Quartic	0.26	0.89	0.33	0.68	0.41	0.73

[z] Mean uptake of 10 cuttings, accumulated since previous date after sticking.

[y] Regression coefficients tested for significant addition to the polynomial model by backward selection F test; *P*-values presented.

Table 2.4. **Water uptake by 'Blue Rug' juniper stem cuttings from a 1 : 1 peat/perlite medium at five moisture levels.**

Medium Moisture	Days after sticking									
	4	8	12	16	24	28	32	38	42	46
(%)	*Uptake (g)*									
125	0.70[z]	0.15	0.23	0.28	0.32	0.21	0.18	0.14	0.14	0.22
250	0.50	0.19	0.19	0.18	0.36	0.19	0.23	0.25	0.11	0.28
375	1.11	0.38	0.46	0.39	0.59	0.32	0.33	0.52	0.50	0.62
500	1.04	0.34	0.52	0.51	0.77	0.42	0.34	0.39	0.29	0.51
625	2.18	0.42	0.67	0.92	1.16	0.82	0.62	1.34	1.25	1.87
Regression[y]										
Linear	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Quadratic	0.01	0.48	0.30	0.01	0.05	0.01	0.25	0.02	0.02	0.04
Cubic	0.26	0.88	0.10	0.98	0.93	0.52	0.43	0.05	0.14	0.17
Quartic	0.01	0.08	0.04	0.31	0.52	0.40	0.49	0.10	0.04	0.24

[z] Mean uptake of 16 cuttings, accumulated since previous date after sticking.

[y] Regression coefficients tested for significant addition to the polynomial model by backward selection F test; *P*-values presented.

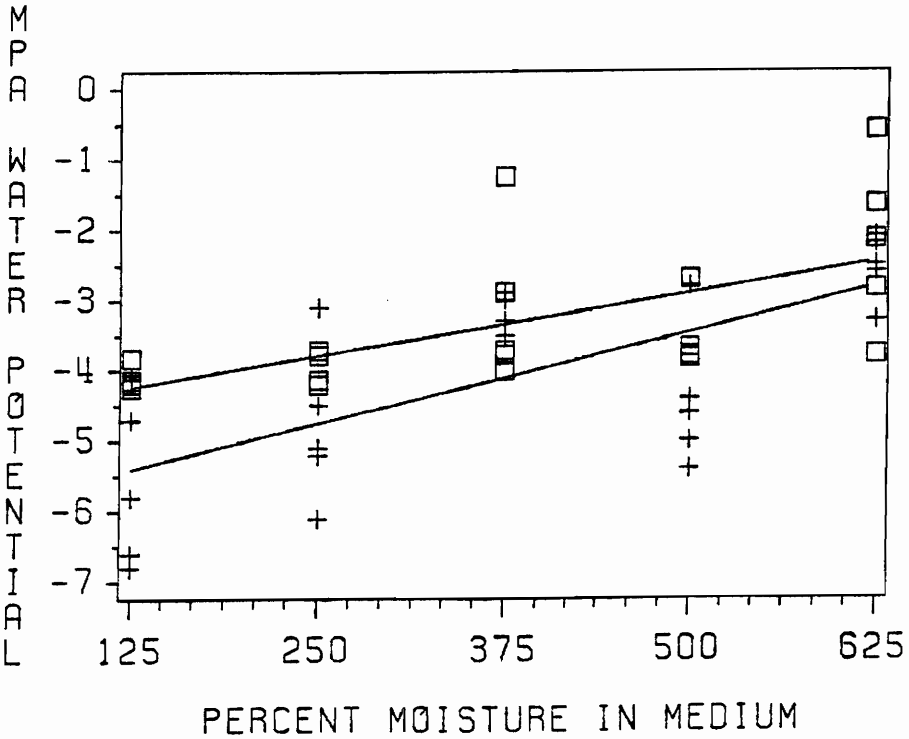


Figure 2.1. Water potential of Blue Rug juniper stem cuttings: 14 and 28 days after sticking into a 1:1 peat/perlite propagation medium maintained at five moisture levels. Five cuttings per moisture level were measured on each date. Regression equations: 14 day (+-+) $Y = -6.048 + 0.005x$, $R^2 = 0.45$, $p = 0.0002$; 28 day (□-□) $Y = -4.667 + 0.0035x$, $R^2 = 0.41$, $p = 0.0006$.

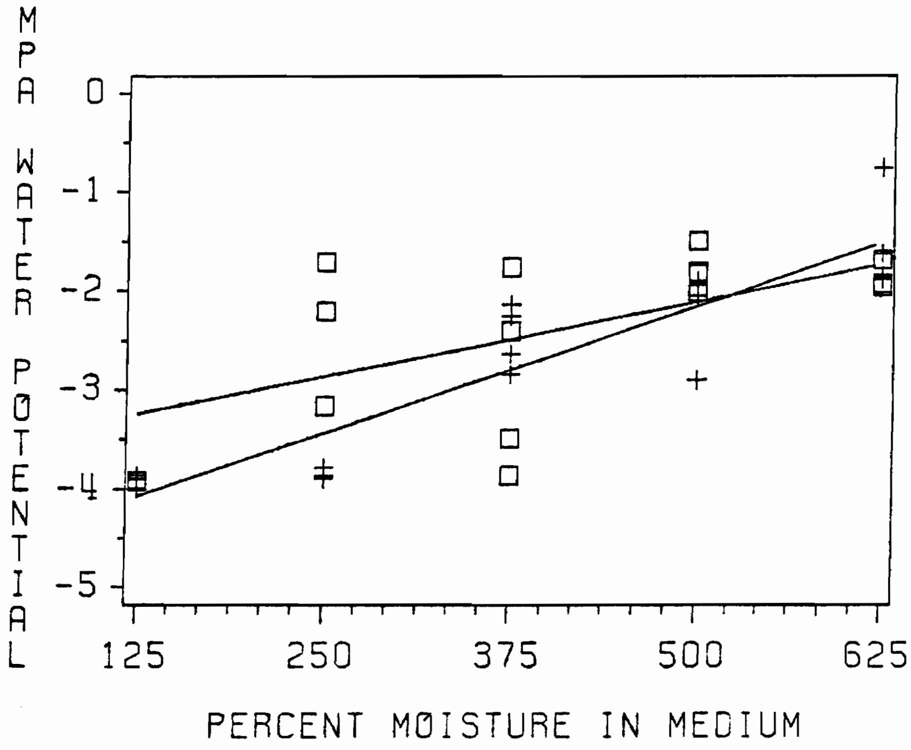


Figure 2.2. Water potential of 'Hino-crimson' azalea stem cuttings: 5 and 14 days after sticking into a 1:1 peat/perlite propagation medium maintained at five moisture levels. Four cuttings per moisture level were measured on each date. Regression equations: 5 days (+-+) $Y = -4.71 + 0.005x$, $R^2 = 0.84$, $p = 0.0001$; 14 days (□-□) $Y = -3.61 + 0.003x$, $R^2 = 0.34$, $p = 0.01$.

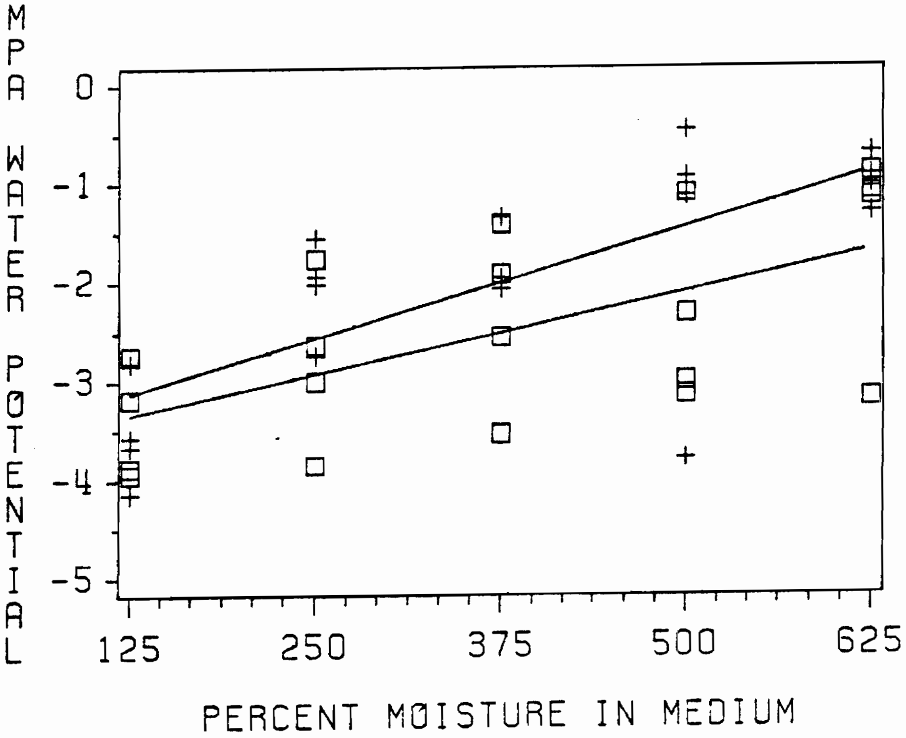


Figure 2.3. Water potential of 27 April 'Helleri' holly stem cuttings: 4 and 10 days after sticking into a 1:1 peat/perlite medium maintained at five moisture levels. Four cuttings per moisture level were measured on each date. Regression equations: 4 days (+-+) $Y = -3.68 + 0.004x$, $R^2 = 0.54$, $p = 0.0007$; 10 days (□-□) $Y = -3.75 + 0.003x$, $R^2 = 0.35$, $p = 0.005$.

Literature Cited

Dirr, M.A. and C.W. Heuser. 1987. *The Reference Manual of Woody Plant Propagation*. Varsity Press, Athens, GA.

Elstrodt, C.J. and D.C. Milbocker. 1983. Improving propagation with vhhp. Nursery Business 8:36-38,55.

Evans, H. 1952. Physiological aspects of the propagation of cacao from cuttings. Rpt. XIII Intl. Hort. Congr. 2:1179-1190.

Gislerød, H.R. 1983. Physical conditions of propagation media and their influence on the rooting of cuttings. Plant and Soil 75:1-14.

Grange, R.I. and K. Loach. 1983. The water economy of unrooted leafy cuttings. J. Hort. Sci. 58:9-17.

Hess, C.E. and W.M. Snyder. 1955. A physiological comparison of the use of mist with other propagation procedures used in rooting cuttings. Rpt. XIV Intl. Hort. Congr. 2:1133-1139.

Hsaio, T.C. 1973. Plant responses to water stress. Annu. Rev. Plant Physiol. 24:519-570.

Loach, K. 1977. Leaf water potential and the rooting of cuttings under mist and polythene. Physiol. Plantarum 40:191-197.

Loach, K. 1985. Rooting of cuttings in relation to the propagation medium. Proc. Intl. Plant Prop. Soc. 35:472-485.

Long, J.C. 1933. The influence of rooting media on the character of roots produced by cuttings. Proc. Amer. Soc. Hort. Sci. 29:352-355.

Scholander, P.F., H.T. Hammel, E.D. Bradstreet, and E.M. Hermmingsen. 1965. Sap pressure in vascular plants. Science 148:339-346.

Slezinski, J. and H. Davidson. 1973. Vapor pressure deficit and polyethylene in plant propagation. Proc. Intl. Plant Prop. Soc. 23:238-241.

Soffer, H. and D.W. Burger. 1988. Effects of dissolved oxygen concentrations in aero-hydroponics on formation and growth of adventitious roots. J. Amer. Soc. Hort. Sci. 113:218-221.

Tilt, K.M. and T.E. Bilderback. 1987. Physical properties of propagation media and their effects on rooting of three woody ornamentals. HortScience 22:245-247.

Chapter Three: Stock Plant Nutrition and Adventitious Rooting of 'Rotundifolia' Holly Stem Cuttings

Abstract

Containerized stock plants of *Ilex crenata* Thunb. 'Rotundifolia' were liquid-fertilized at least three times per week with nitrogen (N) at five rates (25, 50, 100, 200, and 300 mg N • liter^{-1}) and in two forms (100% NH_4NO_3 or 50% NH_4NO_3 + 50% urea) in a factorial treatment design. Phosphorus and potassium were included in a constant ratio with N (100 N : 15 P : 50 K). Percent rooting of stem cuttings decreased linearly with fertilizer rate. Leaf and stem percent N increased from optimal to excessive levels with fertilizer rate. With increasing fertilizer rates, total nonstructural carbohydrate (TNC) levels increased in leaves and remained constant in stems. The stem TNC : N ratio decreased linearly with increasing fertilizer rate, but was only weakly related to

rooting. Stem cutting percent moisture increased and was highly correlated with fertilizer rate. The form of N applied made no statistical difference in these trends. The decrease in percent rooting with increasing fertilizer rates was attributed to increases in shoot growth activity and decreased tissue maturation.

Introduction

Assimilation of inorganic nitrogen (N) by plant tissue is a carbohydrate-utilizing process. It has therefore been assumed that the amount of carbohydrate available in stems can be manipulated by varying the level of N applied to plants. To increase adventitious rooting success, propagators have been encouraged to maintain high carbohydrate levels in stem cuttings by limiting fertility, especially N nutrition, of the stock plants which supply those cuttings (Haissig, 1986; Hartmann and Kester, 1983; Struve, 1981).

Kraus and Kraybill (1918) concluded that a high ratio of carbohydrate : N was responsible for improved adventitious rooting of tomato cuttings. Pearse (1943) showed that cuttings from unfertilized or poorly-fertilized grape (*Vitis vinifera*) vines responded better to synthetic auxin treatment and rooted more easily than cuttings from adequately- or over-fertilized vines. Later, Haun and Cornell (1951) reported that geranium stock plants receiving low levels of N produced significantly higher percentages of rooted cuttings than plants given a higher rate of N; varied levels of P and K had no effect. Preston et al. (1953) found similar trends for azalea.

More recent studies indicate that carbohydrate and N levels alone do not necessarily control rooting. Total nonstructural carbohydrate (TNC) levels in stem cuttings have been positively correlated with rooting in plum (Breen and Muraoka, 1974), pine (Haissig, 1986), palm (Reuveni and Adato, 1974) and chrysanthemum (Stoltz, 1968). However, TNC was not significantly correlated with rooting of *Prosopis alba* stem cuttings (De Souza and Felker, 1986). The particular forms of carbohydrate and their translocation and proximity to the site of adventitious rooting may be more important for propagation than overall TNC levels (Breen and Muraoka, 1974; Haissig, 1984; Veierskov et al., 1982). Haissig (1982) concluded that there was no obvious cause-effect relationship between carbohydrate levels in basal segments of *Pinus banksiana* (Jack pine) stem cuttings and rooting.

Carbohydrate and N levels vary within plants according to tissue age and stem region. An actively growing terminal stem cutting would be lower in carbohydrates than a cutting taken further down the shoot (Dirr and Heuser, 1987). The rooting response of these cuttings may or may not be similar and the reasons for a differential response have not been elucidated. With azalea, cuttings taken from young, succulent growth responded to N treatments differently than mature, hardened stem tissue (Preston et al., 1953). The seasonal rooting response of cuttings from some plants may be related to tissue maturation. Competition for carbohydrates and other growth substances between shoot growth and root formation may differentiate easily-rooted dormant cuttings from difficult-to-root actively-growing cuttings (Ali and Westwood, 1966; Reuveni and Adato, 1974). It is recommended that cuttings of woody plants such as Japanese holly be taken between flushes of shoot growth (Dirr and Heuser, 1987). Cuttings taken from actively growing shoots may be too succulent and may rot during propagation.

Evidence that N can influence the rooting of cuttings possibly by interacting with tissue maturation or dormancy warrants further investigation into the upper and lower limits of stock plant nutrition. Nitrogen must be provided to stock plants at some baseline concentration because of its importance in cellular metabolism. Furthermore, stock plants are often the same plants grown for later sale, and they often receive the heavy fertilization regime required for efficient plant production. Growers sometimes experience little or no rooting of cuttings from plants treated in this manner. Therefore, this study was designed to determine whether the rate of fertilizer applied to 'Rotundifolia' holly stock plants would control the quality and adventitious rooting of stem cuttings, and whether rooting was correlated with tissue N and TNC concentrations.

Materials and Methods

Rooted stem cuttings of 'Rotundifolia' holly were overwintered in a greenhouse and then planted in a 9:1 mix of pine bark and sand in 9 liter (10 quart) plastic containers. The plants were placed outdoors in a gravel bed nursery. Starting 19 May 1989, the plants were fertilized with one of two forms of N: 100 % NH_4NO_3 or a combination of 50 % NH_4NO_3 + 50 % urea (Uran 30, Arcadian Corp., Parsippany, N.J.). Nitrogen was applied at one of five rates (25, 50, 100, 200, and 300 mg $N \bullet liter^{-1}$) along with P (as ammonium polyphosphate) and K (as KCl) in a constant ratio of 100 N : 15 P : 50 K. Treatments were arranged in a 2x5 factorial with four plants per treatment in four randomized complete blocks. The plants were irrigated with the liquid fertilizer solution using a Hozon siphon proportioner (E.C. Geiger Co., Harleysville, Pa.). Usually, plants were fertilized every other day except Saturday and Sunday, when plain water irrigation was used to leach the medium. The medium solution was extracted every other week via the pour-through technique (Wright, 1986) to monitor electrical conductivity (EC) of the container medium. Fertilization frequency was increased if the average EC level of the medium solution from the 300 mg N • liter^{-1} treatment did not reach 2.0 dS • m^{-1}, a level twice that which is recommended for optimal growth of woody nursery crops (Wright, 1987).

Twice during the experiment, in addition to monitoring EC, the levels of NO_3-N and NH_4-N in the pour-through extracted medium solution were analyzed with ion-selective electrodes. One plant per treatment per block was used in each extraction.

On 19 September, four stem cuttings were taken from each plant and placed in plastic bags on ice in a cooler. The cuttings were separated into two groups -- half were for propagation, the other half were for tissue analysis. For propagation, cuttings were trimmed acropetally to 10 cm, stripped of leaves from the basal 4 cm, and dipped basally into 5000 mg 1*H*-indole-3-butyric acid (IBA) • liter^{-1} for five sec. The cuttings were then inserted singly into 0.2 liter pots filled with a 1 : 1 mix of steam-pasteurized sphagnum peat and perlite. The cuttings were placed under intermittent mist in a generalized randomized complete block design consisting of eight blocks; each block contained one cutting per treatment per nursery block (40 cuttings total per block).

On 26 October, the cuttings were evaluated for adventitious root number and length, rootball diameter, and percentage of rooted cuttings. The mean response of four cuttings per block was used for each treatment.

On 20 September, stem cuttings saved for tissue analysis were weighed, rinsed with distilled water, and then dried in a 70°C oven for 72 hr. Stem cutting fresh and dry weight and percent moisture were determined. Stems and leaves were separated and ground in a Cyclone mill (U-D Corporation, Boulder, Colo.). Percent N of the stem and leaf tissue was determined using a modified micro-Kjeldahl technique (Bremner and Breitenbeck, 1983). Percent TNC was determined using an acid hydrolysis and enzymatic starch extraction as described by Smith (1969), followed by an automated colorimetric total sugar analysis (Davis, 1976; Lever, 1972).

Stock plant growth was measured using a total shoot growth index [(plant height + plant width)/2] after the experiment had been terminated. Plant height was measured on the tallest shoot, and plant width was the average of the two widest points measured across the plant. The mean index of four plants per block was used for each treatment.

The General Linear Models (GLM) procedure of SAS® (SAS Institute, Cary N.C.) was used for all regression analyses and to determine significant main effects and interactions due to N rate and form. For tabular rate data, significant components of the full polynomial model were determined by backward selection. Best-fit regression equations are presented in figures. Strength of the linear relationship among various dependent variables was described by Pearson correlation coefficients via the SAS® Correlation procedure. Percentage data was deemed normal by the Shapiro-Wilks test (via the Univariate procedure of SAS®) and was not transformed prior to statistical analysis.

Results

The percentage of rooted cuttings decreased with increasing fertilizer application to the stock plants (Fig. 3.1). This is in agreement with previous work with grape (Pearse, 1943), geranium (Haun and Cornell, 1951), and azalea (Preston et al., 1953). There were no significant differences in root count, root length, and rootball diameter due to treatment (data not shown). Form of applied N made no significant difference in rooting or in the trends in tissue N and TNC content, except for an interaction between N form and rate in leaf percent N (Table 3.1). However, this interaction was not considered practically important and therefore data from both N forms were pooled for regression analysis.

Percent N in the leaves and stems increased with fertilizer rate (Table 3.1) and was negatively correlated with rooting (Table 3.2). The highest rate of applied N (300 mg $N \bullet liter^{-1}$) produced cuttings with tissue N levels that were above 2.5 % N, the level considered optimal for growth of 'Rotundifolia' holly (Table 3.1) (Gilliam and Wright, 1977). In a preliminary study, 'Rotundifolia' holly stock plants were fertilized with between 30 mg $N \bullet liter^{-1}$ and 180 mg $N \bullet liter^{-1}$ in the irrigation water. Stem cutting N concentrations ranged from 1.0 % to 2.5 %, but there was no significant difference in rooting percentages (data not shown).

Percent TNC of the leaf was increased with fertilizer rate, whereas percent TNC of the stem was not changed with fertilizer rate (Table 3.1). Rooting was negatively correlated with leaf percent TNC (Table 3.2). There was no statistical correlation between rooting and stem percent TNC. There was a positive linear relationship between leaf

N and leaf TNC (Fig. 3.2). The ratio of TNC to N in the stem was negatively correlated with applied N rate (Fig 3.3).

Stem cutting succulence, measured as percent moisture, was increased with fertilizer rate (Table 3.1), and was correlated with percent N in both stem and leaf (Table 3.2). This may be an indication of the effect of N on tissue maturity. Shoots continued to elongate on plants receiving high N rates (data not shown). Tissue maturation may have been a factor in the negative correlation of stem cutting moisture levels with adventitious rooting (Table 3.2).

Total shoot growth of the stock plants increased with increasing fertilizer rates (Table 3.3). At N rates above 25 mg N • liter^{-1}, plants fertilized with 100 % NH_4NO_3 were larger than plants fertilized with Uran 30. Increases in plant size generally decreased in magnitude when N rates reached 100 to 200 mg N • liter^{-1}. Stock plants were visibly smaller and shoots available for cuttings were much fewer at the two lowest fertilizer rates than on plants receiving higher fertilizer rates. Fertilizer rates of 100 mg N • liter^{-1} and higher resulted in EC, NO_3-N, and NH_4-N at levels in the medium solution which have been associated with vigorous growth of *Ilex crenata* nursery stock (Wright, 1986) (Table 3.3).

Discussion

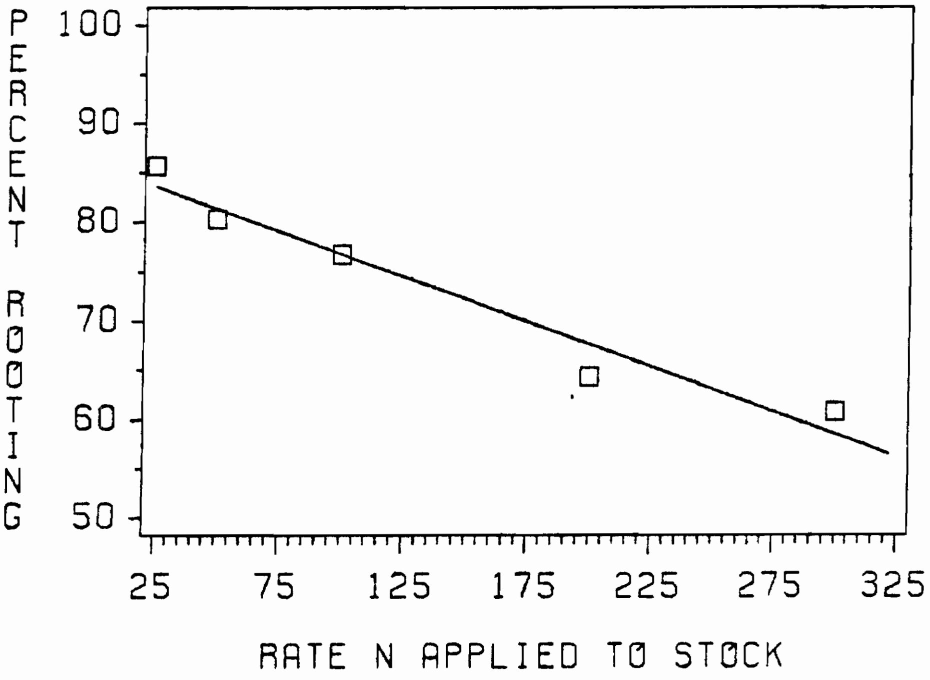
There is a negative relationship between adventitious rooting of 'Rotundifolia' holly stem cuttings and fertilizer rates. The exact reason for this response is difficult to ascertain. Increasing the rate of N applied to stock plants resulted in a concomitant increase in leaf percent TNC but no change in stem percent TNC. The ratio of carbohydrate : N decreased in the stem with increasing fertilizer rates, but was weakly related to rooting success ($r = 0.37$, $p = 0.02$). Reductions in adventitious rooting as a result of high fertility has in the past been attributed to a reduction in the cutting C : N ratio (Kraus and Kraybill, 1918; Pearse, 1943). Whether the C : N ratio has a direct effect on rooting is questionable.

The relationship between fertilizer rates, stem cutting percent moisture, stock plant growth, and adventitious rooting suggests that N may control rooting of 'Rotundifolia' holly cuttings by influencing tissue maturation and associated physiological changes. The moisture content of cuttings may indicate tissue maturity because new shoot growth tends to be higher in moisture content than shoots which have hardened or matured. Greater total growth of 'Rotundifolia' holly stock plants at higher fertilizer rates was probably the result of a more active growth state maintained during the growing season. Gilliam and Wright (1978) showed that high rates of applied N-P-K fertilizer increase the frequency of flushes of shoot growth on *Ilex crenata* 'Helleri' and maintain a more active growth state overall. Adventitious rooting of 'Rotundifolia' holly may have been limited by the increased shoot growth activity of more heavily fertilized plants. Cuttings of actively growing shoots of *Ilex crenata* are more difficult to root than inactive or dormant shoots (Dirr and Heuser, 1987).

The mechanism by which rooting may be limited by shoot growth activity or tissue maturation is unknown. Decreased survival and rooting of succulent cuttings may be directly due to increased water stress, from excessive water loss either through stomata which have not fully developed or through incomplete cuticle on younger tissue.

Changes in the balance of endogenous rooting promoters or inhibitors and other growth substances may occur with tissue maturation (De Souza and Felker, 1986; Haissig, 1986; Reuveni and Adato, 1974). These substances may interact with carbohydrates and N in controlling the adventitious rooting process. The distribution of carbohydrates may change with cutting maturity and may indicate a redistribution of other substances in the cutting which can influence rooting.

Leaf N levels maintained at about 2.5 %, along with medium solution EC and N levels maintained at about 0.75 dS • m^{-1} and 75 - 100 mg N • $liter^{-1}$, respectively, can provide an acceptable balance between stock plant growth and stem cutting rooting ability for 'Rotundifolia' holly. If these limits are exceeded, resultant minor increases in plant growth will likely be outweighed by subsequent decreases in the rooting of cuttings.



(ppm)

Figure 3.1. **Percentage of rooted stem cuttings of 'Rotundifolia' holly stock plants fertilized with 2 forms and 5 rates of N:** There was no significant difference in rooting between N forms (NH_4NO_3 and Uran 30) so data were pooled. Each point is the percentage of 64 cuttings. Percentage data were not transformed because data were deemed normal by the Shapiro-Wilks test ($p=0.299$). Regression equation: $y = 85.9 - 0.09x$, $R^2 = 0.95$, $p=0.004$.

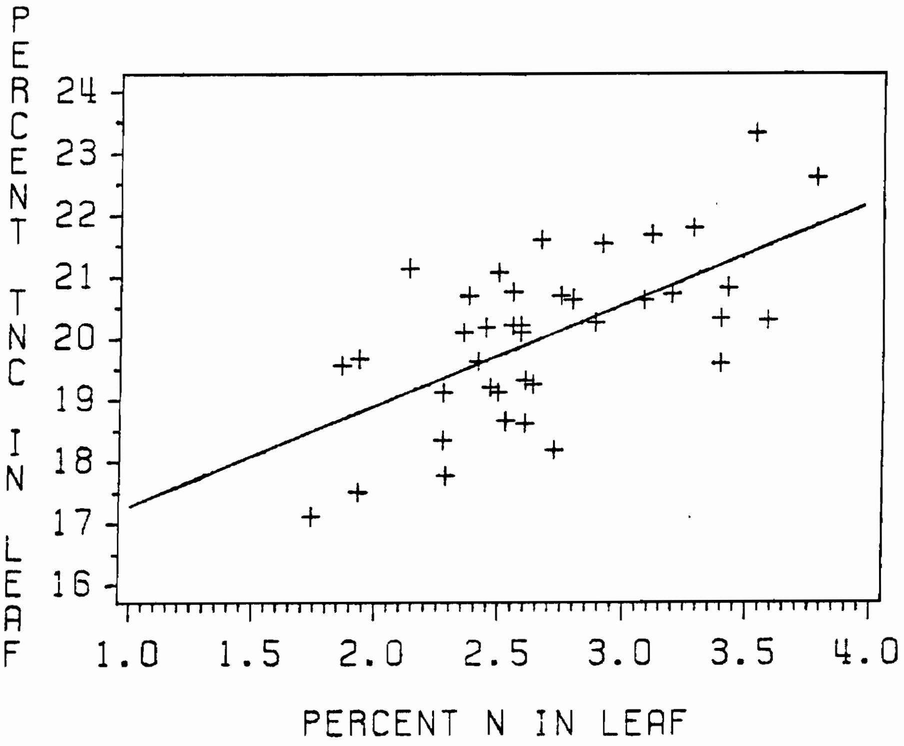


Figure 3.2. The relationship between percent TNC and percent N in leaf tissue of stem cuttings of 'Rotundifolia' holly: Stock plants fertilized with two forms of N at five rates. TNC=Total Nonstructural Carbohydrate. Each point represents the percent TNC at a given percent N of the combined leaf tissue of eight cuttings. There was no significant difference between N form applied (NH_4NO_3 and Uran 30) so data were pooled. Regression equation: $y = 15.6 + 1.63x$, $R^2 = 0.39$, $p = 0.0001$.

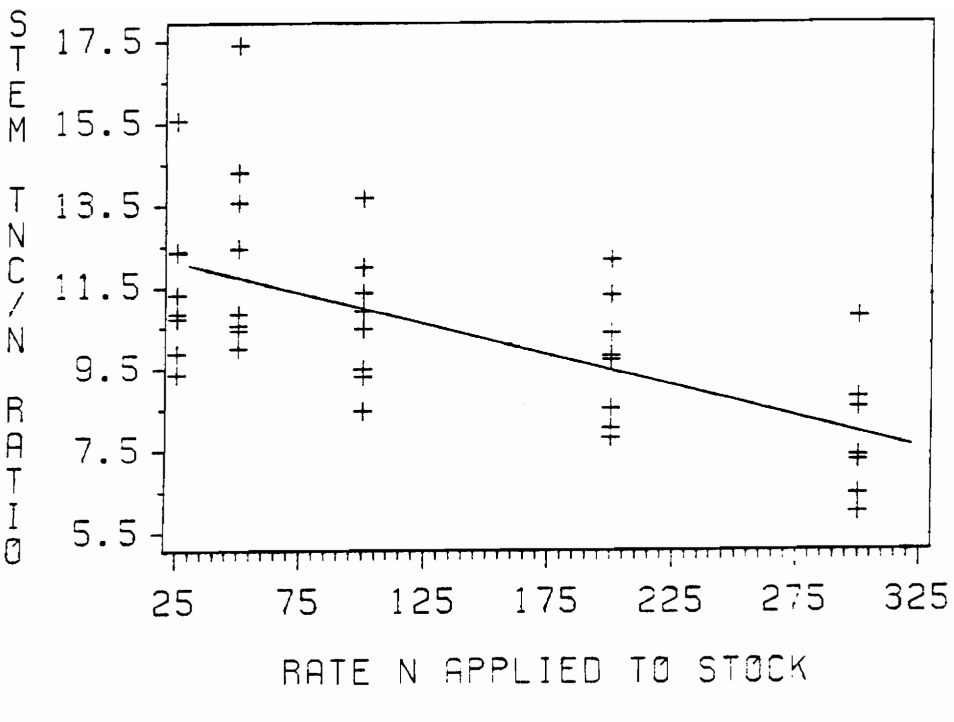


Figure 3.3. **The carbohydrate : N ratio in stem tissue of cuttings taken from 'Rotundifolia' holly stock plants fertilized with 5 rates of N:** Pooled data from stock plants fertilized with two forms of N (NH_4NO_3 or Uran 30) are presented since there was no statistical difference due to N form. TNC=Total Nonstructural Carbohydrate. The ratio of percent TNC to percent N in stems is from the combined stem tissue of eight cuttings. Regression equation: $y = 12.5 - 0.02x$, $R^2=0.42$, $p=0.0001$.

Table 3.1. **Nitrogen (N) and carbohydrate (TNC) levels in stem cuttings of 'Rotundifolia' holly stock plants fertilized with 5 rates and 2 forms of N.**

N Treatment		N Level		T.N.C. Level		
Rate[z]	Form	Leaf	Stem	Leaf	Stem	Moisture
		(%)		(%)		(%)
25	NH_4NO_3	2.4[y]	0.79	18.4	8.9	63
50		2.5	0.77	19.6	9.6	63
100		2.4	0.95	20.9	9.8	64
200		2.6	0.91	20.8	9.4	66
300		3.4	1.06	21.6	8.5	67
25	Uran 30	2.4	0.75	18.5	8.6	63
50		2.2	0.86	19.6	10.0	62
100		2.4	0.95	19.6	9.9	64
200		3.1	1.04	20.8	9.2	66
300		3.5	1.20	20.7	8.8	68
Statistics[x]						
Rate	Linear	****	****	****	NS	****
	Quadratic	*	NS	*	NS	NS
	Cubic	NS	NS	NS	*	*
	Quartic	NS	NS	NS	NS	*
Form		NS	NS	NS	NS	NS
Rate x Form		**	NS	NS	NS	NS

[z]mg N • liter^{-1}.

[y]Mean of four blocks. Eight cuttings were pooled for tissue analysis of each block. Since N form was nonsignificant, data were pooled for regression analysis.

[w]NS , *, **, ***, ****Nonsignificant and significant at the .05, .01, .001, and .0001 levels, respectively. For rate data, regression coefficients were tested for significant addition to the full polynomial model by backward selection.

Table 3.2. Correlation coefficients for percentage of rooted cuttings, N, TNC, and moisture in 'Rotundifolia' holly stem cutting tissue.

Variable	Leaf N[z] (%)	Stem N (%)	Leaf TNC (%)	Stem TNC (%)	Moisture (%)
Rooting[y]	-0.42 **	-0.38 *	-0.42 **	-0.09 NS	-0.57 ****
Leaf N		0.66 ****	0.62 ****	-0.38 *	0.82 ****
Stem N			0.61 ****	0.09 NS	0.63 ****

[z] Based on four blocks. Eight cuttings per block were pooled for tissue analysis.

[y] Adventitious rooting percentages were based on the number of rooted cuttings per block.

NS, *, **, ***, **** = Nonsignificant and significant at the .05, .01, .001, and .0001 levels, respectively.

Table 3.3. Electrical conductivity (EC) and nitrogen (N) in the medium solution, and growth of containerized 'Rotundifolia' holly stock plants.

N Treatment			E.C.	NO_3	NH_4	Growth[y]
Rate[z]	Form		*($dS \bullet m^{-1}$)*	*($mg \bullet liter^{-1}$)*	*($mg \bullet liter^{-1}$)*	*(cm)*
25	NH_4NO_3		0.12[x]	3.6	1.3	29.7
50			0.40	14.3	16.1	32.2
100			0.77	31.8	41.3	35.3
200			1.80	80.5	123.8	35.6
300			2.18	104.0	175.8	38.4
25	Uran 30		0.17	4.6	8.2	30.1
50			0.35	12.4	19.2	31.5
100			0.76	32.9	57.1	33.2
200			1.44	67.1	120.5	35.0
300			1.87	89.3	168.8	35.8
Statistics[w]						
Rate		Linear	****	****	****	****
		Quadratic	NS	NS	NS	*
		Cubic	NS	NS	NS	*
		Quartic	NS	NS	NS	NS
Form			NS	NS	NS	*
Rate x Form			NS	NS	NS	NS

[z] mg N • liter^{-1}. Stock plants were fertilized at least three times per week. Leachate was collected from each block periodically throughout the experiment; presented here is the analysis of leachate collected 16 weeks after fertilization began.

[y] Growth expressed as (plant ht. + plant width)/2, measured at the termination of this experiment.

[x] Mean of four blocks.

[w] NS , *, **, ***, ****Nonsignificant and significant at the .05, .01, .001, and .0001 levels, respectively. For rate data, regression coefficients were tested for significant addition to the full polynomial model by backward selection.

Literature Cited

Ali, N. and M.N. Westwood. 1966. Rooting of pear cuttings as related to carbohydrates, nitrogen, and rest period. Proc. Amer. Soc. Hort. Sci. 88:145-150.

Breen, P.J. and T. Muraoka. 1974. Effect of leaves on carbohydrate content and movement of ^{14}C-assimilate in plum cuttings. J. Amer. Soc. Hort. Sci. 99:326-332.

Bremner, J.M. and G.A. Breitenbeck. 1983. A simple method for determination of ammonium in semimicro-kjeldahl analysis of soils and plant materials using a block digestor. Commun. Soil Sci. Plant Anal. 14:905-913.

Davis, R.E. 1976. A combined automated procedure for the determination of reducing sugars and nicotine alkaloids in tobacco products using a new reducing sugar method. Tobacco Sci. 20:139-144.

De Souza, S.M. and P. Felker. 1986. The influence of stock plant fertilization on tissue concentrations of N, P, and carbohydrates and the rooting of *Prosopis alba* cuttings. For. Ecol. Mgt. 16:181-190.

Dirr, M.A. and C.W. Heuser. 1987. *The Reference Manual of Woody Plant Propagation.* Varsity Press, Athens, GA.

Gilliam, C.H. and R.D. Wright. 1977. Effect of four nitrogen levels on soil, soil solution, and tissue nutrient levels in three container-grown *Ilex* cultivars. J. Amer. Soc. Hort. Sci. 102:662-664.

Gilliam, C.H. and R.D. Wright. 1978. Effect of three nitrogen levels on tissue nitrogen fluctuation during a flush of growth on 'Helleri' holly (*Ilex crenata* Thunb.). HortScience 13:301-302.

Haissig, B.E. 1982. Carbohydrate and amino acid concentrations during adventitious root primordium development in *Pinus banksiana* Lamb. cuttings. For. Sci. 28:813-821.

Haissig, B.E. 1984. Carbohydrate accumulation and partitioning in *Pinus banksiana* seedlings and seedling cuttings. Physiol. Plantarum 61:13-19.

Haissig, B.E. 1986. Metabolic processes in adventitious rooting of cuttings, pp.154-193. In: *New Root Formation in Plants and Cuttings.* M.B. Jackson, ed. Martinus Nijhoff Pub., Dordrecht.

Hartmann, H.T. and D.E. Kester. 1983. *Plant Propagation, Principles and Practices.* 4th ed. Prentice-Hall, Englewood Cliffs, N.J.

Haun, J.R. and P.W. Cornell. 1951. Rooting response of geranium as influenced by nitrogen, phosphorus, and potassium nutrition of the stock plant. Proc. Amer. Soc. Hort. Sci. 58:317-323.

Kraus, E.J. and H.R. Kraybill. 1918. Vegetation and reproduction with special reference to tomato. Ore. Agr. Coll. Exp. Sta. Bul., p.149.

Lever, M. 1972. A new reaction for colorimetric determination of carbohydrates. Anal. Biochem. 47:273-279.

Pearse, H.L. 1943. Effect of nutrients and phytohormones on rooting of vine cuttings. Amer. J. Bot. n.s. 7:123-132.

Preston, W.H., J.B. Shanks and P.W. Cornell. 1953. Influence of mineral nutrition on production, rooting, and survival of cuttings of azaleas. Proc. Amer. Soc. Hort. Sci. 61:499-507.

Reuveni, O. and I. Adato. 1974. Endogenous carbohydrates, root promoters, and root inhibitors in easy- and difficult-to-root date palm (*Phoenix dactylifera* L.) offshoots. J. Amer. Soc. Hort. Sci. 99:361-363.

Smith, D. 1969. Removing and analyzing total nonstructural carbohydrates from plant tissue. Wisc. Agr. Expt. Sta. Res. Rpt. 41.

Stoltz, L.P. 1968. Factors influencing root initiation in an easy- and difficult-to-root chrysanthemum. Proc. Amer. Soc. Hort. Sci. 92:622-626.

Struve, D.K. 1981. The relationship between carbohydrates, nitrogen, and rooting of stem cuttings. The Plant Prop. 27(2):6-7.

Veierskov, B., A.S. Anderson, and E.N. Eriksen. 1982. Dynamics of extractable carbohydrates in *Pisum sativum* I. Carbohydrate and nitrogen content in pea plants and cuttings grown at two different irradiances. Physiol. Plantarum 55:167-173.

Wright, R.D. 1986. The pour-through nutrient extraction procedure. HortScience 21:227-229.

Wright, R.D. 1987. The Virginia Tech liquid fertilizer system for container grown plants. Virginia Polytechnic Institute and State University College Agr. Life Sci. Info. Ser. 86-5.

Vita

William Henry Rein was born on May 26, 1965, in Philadelphia, Pa. He grew up in nearby Warminster, attending schools in the Centennial School District and the Archdiocese of Philadelphia. In 1983, he graduated from Archbishop Wood High School. Bill then enrolled in Delaware Valley College of Science and Agriculture, Doylestown, Pa., where he received a B.S. in Ornamental Horticulture in May 1987. During college Bill spent his summers employed as a landscaper for a design and installation firm, and worked as an intern in insecticide research at the Rohm and Haas Co. Spring House, Pa., laboratories. In September 1987, he began graduate studies in the M.S. program of the Department of Horticulture at Virginia Polytechnic Institute and State University, Blacksburg.

William H. Rein