RELATIONSHIP BETWEEN ROOTING SUBSTANCES AND THE ROOTING RESPONSE OF COLORADO BLUE SPRUCE

by

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LITERATURE REVIEW

The native habitat of the Colorado blue spruce, Picea pungens Engelm., is the southwestern United States. The blue-foliaged cultivars, especially, are used extensively in landscape plantings as specimen trees (26). Their propagation, however, is a difficult task.

Most spruce are easily propagated by seed. Unfortunately, the Colorado blue spruce does not come true from seed. Only a small percentage of the seedlings have the desirable blue color. Propagation of spruce from cuttings is also difficult (24). The percentages which root are generally low (32), making this method of propagation economically unfeasible. Because of these difficulties, the majority of nurserymen propagate the cultivars of Colorado blue spruce by grafting. The desirable scion is grafted onto rootstock of Norway spruce, Picea abies (L.) Karst. (11,23). However, the grafting procedure also has drawbacks. The method tends to be slow and the union commonly fails to heal properly (11,34).

The propagation of blue spruce cultivars by cuttings could simplify their production if the problems encountered could be understood and overcome. Previous attempts to root cuttings have met with varied results (23,27,32,34), generally indicating that rooting capacity varies with the cultivar. Additionally, no one procedure has been developed which will result in satisfactory rooting of the various cultivars. Discrepancies in procedure can be seen in two examples. Teuscher (32) reported that the best time to take cuttings of Picea pungens var. glauca 'Montgomery' was toward the end of February or early March in Montreal, Canada. Softwood

cuttings taken in June also rooted well. He found that the needles at the base of the cutting must not be removed. Application of rooting hormones and additional wounding also proved detrimental. Wells (34), working in New Jersey with the cultivar 'Koster', found that cuttings should be taken in March. Wells reported that the application of indole-butyric acid (IBA) proved beneficial, as did the removal of the lower needles on the cutting. Wounding also increased rooting (34).

Several factors could account for the difficulty in rooting Colorado blue spruce cuttings in general, and for the differences in rooting response between cultivars. Juvenility is one factor which profoundly affects the rooting of cuttings. Generally, cuttings taken from young plants (in the juvenile phase) will root better than cuttings taken from mature, seed-bearing plants (28). Gardner (7) found that 90% of the cuttings taken from two-year-old seedlings of Norway spruce rooted, while only 50% of the cuttings from older trees rooted. The same relationship was found in Eastern white pine (Pinus strobus L.): 98% of the cuttings from one-year-old plants rooted, 50% from two-year-old plants, and 12% from three-year-old plants. Similar relationships were noted in Fraser fir, Abies fraseri (Pursh) Poir., and Douglas-fir, Pseudotsuga menziesii (Mirb.) Franco (3,16). Also as the plant matures, there is a tendency for the lower portion of many trees and shrubs and their root system to remain juvenile or to maintain the ability to produce juvenile shoots (14). This phenomenon is known as topophysis. The oldest portion of a tree or shrub from the standpoint of

chronological age, represents the juvenile portion of the plant. The mature phase is present in the upper, youngest portion of the tree or shrub (19). Grace (9) found that 48% of the cuttings taken from the upper portion of Norway spruce rooted, while 86% of the lower cuttings rooted.

Since cuttings from juvenile plant material initiate adventitious roots more readily than mature material, vegetative propagation may be simplified by prolonging or reestablishing the juvenile form of growth. Heavy pruning or shearing of stock plants is one method utilized to obtain easy-to-root plant material (3,19,31,35). The reversion to the juvenile phase from the adult phase may be accomplished by applying chemicals such as gibberellins (19,31). Another method by which reversion has been produced is by grafting. Grafting of mature scions to juvenile rootstocks may promote a return to juvenile growth (19,31). The juvenile stage of growth may be prolonged by reduced light (31).

Juvenility in relation to rooting may be caused by high levels of phenolic compounds in juvenile compared to mature tissue. In English ivy, Hedera helix L., the levels of phenolic compounds in the mature tissue are lower than those in the juvenile tissue (8). Rooting inhibitors may also be involved in juvenility. A direct association between decreased rooting and the production of rooting inhibitors has been noted in Eucalyptus grandis W. Hill ex Maiden (25). Rooting inhibitors were absent in easily-rooted seedling cuttings, whereas they were present in older, mature plants.

Another factor which may affect the rooting ability of cuttings is the C/N ratio (14). A high level of nitrogen in

cutting material is generally detrimental to rooting because it is associated with soft, succulent growth which is low in carbohydrates. While adequate levels of carbohydrates are essential for root development, they do not effect root initiation (33).

The degree of lignification within stem cuttings may also affect rooting. Many difficult-to-root cuttings contain a high amount of primary phloem fibers. Since adventitious root initiation occurs in the phloem, a phloem fiber ring may inhibit rooting (11,14).

In the mid-1930s, indoleacetic acid (IAA) was identified as a naturally occurring auxin which stimulated adventitious root initiation. Through subsequent research it has been proven that auxin, natural or artificially applied, is a requirement for initiation of adventitious roots on cuttings. Other plant hormones, in addition to auxin, may also affect rooting. Both gibberellins and cytokinins inhibit root formation (5,33,35). The gibberellins limit root development by stimulating competing growth, although they have no direct effect upon root initiation (5,33). Abscisic acid (ABA) promotes rooting. ABA may act as an auxin synergist or as an gibberellin antagonist (35).

Hess (12) has demonstrated that the presence or absence of root-promoting substances in plant tissue may also play a role in root initiation. Four root-promoting substances were detected in methanolic extracts of the easy-to-root, juvenile form of English ivy. These root-promoting substances, termed cofactors, are naturally occurring compounds, other

than auxin, which are essential for root initiation (11). They act as auxin synergists.

The cofactors reported by Hess (13) are referred to by numbers on the basis of their relative position on a chromatogram developed in isopropanol and water (8:2 v/v). The R_f values of the cofactors are: cofactor 1, 0-0.13; cofactor 2, 0.33-0.53; cofactor 3, 0.60-0.73; and cofactor 4, 0.80-0.93. Methanolic extracts of lyophilized tissue of red and white flowering Hibiscus rosa-sinensis L. indicated the presence of four cofactors in the easy-to-root red Hibiscus, while in the extract of the difficult-to-root white form, there are only two root-promoting substances (12).

Lee et al. (21) also found a relationship between rooting cofactors and the rooting response of three clones of Rhododendron sp. L. A methanolic extract of an easy-to-root clone of Rhododendron 'Cunningham's White', intermediate-to-root clone of 'English Roseum', and a difficult-to-root clone of 'Dr. H. C. Dresselhuys' were obtained in July from terminal stem cuttings. A mung bean bioassay indicated the presence of four cofactors in the extract of 'Cunningham's White'. Two root-promoting substances were detected in extracts from stem tissue of 'English Roseum' while only one cofactor was found in the extract of 'Dr. H. C. Dresselhuys'.

Kawase (18) isolated four water soluble rooting substances in several woody ornamentals by centrifugal diffusion and water extraction. In a similar study, Kawase (17) detected the presence of four rooting cofactors in easy-to-root willow cuttings. The location of these cofactors corresponds with those found by Hess.

A correlation between the seasonal (more accurately the stage of growth of the stock plant) rooting response and the presence of rooting cofactors has not been completely substantiated (1,6,20,22,30). Bassuk and Howard (1) identified four rooting cofactors in sap extracted from one-year-old shoot growth of M 26 apple trees and found a strong correlation between the seasonal rooting response of the cuttings and the root-promoting activity of the sap. Lipecki and Dennis, working with softwood apple cuttings of MM 106, MM 109, and EM IX, found no such correlation (22). The level of rooting cofactors in cuttings taken in late summer was higher than cuttings taken in late spring, while the rooting capacity actually declined through the spring and summer. A correlation was noted, however, between growth inhibitor levels and the seasonal rooting response. Good rooting response was associated with high levels of a growth inhibitor at the base of the cuttings. The inhibitor of wheat coleoptile elongation occurred at R_f 0.7-1.0. Phloridzin was thought to be responsible for the inhibition of growth. Phloridzin will also promote root initiation in mung bean cuttings. While some of the results are contradictory, most of the evidence does seem to indicate a correlation between the presence of rooting cofactors and ease of rooting.

Purified cofactor 4 contains a number of oxygenated terpenoids (8,15). They form an oily, yellow liquid at room temperature which is only slightly soluble in water (13). Girouard (8) fractionated cofactor 4 from juvenile English ivy into five major areas of activity and several minor ones. Cofactor 3 consists of chlorogenic and isochlorogenic acids,

and an unknown compound termed P-257 by Girouard (8). Chlorogenic acid also has been identified as a component of cofactor 2. There are three active constituents of rooting cofactor 1; however, none have been identified to date (1).

The role of the rooting cofactors in root initiation is presently not clear. They may protect indoleacetic acid (IAA) from destruction by indoleacetic acid oxidase (11). Phenolic compounds such as catechol (in the presence of IAA) have been found to promote rooting of mung bean (10,12). This strong synergism could be due to the decreased degradation of IAA in the presence of catechol. Phenols such a catechol do inhibit the peroxidase-like indoleacetic acid oxidase system in peas and wheat.

Other researchers have proposed that rooting is stimulated by the formation of an auxin-cofactor complex in the presence of a polyphenol oxidase enzyme (4). The cofactors, thought to be orthodiphenols, react with auxin in the presence of polyphenol oxidase to give rise to the complex "rhizocaline" (11). Rhizocaline formation may be considered as one step in a chain of reactions which culminates in the differentiation of cells and tissues and finally in the emergence of adventitious roots. Evidence to support this has been presented by Bassuk et al. (2), who noted increased rooting of apple cuttings by treating the bases of the cuttings with polyphenol oxidase and phloridzin.

Root initiation in cuttings occurs in parenchyma cells near or just outside the phloem, in phloem rays, or in the interfascicular region between vascular bundles (33). The process of root initiation is thought to consist of four

distinct phases (29). The first phase begins with the swelling of a single parenchyma cell containing polyphenol oxidase. Cytoplasmic migration in the surrounding cells, followed by asymmetric cell division, occurs in the second phase. The third phase includes continued division of the surrounding cells to form groups of meristematic tissue or meristemoids. Finally, the meristemoids differentiate into root primordia. The interruption of the phloem when making a cutting allows the accumulation of auxin and cofactors at the base of the cutting. These materials accumulate in the phloem and nearby parenchyma cells. The presence of auxin, cofactors, and polyphenol oxidase in parenchymatous cells stimulates the cells to become meristematic and form root initials.

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RELATIONSHIP BETWEEN ROOTING SUBSTANCES AND THE ROOTING RESPONSE OF COLORADO BLUE SPRUCE[1]

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Abstract

There is no significant difference in the level of endogenous root-promoting substances in upper and lower stem cuttings of Colorado blue spruce (Picea pungens Engelm.). Mung bean bioassays of extracts from both upper and lower stem cuttings produced a relatively linear increase in rooting with the relative concentration. Chromatographed extracts of both the upper and lower stem cuttings appear to contain three root-promoting fractions. The root-promoting fractions were located at R_f's 0.3-0.4, 0.7-0.8, and 0.9-1.0. The addition of IAA enhanced the rooting response at these fractions. The root-promoting fractions, therefore, may contain compounds which serve as cofactors of IAA. Mung bean bioassays of chromatographed water extracts of upper and lower cuttings failed to detect any root-enhancing substances. These results indicate that the rooting cofactors in Colorado blue spruce are not significantly water soluble.

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While the blue-foliaged cultivars of Colorado blue spruce (Picea pungens Engelm.) are valuable landscape trees, their propagation is difficult. The majority of nurserymen propagate these cultivars by grafting the desirable scion onto rootstockes of Norway spruce, Picea abies (L.) Karst. (10,19). The grafting procedure, however, is not without problems. Grafting tends to be a slow, costly process and the graft union commonly fails to heal properly (10,25). Attempts to root cuttings of blue spruce cultivars have met with varied results (19,20,23,25). Generally, the percentages which root are poor, with no one procedure developed which would permit large scale propagation of the various blue spruce cultivars by cuttings. Currently the variables which affect the rooting of cuttings are not completely understood. If these factors could be identified and overcome, the propagation of blue spruce cultivars by cuttings may become economically feasible.

Several factors could account for the difficulty in rooting Colorado blue spruce cuttings. One factor which may affect the rooting ability of cuttings is the C/N ratio (14). Adequate levels of carbohydrates are apparently essential for optimum root development (24). The degree of lignification within stem cuttings may also affect rooting. Many difficult-to-root cuttings contain a high amount of primary phloem fibers which may inhibit rooting (10,14). The positive role of auxins, both natural and synthetic, in root initiation has been established. Indolebutyric acid (IBA) promoted root initiation in cuttings of Koster blue spruce (25), although another attempt to pro-

mote rooting of Colorado blue spruce cuttings by applying auxins proved detrimental (23). Gibberellins and cytokinins, such as GA_3 and kinetin respectively, seem to inhibit root formation (5,10,24,26). Abscisic acid (ABA) has been reported to promote rooting (26).

Juvenility is another factor which may affect rooting. Generally, cuttings taken from young plants (in the juvenile phase) will root better than cuttings taken from plants in the mature or adult phase (21). This has been demonstrated in both conifers and deciduous trees. In one study (7), 90% of the cuttings taken from two-year-old seedlings of Norway spruce rooted, while only 50% of the cuttings from older trees rooted. The same relationship was true for Eastern white pine (Pinus strobus L.): 98% of the cuttings from one-year-old plants rooted, 50% from two-year-old plants, and 12% from three-year-old plants. Similar relationships were noted in Fraser fir, Abies fraseri (Pursh) Poir., and Douglas-fir, Pseudotsuga menziesii (Mirb.) Franco (4,15).

The rooting response of cuttings may also be affected by their position on an individual branch of a tree (topophysis) or their relative location on the entire tree 'cyclophysis' (4). Only 48% of the cuttings taken from the upper portion of Norway spruce rooted, while the rooting percentage of lower cuttings was 86% (8).

The relationship between juvenility and rooting may be due to the presence or absence of root-promoting substances in the plant tissue. Several root-promoting substances, termed co-

factors, have been isolated from English ivy using a mung bean bioassay. A direct correlation between rooting response and the number of cofactors has been substantiated (1,12,16,17,18). In Hibiscus rosa sinensis L., there were four cofactors in the easy-to-root red Hibiscus, while there were only two in the difficult-to-root white form (12). The cofactors are typically referred to by numbers on the basis of their relative position on a chromatogram developed in isopropanol and water (8:2 v/v). The R_f values of the cofactors are: cofactor 1, 0-0.13; cofactor 2, 0.33-0.53; cofactor 3, 0.60-0.73; and cofactor 4, 0.80-0.93 (13).

The objective of this study was to determine whether there is a relationship between the ease of rooting in cuttings from the upper and lower portion of Colorado blue spruce and the presence of root-promoting substances in the tissue.

Materials and Methods

Sampling Procedure. Terminal softwood cuttings were collected from three Colorado blue spruce at the Plant Material Center of the Soil Conservation Service, Manhattan, Kansas. Cuttings were obtained from both the upper and lower one-third portion of each tree on June 17 and 18, 1981. The cuttings were immediately placed in liquid nitrogen. The cuttings were then lyophilized for approximately twenty-four hours and ground in a Wiley mill to a 20 mesh size (12,17,18). The ground tissue was refrigerated in specimen bottles at -20°C prior to extraction.

Presence of Root-Promoting Substances. A 0.25 gram sample of lyophilized, ground tissue was extracted with three succes-

sive 25 ml amounts of chilled 75% (v/v) aqueous methanol (12,18). An ice bath and constant agitation were provided during extraction. Each extraction period was one hour in length for a total of three hours. The extracts were filtered through Whatman No. 1 filter paper, combined, and evaporated to dryness at 40°C in a drying oven. The residue was taken up in 50 ml of distilled water and the following amounts ultilized: (a) 5.0 ml, (b) 2.5 ml, and (c) 1.0 ml (12). The amounts were transferred to a 19 X 48 mm glass vial. Distilled water was added to (b) and (c) to bring their volume to 5 ml. Either 5 ml of 1 X 10^{-5}M IAA or distilled water was then added to each vial and a mung bean bioassay conducted (see page 18).

Fractionation of the Root-Promoting Substances. One-half gram of ground tissue was extracted with three successive 25 ml portions of chilled absolute methanol. Each extraction period was 30 minutes in length for a total of 90 minutes. The extracts were filtered through Whatman No. 1 filter paper, combined, and evaporated to dryness at 40°C. The residue was taken up in 5 ml of absolute methanol (12,18), each ml of solution containing the extract from 0.1 gram of ground tissue.

Alternatively, samples were extracted with distilled water and the residue was taken up in distilled water (16). The other procedural steps were identical to those used for the extraction with absolute methanol.

Chromatographic Separation. Both absolute methanol and distilled water extracts (see above) were fractionated by paper chromatography (12,17,18). Whatman No. 3 MM chromatographic paper was prewashed by ascending chromatography in isopropanol:

water (8:2 v/v) and air-dried. One-half ml of the concentrated extract, representing 0.05 gram of ground tissue, was applied by a 100 µl Lang Levy micropipette as a band 4 cm from the ends of the strips. After drying, the strips were equilibrated in the chromatography chamber for one hour before immersion in the solvent. Solvent flow was terminated when the front had ascended 20 cm beyond the starting band. The air-dried chromatograms were cut into 2 cm sections, each corresponding to an R_f increment of 0.1. These chromatogram segments were then placed in glass vials. Either 10 ml of distilled water or a solution containing 5×10^{-6}M IAA was then added to each vial. A mung bean bioassay was conducted immediately thereafter.

Mung Bean Bioassay. Mung bean, Vigna radiata L., seeds (George W. Park Seed Co. Inc., lots B-1398 and B11-78) were planted in moist vermiculite and germinated in a growth chamber at 21-26°C with a 16 hour photoperiod. The light source (General Electric Power Groove Fluorescent Lamps) provided a light intensity of 9,000 lux at plant level. The temperature was maintained at 21-26°C. The seedlings, which were ready for use in 9-10 days were cut 3 cm below the cotyledonary node. The cotyledons were also removed. The cutting thus consisted of two primary leaves, the trifoliate bud, the epicotyl, and 3 cm of hypocotyl (12,18). Five uniform cuttings were placed in each vial containing a section of chromatogram or extract. The original level of the solution was maintained by daily additions of distilled water. The cuttings were placed in a growth chamber with a 16 hour photoperiod at a temperature of 21-26°C. The

visible roots per cutting were counted after seven days.

The cotyledons were removed to increase the sensitivity of the mung bean bioassay to root-promoting substances. Bassuk and Howard (2) concluded that cotyledons were a major source of endogenous root-promoting substances. Additionally, they concluded that a consistent and sensitive bioassay could only be obtained by removing the cotyledons from the seedlings. Removing the cotyledons four days after sowing produced the most sensitive bioassay. The proper time to remove the cotyledons, however, is ultimately dependent on the germination conditions under which they are grown.

Results and Discussion

75% Methanol Extracts The extracts derived from cuttings taken from both the upper and lower portions of Colorado blue spruce trees stimulated mung bean root initiation in the absence of IAA (Figure 1A). The average number of roots per cutting increased relatively linearly with the relative concentration of extract. Similar increaes were noted in bioassays of extracts containing 10^{-5}M IAA (Figure 1B). There was, however, no significant difference in rooting response in regard to location of cutting material or presence of IAA (Table 1). Since the addition of IAA did not significantly increase root initiation, the possibility exists that IAA is a constituent of the extracts. A similar finding was noted in English ivy where Hess (12) detected three growth-promoting substances in juvenile and mature English ivy using an Avena coleoptile bioassay. One of the growth-promoting substances was located at the position of

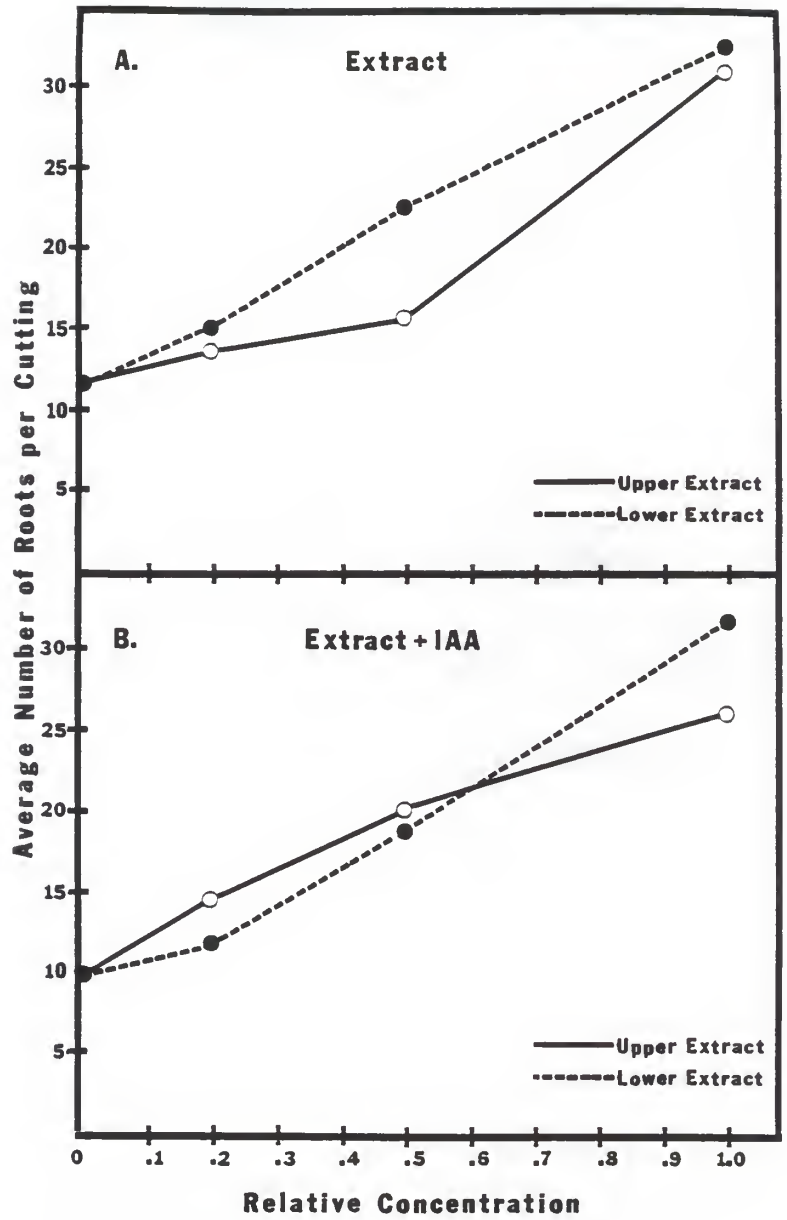

Fig. 1. The effect of 75% (v/v) aqueous methanolic extracts obtained from upper and lower cuttings of Picea pungens upon root initiation in the mung bean. Rooting response to extracts only (A) or extract plus IAA (B). The relative concentration represents the amount of extract in milliliters utilized from 0.25 gram dry weight of tissue.

Table 1. Analysis of variance of mung bean rooting responses to methanolic extracts (75% v/v aqueous methanol) obtained from cuttings of Colorado blue spruce. Data was analyzed as a complete factorial, interactions of interest below.

Source	d.f.	ANOVA SS	F Value	Pr>F[z]
Tree	1	30.67	0.42	0.5183
Position	1	78.75	1.08	0.3013
Tree*Position	1	0.03	0.00	0.9841
IAA	1	78.75	1.08	0.3013
Rel. Concentration	3	7638.07	34.84	0.0001*
IAA*Rel. Con.	3	55.13	0.25	0.8603
Position*IAA	1	34.01	0.47	0.4964
Position*Rel. Con.	3	108.13	0.49	0.6916
Position*IAA*Rel. Con.	3	211.47	0.96	0.4133

[z]Probability of greater F. Starred numbers are less than 5%.

IAA. Since both juvenile and mature English ivy contained similar growth-promoting substances, Hess concluded that there was no relationship between auxin activity and the rooting ability of juvenile and mature English ivy, other root-promoting substances must be involved.

The above results demonstrate the existence of root-promoting substance(s) in the extracts of stem cuttings in Colorado blue spruce (Figure 2). The precise number of root-promoting substances in the extract is unknown. Also, the presence of rooting inhibitors within the extract is possible. Paper chromatography is one method by which the extracts can be separated into their various components. Paper chromatography and mung bean bioassays were used to determine the number of root-promoting substances within the extracts. The mung bean bioassay was utilized because of its relative insensitivity to IAA and its positive response to the presence of rooting cofactors (11). The test, therefore, is a method to detect root-promoting substances (other than IAA) thought to be present in easy-to-root cuttings.

The above results and subsequent studies were conducted with data collected from only two of the three trees (see page 38).

Fractionated Methanol Extracts An analysis of variance of mung bean rooting responses to methanolic extracts chromatographed with isopropanol:water (8:2 v/v) indicated several significant variables and interactions at the 5% level of significance (Table 2).

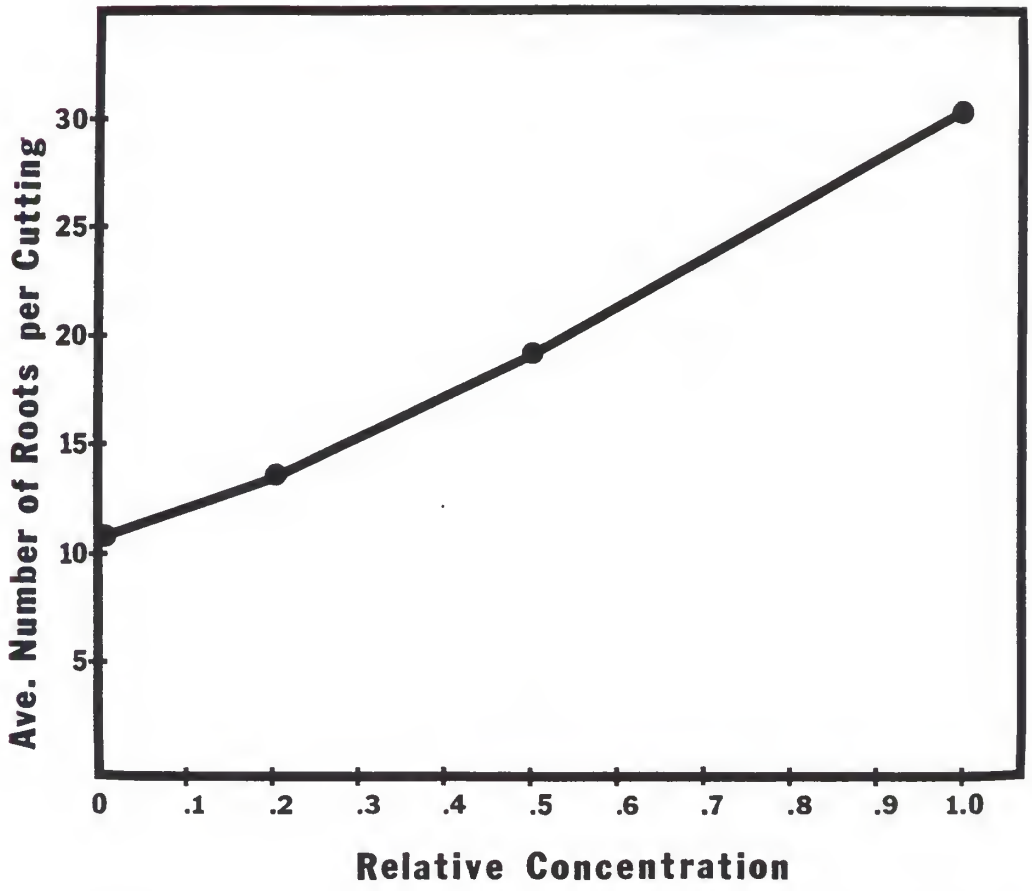

Fig. 2. The effect of 75% (v/v) aqueous methanolic extracts obtained from cuttings of Picea pungens upon root initiation in the mung bean. Represents the average rooting response to extract and extract plus IAA.

Table 2. Analysis of variance of mung bean rooting responses to methanolic extracts obtained from cuttings of Colorado blue spruce. The extracts were chromatographed with isopropanol: water (8:2 v/v). Data was analyzed as a complete factorial, interactions of interest below.

Source	d.f.	SS	F Value	Pr>F[z]
Tree	1	7.91	0.15	0.6962
Tree*R_f	9	223.03	0.48	0.8897
Tree*IAA	1	58.64	1.13	0.2880
Tree*Position	1	71.42	1.38	0.2410
Position	1	248.93	4.80	0.0289*
Position*IAA	1	120.84	2.33	0.1274
Position*R_f	9	781.41	1.68	0.0917
Position*IAA*R_f	9	449.36	0.96	0.4700
IAA	1	4,346.93	83.90	0.0001*
IAA*R_f	9	1,849.32	3.97	0.0001*
R_f	9	10,286.91	22.06	0.0001*

[z]Probability of greater F. Starred numbers are less than 5%.

The presence or absence of IAA significantly affected the rooting response of mung bean cuttings. An average of 10.20 roots per cutting were noted in controls containing distilled water. Alternatively, controls containing 5 X 10^{-6}M IAA produced an average of 12.70 roots per cutting, indicating that IAA alone promotes root initiation in mung bean.

Mung bean bioassays of chromatograms without IAA produced an average of 11.31 roots per cutting, demonstrating that the extract alone also promoted root initiation. The addition of IAA to chromatograms of extracts of Colorado blue spruce produced a synergistic rooting response. An average of 17.75 roots per cutting were noted in mung bean bioassays conducted with chromatographed extracts plus IAA.

The extracts from cuttings of Colorado blue spruce contain root-promoting substances which react synergistically with IAA. In an effort to characterize the root-promoting substances extracted from English ivy, Hess (13) demonstrated that certain phenolic compounds promote rooting of mung bean. These compounds stimulate root initiation alone, but react synergistically with IAA. Hess concluded that the root-promoting substances in English ivy, as well as certain dihydroxy phenols, serve as cofactors of IAA. Since the chromatographed extracts of stem cuttings of Colorado blue spruce also react synergistically with IAA, the root-promoting substances in these extracts may be rooting cofactors.

The location or position of the stem cuttings also had a significant effect on root initiation in mung bean cuttings

(Table 2). Chromatographed extracts of upper cuttings produced an average of 15.17 roots per cutting, while lower cuttings produced 13.89 roots per cutting. This difference in rooting response to extracts of upper and lower stem cuttings contradicted earlier results and expectations. There was no significant difference in rooting response in regard to location of cutting material when complete extracts were examined. This discrepancy could possibly be explained by the different amounts of tissue utilized for each experiment. The equivalent of a 0.025 gram sample per assay was used to detect the presence of root-promoting substances in the initial study, while the equivalent of a 0.05 gram sample was used when the extracts were fractionated. Another possible explanation is that 75% methanol was used to elute the complete extract, while absolute methanol was used to determine the number of root-promoting substances in the stem cuttings via paper chromatography. The absolute methanol may have been more efficient in extracting the root-promoting substances. Also, the chromatographic procedure separates root-inhibiting and promoting compounds. Different levels of inhibitors in the complete extracts of upper and lower stem cuttings may also have accounted for the initial lack of an effect of position on mung bean rooting response.

In terms of rooting response, an interaction existed between IAA and R_f (Table 2). Chromatographed methanolic extracts in the presence of IAA produced three obvious rooting peaks (Table 3). IAA enhanced rooting at R_f's 0.3-0.4, 0.7-0.8, and

Table 3. Mung bean rooting responses to methanolic extracts of blue spruce in the presence and absence of IAA.

R_f	Mean number of roots per cutting		
	IAA	No IAA	Combination
0 - .1	10.90	9.60	10.250
.1 - .2	9.80	8.75	9.275
.2 - .3	13.60	9.15	11.375
.3 - .4	21.10	10.55	15.825
.4 - .5	14.45	10.10	12.275
.5 - .6	14.15	9.55	11.850
.6 - .7	18.45	8.90	13.675
.7 - .8	21.65	9.55	15.600
.8 - .9	12.70	8.40	10.550
.9 -1.0	43.25	29.35	36.300
Control	15.25	10.50	12.875

0.9-1.0. Chromatographed methanolic extracts without IAA produced a slight root-promoting effect at R_f 0.3-0.4 and a more pronounced peak at 0.9-1.0. The fraction at 0.7-0.8 had little effect on root initiation.

While the root-promoting substances alone increased the rooting of mung bean, the rooting response was enhanced in the presence of IAA. The substances at R_f's 0.3-0.4, 0.7-0.8, and 0.9-1.0 may serve as cofactors of IAA. These root-promoting fractions correspond to cofactors 2, 3, and 4, respectively, found by Hess (12,13) in the easy-to-root, juvenile form of Hedera helix and the red-flowering Hibiscus rosa-sinensis. The R_f values of cofactors 2, 3, and 4 were: 0-0.13, 0.33-0.53, and 0.80-0.93 respectively.

Some compounds within the extract also significantly affected root initiation in mung bean in the absence of IAA (Table 3). Again there are three major rooting peaks at R_f's 0.3-0.4, 0.7-0.8, and 0.9-1.0. Similar root-promoting activity was noted by Kawase (17) in centrifugal diffusates of several species of woody ornamentals. Four root-promoting fractions were located at R_f's 0.01, 0.2-0.4, 0.6-0.8, and 0.9-1.0 in mung bean bioassays conducted without IAA.

While the extracts of stem cuttings of Colorado blue spruce contain three root-promoting substances, their distribution within the plant is of prime concern. Methanolic extracts of stem cuttings from the upper one-third portion of the trees contained three areas which enhanced rooting in the presence of IAA (Table 4). These fractions were located at R_f's 0.3-0.4,

Table 4. Mung bean rooting responses to methanolic extracts of upper and lower stem cuttings of blue spruce in the presence and absence of IAA (5×10^{-6}M). The total extract applied to each chromatogram represents 0.50 gram dry weight of plant tissue.

	Upper cutting extract				Lower cutting extract		
	Mean number of roots per cutting				Mean number of roots per cutting		
R_f	IAA	No IAA	Difference[z]	R_f	IAA	No IAA	Difference[z]
0- .1	10.1	10.4	-0.3	0- .1	11.7	8.8	2.9
.1- .2	8.0	8.6	-0.6	.1- .2	11.6	8.9	2.7
.2- .3	15.6	8.6	7.0	.2- .3	11.6	9.7	1.9
.3- .4	23.6	11.1	12.5*[y]	.3- .4	18.6	10.0	8.6
.4- .5	15.7	9.3	6.4	.4- .5	13.2	10.9	2.3
.5- .6	15.9	9.7	6.2	.5- .6	12.4	9.4	3.0
.6- .7	17.6	8.3	9.3	.6- .7	19.3	9.5	9.8
.7- .8	22.7	8.3	14.4*	.7- .8	20.6	10.8	9.8
.8- .9	12.1	9.0	3.1	.8- .9	13.3	7.8	5.5
.9-1.0	50.1	30.9	19.2*	.9-1.0	36.4	27.8	8.6
Control	16.7	11.5	5.2	Control	13.8	9.5	4.3

[z]Mean number of roots per cutting with IAA minus the mean number of roots per cutting without IAA.

[y]Mean seperation by protected LSD. Overall error rate of five percent by using the Bonferroni procedure (12), comparison significant at .0025*.

0.7-0.8, and 0.9-1.0. Chromatographed extracts of upper cuttings without IAA promoted rooting at 0.3-0.4 and 0.9-1.0. The fraction at 0.7-0.8 had no appreciable effect on root initiation.

While not statistically significant at an overall error rate of 5%, the extracts of lower cuttings produced three rooting peaks which correspond to the root-promoting fractions of the upper extracts. Chromatographed extracts in the presence of IAA enhanced rooting at R_f's 0.3-0.4, 0.7-0.8, and 0.9-1.0. Fractions at R_f's 0.3-0.5, 0.7-0.8, and 0.9-1.0 promoted rooting of cuttings in the absence of IAA. Since methanolic extracts of both upper and lower stem cuttings contain three root-promoting fractions, these results provide no basis for the difference in rooting response.

The high root-promoting activity at R_f 0.9-1.0 in mung bean bioassays conducted without IAA may be due to the presence of endogenous IAA in the stem cuttings. Another possible factor affecting rooting in the last fraction may be the chromatography paper. Bassuk and Howard (1) reported that Whatman No. 3 MM chromatography paper may contain a root-promoting compound which travels with the solvent (n-butanol:acetic acid:water, 63:10:27 v/v) front. Evidence to support this hypothesis was also reported by Kawase (16).

Fractionated Water Extracts An analysis of variance of mung bean rooting response to water extracts chromatographed with isopropanol:water (8:2 v/v) indicated that the compounds within the various R_f fractions significantly affected the rooting response of mung bean (Table 5). However, there were

Table 5. Analysis of variance of mung bean rooting responses to water extracts obtained from cuttings of Colorado blue spruce. The extracts were chromatographed with isopropanol: water (8:2 v/v). Data was analyzed as a complete factorial, interactions of interest below.

Source	d.f.	SS	F Value	Pr > F[z]
Tree	1	16.00	0.68	0.4104
Tree*R_f	9	259.90	1.23	0.2765
Tree*IAA	1	0.04	0.00	0.9672
Tree*Position	1	33.64	1.43	0.2328
Position	1	38.81	1.65	0.2000
Position*IAA	1	0.01	0.00	0.9836
Position*R_f	9	185.51	0.87	0.5489
Position*IAA*R_f	9	173.59	0.82	0.6006
IAA	1	0.01	0.00	0.9836
IAA*R_f	9	413.29	1.95	0.0434*
R_f	9	957.32	4.51	0.0001*

[z]Probability of greater F. Starred numbers are less than 5%.

no major root-promoting fractions (Table 6). There were small rooting peaks at R_f's 0.0-0.1 and 0.7-0.9 in the presence of IAA. A slight inhibition of rooting was noted at R_f's 0.1-0.3 and 0.5-0.6.

Also, in terms of rooting response in mung bean, an interaction existed between IAA and R_f (Table 5). Yet there is no apparent IAA ehancement of rooting, with the possible exception of the fraction at R_f 0.0-0.1. However, even this fraction is not significantly different when compared to the corresponding fraction without IAA (Table 7).

There was no IAA enhancement of rooting of mung bean cuttings even in the controls (Table 7). This may be due to the natural variability in rooting response within the mung bean cuttings.

The results of chromatograms of upper and lower water extracts are somewhat inconclusive. In the presence of IAA, there was a slight enhancement of rooting at R_f 0.0-0.1. This was noted in chromatographed extracts of both upper and lower stem cuttings. Inhibition of rooting in the presence of IAA occurred at R_f's 0.3-0.4 and 0.9-1.0. Both the increases and declines in rooting were rather small and none were significantly different (Table 7).

The results suggest that the root-promoting substances in Colorado blue spruce are not significantly water soluble. Since rooting cofactors have been characterized by Hess (13) as phenols or lipid-like compounds, they would be, at best, only slightly soluble in water. By isolating and purifying cofactor 4 from

Table 6. Mung bean rooting responses to water extracts of Colorado blue spruce in the presence and absence of IAA.

R_f	Mean number of roots per cutting		
	IAA	No IAA	Combination
0- .1	14.30	10.75	12.525
.1- .2	8.15	9.70	8.925
.2- .3	10.05	8.85	9.450
.3- .4	8.60	11.60	10.100
.4- .5	11.90	10.50	11.200
.5- .6	8.70	9.90	9.300
.6- .7	12.75	12.25	12.500
.7- .8	14.40	13.30	13.850
.8- .9	14.40	13.35	13.875
.9-1.0	11.55	14.70	13.125
Control	12.35	12.45	12.400

Table 7. Mung bean rooting responses to water extracts of upper and lower stem cuttings of blue spruce in the presence and absence of IAA (5×10^{-6}M). The total extract applied to each chromatogram represents 0.50 gram dry weight of plant tissue.

	Upper cutting extract				Lower cutting extract		
	Mean number of roots per cutting				Mean number of roots per cutting		
R_f	IAA	No IAA	Difference[z]	R_f	IAA	No IAA	Difference[z]
0- .1	14.2	10.1	4.1	0- .1	14.4	11.4	3.0
.1- .2	7.8	7.7	0.1	.1- .2	8.5	11.7	-3.2
.2- .3	10.8	9.0	1.8	.2- .3	9.3	8.7	0.6
.3- .4	8.1	11.5	-3.4	.3- .4	9.1	11.7	-2.6
.4- .5	10.4	10.1	0.3	.4- .5	13.4	10.9	2.5
.5- .6	7.1	9.9	-2.8	.5- .6	10.3	9.9	0.4
.6- .7	14.4	11.9	2.5	.6- .7	11.1	12.6	-1.5
.7- .8	15.8	13.7	2.1	.7- .8	13.0	12.9	0.1
.8- .9	14.1	13.7	0.4	.8- .9	14.7	13.0	1.7
.9-1.0	9.7	15.0	-5.3	.9-1.0	13.4	14.4	-1.0
Control	12.9	12.1	0.8	Control	11.8	12.8	-1.0

[z] Mean number of roots per cutting with IAA minus the mean number of roots per cutting without IAA. No significant difference by protected LSD with an overall error rate of five percent by using the Bonferroni procedure (12).

the easy-to-root, juvenile form of English ivy, Hess found that the cofactor was soluble in various alcohols but only slightly soluble in water.

Other evidence has suggested that root-promoting compounds are water soluble. Kawase (16,17) detected four root-promoting substances in several deciduous and evergreen shrubs by water extraction and by centrifugal diffusion. The cofactors corresponded to those found in methanolic extracts of Hedera helix and Hibiscus rosa-sinensis by Hess. The chemical nature of these water soluble, root-promoting substances has not been determined. To date, no direct comparison has been made between those cofactors tentatively identified by Kawase and those identified by Hess.

The results from these studies strongly suggest that the methanolic extracts from upper and lower cuttings of Colorado blue spruce contain three root-promoting substances located at R_f's 0.3-0.4, 0.7-0.8, and 0.9-1.0. Since these substances react synergistically with IAA, one might conclude that they may be cofactors of IAA. Further evidence suggests that there are three root-promoting substances in both upper and lower stem cuttings.

The presence of three root-promoting substances in both upper and lower stem cuttings provides no basis for the difference in rooting response between upper and lower cuttings of Colorado blue spruce. Other factors must be involved to account for this difference.

Previous research has detected the presence of four root-

promoting substances in easy-to-root plants. Hess (12,13) found four root-promoting substances or cofactors in the juvenile form of English ivy. Bassuk and Howard (1) tentatively identified four cofactors in sap extracted from apple stem cuttings. This rooting activity was closely correlated with the natural seasonal rooting response. The results from Colorado blue spruce indicate the presence of only three root-promoting substances or cofactors. The presence of three root-promoting substances, rather than four, may play a part in preventing the propagation of Colorado blue spruce from cuttings. However, it is believed that other, more important factors are involved.

Cuttings may fail to root because of the absence of polyphenol oxidase. Researchers have proposed that cofactors react with auxin in the presence of polyphenol oxidase to form the complex "rhizocaline" (10). The presence of the auxin-cofactor complex in parenchymatous cells stimulates the cells to become meristematic and form root initials. Research has confirmed that the absence of polyphenol oxidase may inhibit rooting. Bassuk et al. (3) noted increased rooting of apple cuttings by treating the bases of the cuttings with polyphenol oxidase. Alternately, polyphenol oxidase may be present but inactivated by enzyme inhibitors.

Another factor which may prevent the successful propagation of blue spruce cuttings is the presence of rooting inhibitors. Fadl and Hartmann (6) reported high levels of root-promoting activity in extracts of easy-to-root 'Old Home' pear (Pyrus) cuttings, while the difficult-to-root 'Bartlett' pear contained large amounts of inhibitors. Two plant hormones

known to inhibit root initiation are cytokinins and gibberellins.

Another factor which may affect the rooting ability of cuttings is the C/N ratio (10). High levels of carbohydrates in stem cuttings favor root development, while high levels of nitrogen in cuttings are not conducive to good rooting. Generally, the best rooting response is obtained from cuttings high in carbohydrates but rather low in nitrogen. In rooting studies conducted with grape cuttings, 63% of cuttings with high levels of starch rooted, 35% of the cuttings with medium levels of starch rooted, while only 17% of the cuttings with a low starch content rooted. The amount of lignification may also be involved. There is a correlation between the rooting of apple stem cuttings and the amount of fiber formation (14). Further studies need to be done to ascertain the factors which directly effect rooting of Colorado blue spruce cuttings.

In this study softwood terminal cuttings were taken from Colorado blue spruce. According to Black (4) however, the position on a branch from which cuttings are taken does influence rooting capacity. This rooting effect is termed branch order position or topophysis. The terminal cuttings utilized in this study would be classified as first order terminal cuttings. Black noted that first order terminal cuttings are somewhat difficult to root. According to his study the first order lateral and second order terminal positions gave the highest rooting response. The phenomenon of topophysis may be responsible for the variable rooting responses reported by

nurserymen.

Lee et al. (18) added boron to the solution prior to the mung bean bioassay. It appears the inclusion of boron promotes root growth, rather than root initiation (9). Although boron was not used in this study, addition of a small quantity may have proved beneficial. The main benefit being faster root growth, possibly resulting in a more sensitive mung bean bioassay.

Although IBA and NAA are more stable than IAA in plant tissue, all auxins seem to have similar modes of action. All auxins promote adventitious rooting in cuttings, with IBA and NAA generally more effective than IAA. The addition of only a small amount of IAA (10^{-5} or 5×10^{-6}M) resulted in a reliable mung bean bioassay. IBA has also given reliable results (1,3). Quantity of auxin would seem to be the important factor.

It was previously mentioned that a tree was omitted from this study. The extracts of lower cuttings produced a greater rooting response than extracts of upper cuttings in the majority of trees. However, the extract obtained from the upper cuttings of one tree exhibited a higher rooting response than the extract of lower cuttings (Table 8). Therefore, the data obtained from this tree was omitted from the statistical analysis because of its distorted rooting response.

Table 8. Analysis of variance of mung bean rooting responses to methanolic extracts (75% v/v aqueous methanol) obtained from cuttings of Colorado blue spruce. Data was analyzed as a complete factorial, interactions of interest below.

Source	d.f.	ANOVA SS	F Value	Pr $>F^z$
Tree	2	163.18	1.13	0.3259
Position	1	10.58	0.15	0.7025
Tree*Position	2	448.22	3.10	0.0475*
IAA	1	19.22	0.27	0.6068
Rel. Concentration	3	8266.48	38.11	0.0001*
IAA*Rel. Con.	3	61.78	0.28	0.8375
Position*IAA	1	551.12	7.62	0.0064*
Position*Rel. Con.	3	52.15	0.24	0.8679
Position*IAA*Rel. Con.	3	173.41	0.80	0.4985

[z]Probability of greater F. Starred numbers are less than 5%.

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RELATIONSHIP BETWEEN ROOTING SUBSTANCES AND THE ROOTING RESPONSE OF COLORADO BLUE SPRUCE

by

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ABSTRACT

There is no significant difference in the level of endogenous root-promoting substances in upper and lower stem cuttings of Colorado blue spruce (Picea pungens Engelm.). Mung bean bioassays of extracts from both upper and lower stem cuttings produced a relatively linear increase in rooting with the relative concentration. Chromatographed extracts of both the upper and lower stem cuttings appear to contain three root-promoting fractions. The root-promoting fractions were located at R_f's 0.3-0.4, 0.7-0.8, and 0.9-1.0. The addition of IAA enhanced the rooting response at these fractions. The root-promoting fractions, therefore, may contain compounds which serve as cofactors of IAA. Mung bean bioassays of chromatographed water extracts of upper and lower cuttings failed to detect any root-enhancing substances. These results indicate that the rooting cofactors in Colorado blue spruce are not significantly water soluble.