Affect of Foliage on Uptake and Movement of IAA-2-^{14}C

A comparison was made between cuttings with all leaves removed from the uppermost inch and cuttings with no terminal foliage removed to determine the affect of additional leaf scars and absence of photosynthetic surface on uptake and subsequent movement in the stem. Four cuttings from each treatment were harvested after 2 days and auxin extracts were made as previously described. Four more cuttings were harvested from each group after 7 days.

Almost twice as much auxin was taken up in cuttings with defoliated apices as compared to those with foliated apices (Table 3). It was found

Table 3. Effect of defoliation on uptake and movement of ^{14}C in cuttings of *Ilex crenata* 'Convexa' after 10 second terminal dip in IAA-2-^{14}C.

Treatment	Locus (inches)	Number of days after application	
		Counts per minute 2	7
foliated apex.....	1st	3630	4511
	2nd	1214	746
	3rd	537	299
	4th	2275	1372
	Total	7656	6928
defoliated apex.....	1st	9513	14390
	2nd	2299	3464
	3rd	608	555
	4th	220	612
	Total	12530	19021

that less isotope was carried to the base of defoliated cuttings. Cuttings with defoliated apices had less than ⅔ of the total leaf area of the other cuttings. Perhaps this partly explains the lack of polar movement of auxin if it is in some way connected with movement of photosynthate. Defoliated treated cuttings exhibited a strong inhibition of terminal and lateral shoot growth during and after rooting. This was not observed when terminal applications were made to foliated cuttings. It will be noted that there is approximately 3 times as much label in the upper inch of the defoliated cuttings. It is also possible that some of the label was held in the xylem in the upper segments of those cuttings and therefore was not in a position to be readily translocated to the basal area. Cuttings with defoliated apices were found to develop roots at approximately the same time as foliated cuttings but the root system was not as large. The additional auxin in the defoliated cuttings must have been taken up through the freshly exposed leaf scars since there was no other port of entry which was not previously available in cuttings with foliated apices. Basally treated cuttings were also partially defoliated at the base when they were prepared for treatment and it was possible that auxin absorbed in basal dips was partly absorbed through leaf scars. It could be possible that leaf scars are one of the primary ports of entry of auxin in stem cuttings.

The Relationship between Rooting Cofactors of Easy and Difficult-to-root Cuttings of Three Clones of *Rhododendron*[1]

Choong Il Lee,[2] John J. McGuire, and John T. Kitchin,
Rhode Island Agricultural Experiment Station, Kingston

Abstract. The level of endogenous root-promoting and inhibiting substances in 3 clones of rhododendron were compared at seasonal intervals in order to study the clonal and seasonal variation in rooting response of cuttings. The highest levels of 4 rooting cofactors in any season were found in both stem and leaf tissue of *Rhododendron* cv. 'Cunningham's White' followed by 'English Roseum'. The clone 'Dr. H. C. Dresselhuys' contained the least amount of the rooting cofactors. An inhibitor was often found in all clones, but it appeared less responsible for clonal differences in rooting response than variation in levels of the rooting cofactors. Rooting cofactor levels contained in the stem tissue of 'Cunningham's White' were not less than those in the leaf tissue. In contrast, cofactor levels present in the stem tissue of 'English Roseum' and 'Dr. H. C. Dresselhuys' were less than those in the leaf tissue. The promoting activity of rooting cofactors in all tissues of the clones increased in September and decreased again in November to the level of July extract. The inhibitor found in the July extracts disappeared in September and reappeared in November.

Rooting of cuttings of 'Dr. H. C. Dresselhuys' was significantly improved by grafting a leaf and bud scion of 'Cunningham's White'. On the other hand, scions of 'Dr. H. C. Dresselhuys' resulted in decreased rooting of cuttings of 'Cunningham's White'. Rooting capacity of 'English Roseum' was less affected by a leaf and bud scion of other clones of *Rhododendron.*

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Introduction

EXTREMELY poor rooting of cuttings of some clones of *Rhododendron* is one of the factors which decrease the production efficiency of this ornamental. Some endogenous rooting factors, other than auxin, which control rooting are believed to occur in easy-to-root cuttings of some genera, but to be present in a smaller amount or absent in the difficult-to-root ones (1, 3, 4, 5, 6). Hess (2) suggested that the presence of 4 root-promoting substances, named rooting cofactors, in the extracts obtained from stem tissues of juvenile form of *Hedera helix* L. cuttings was responsible for its high rooting capacity. Rooting cofactors have also been found in chrysanthemum, hibiscus, camellia, and were related to rooting ability (2, 4, 6). They have not previously been studied in *Rhododendron.*

Objectives of this study were to determine: 1) if rooting cofactors or inhibitors exist in the stem and leaf tissue of 3 clones of rhododendron; 2) the relationship between the substances and rooting response of cuttings; 3) differences in content of the substances between stem and leaf tissues; and 4) the seasonal changes in the levels of the substances.

Materials and Methods

Experiment I

Sampling procedure. An easy-to-root clone of *Rhododendron* 'Cunningham's White', intermediate-to-root

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clone of 'English Roseum', and difficult-to-root clone of 'Dr. H. C. Dresselhuys' grown under natural conditions were used in the study. Terminal stem cuttings with leaves were taken on July 10, September 15, and November 15, 1967. Each time they were placed immediately in a cold room at minus 20°C for 24 hr to prevent changes in growth substances. The lower half inch of stem from each frozen cutting was discarded and the remainder was then cut into 5 to 10 mm segments. Stem and leaf segments of each clone were dried separately for 18 to 20 hr until brittle dry in a lyophilizer. Dried samples were then ground, at 0°, in a Wiley intermediate mill to pass a 60 mesh screen and refrigerated at minus 20° until extracted.

Extraction and chromatographic separation. One gram samples of lyophilized ground tissue were extracted with 3 successive 25 ml aliquots of chilled anhydrous methanol at 0°. Each extraction period was for 30 min, with intermittent shaking. The extracts were filtered, combined and taken to dryness in a vacuum oven at 35 ± 2°, which required 10 to 12 hr. The residue was taken up in 4 ml of anhydrous methanol at room temperature.

One-fourth of a ml of the redissolved extract was applied as a band 4.0 cm from the bottom of a 5 cm wide strip of water washed Whatmann No. 3 MM chromatographic paper using a 25 lambda capillary pipette. After streaking, the papers were allowed to equilibrate in the dark for 12 hr at 2°, and then were immersed in a solvent system of isopropanol: water (8:2 v/v) for development at that temperature. There was a minimum of chlorophyll streaking at the low temperature used. The solvent flow was terminated when the front had ascended 30 cm above the starting line, which required 42–44 hr. The chromatograms were then dried in a hood for at least 2 hr.

Preparation of mung bean seedlings. Dry seeds of mung bean, *Phaseolus aureus* Roxb. were treated for 3 to 5 minutes in a solution of 1 part of Clorox (5.25% commercial sodium hypochlorite) to 15 parts of water, they were then rinsed and soaked in running tap water for 24 hr. A volume of 250 ml of the dry seeds was enough to obtain 1500 seedlings.

The seeds were planted in moist horticultural medium grade perlite using a plant tray 56x30x6 cm. The seeds were germinated in a controlled environment chamber maintained at 28 ± 2°, 16 hr photoperiod with a light intensity of 1000 ft-c at plant level, and a relative humidity of approximately 40%. The light source was supplied by eight 40 watt cool white fluorescent lamps and two 75 watt incandescent bulbs. Seedlings were watered lightly every 2 days and were ready for use in 7 to 8 days.

Cuttings were prepared by removing the seedling root system 3 cm below the cotyledonary node. The cuttings consisted of 3 cm of hypocotyl, 4 to 5 cm of epicotyl tissue, primary leaves, and the trifoliated bud. Cotyledons were removed when they had not abscissed at the time the cuttings were prepared.

Mung bean rooting bioassay. The mung bean bioassay developed by Hess (3, 4) was used to determine the root-promoting activity of the separated extracts. The dried chromatograms, as mentioned above, were cut into 15 2-cm sections and a control section was cut from the paper above the solvent front. Each section was then rolled and placed in a 19×65 mm shell vial. After the section had been placed in the vials, 4 ml of 5×10^{-6}M IAA containing 1 ppm boron were added (8). A period of 1 hr was allowed for equilibration before 5 prepared mung bean cuttings were placed in each vial. The vials were placed under the same environmental conditions used for growing the seedlings.

The 4 ml of IAA solution and the eluate from the chromatogram section was mostly taken up by the cuttings within 40 hr and the vials were refilled to the cotyledonary node with distilled water. They were checked once each day and the water level was maintained at the cotyledonary node. At the end of 6 to 7 days cuttings were removed and the number of roots initiated and partially developed root initials per cutting were counted.

Statistical analysis. The mung bean bioassay was repeated 3 times at each of the 3 seasons. This provided 3 replications for each of the sampling dates. Statistical differences were determined by use of two way analysis of variance. Treatment means were compared for significance by the least significant difference method.

The average number of roots initiated in the check vial and those at the region of R_f values, 0.07–0.13, 0.27–0.33, 0.60–0.67, and 0.80–0.87, as reported by Hess (2) to be rooting cofactor 1, 2, 3, and 4 respectively and also at the inhibitory region of R_f 0.00–0.07 were recorded. The amount of each cofactor or inhibitor was indicated by number of roots on the mung bean cutting.

EXPERIMENT II

Reciprocal side grafts were made to determine the effects of transmittable endogenous substances from scion to root stock on rooting response of the cuttings in the 3 clones of rhododendron.

Preparation of cuttings and grafting technique. Terminal 4 inch stem cuttings of rhododendron were taken on September 13, 1967 and immediately placed at 0° for 24 hr. The lowest inch of stem and flower buds if

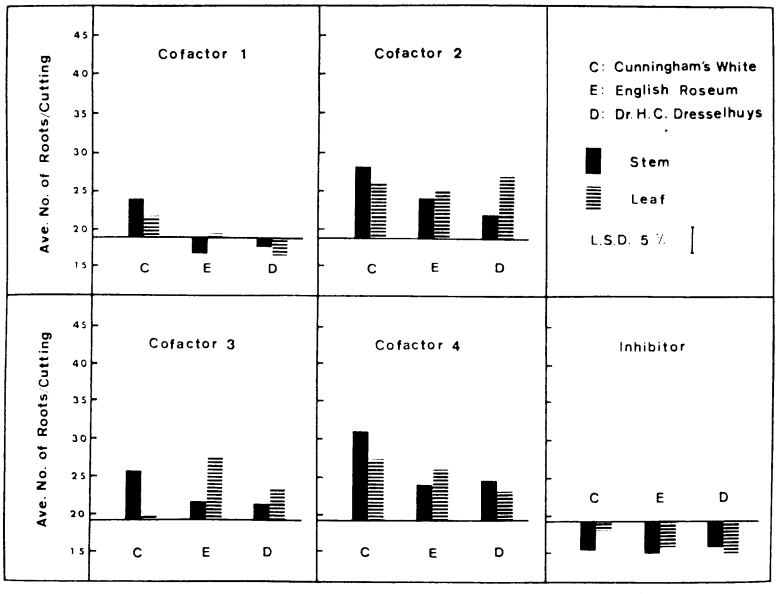

Fig. 1. Number of roots per mung bean cutting treated with rooting cofactors and inhibitor extracted from *Rhododendron* shoots collected on July 10, 1967.

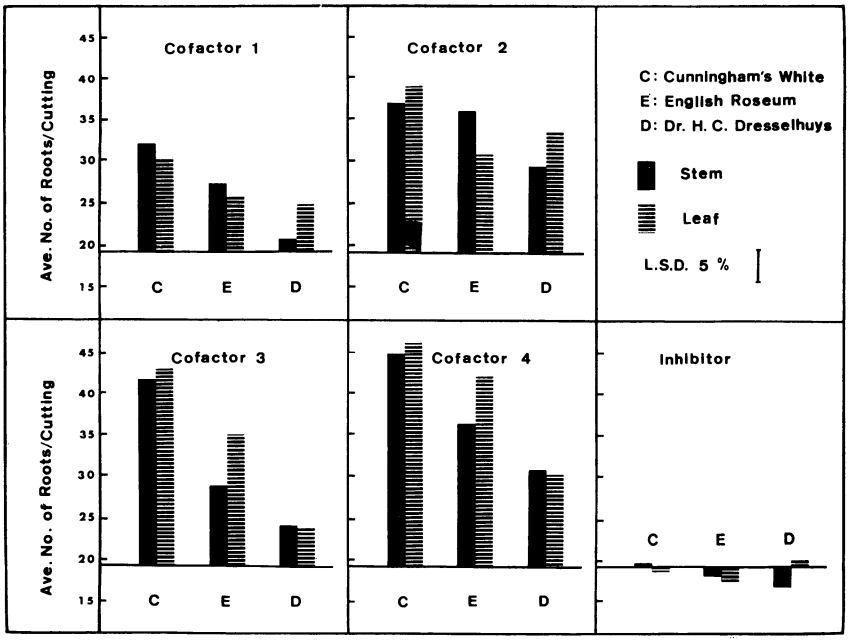

Fig. 2. Number of roots per mung bean cutting treated with rooting cofactors and inhibitor extracted from *Rhododendron* shoots collected on September 15, 1967.

present were removed. Each cutting contained 5 leaves. One-third to one-half of each leaf was removed depending on the leaf size. One of the leaves on the stem was replaced by a leaf and bud scion of another clone using the side graft method. The leaf of the scion was not shortened. All possible combinations of grafts among the 3 clones of 'Cunningham's White', 'English Roseum', and 'Dr. H. C. Dresselhuys' were made. Cuttings in each clone without grafting were used as controls.

Auxin treatment and planting. All cuttings were wounded slightly along 2 sides of the lowest inch of stem and were dipped for 10 sec in an auxin solution of 0.1% IBA, 0.1% NAA, and 50 ppm boron in ethanol. Cuttings were then planted in a randomized block design in a medium of 50% sphagnum peat moss and 50% perlite. Within a month the graft unions were healed. After 3 months the number of cuttings rooted and the diameter of the root ball on the cuttings were recorded.

Results and Discussion

EXPERIMENT I

The root-promoting activity of extracts from stem and leaf tissues of 3 rhododendron clones as indicated by number of roots initiated on mung bean cuttings is shown in Fig. 1, 2, and 3. The horizontal line in the histograms represents the average number of roots per cutting on the control. Columns above the horizontal line and below the horizontal line represent promotion and inhibition, respectively, of root initiation in comparison to controls.

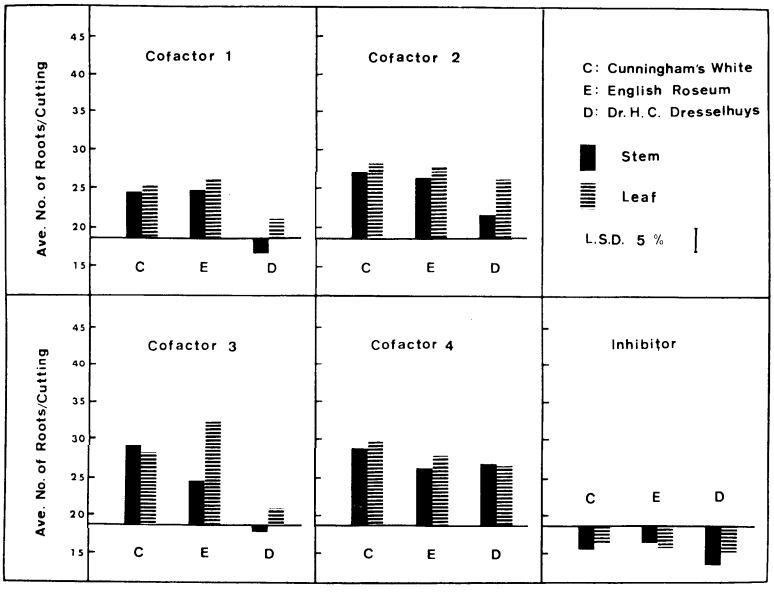

Fig. 3. Number of roots per mung bean cutting treated with rooting cofactors and inhibitor extracted from *Rhododendron* shoots collected on November 15, 1967.

July experiment. Extracts from stem tissue of 'Cunningham's White' contained 4 root-promoting substances found at R_f 0.10, 0.30, 0.63, and 0.83. Two rooting cofactors, 2 and 4, were found in significant amounts in extracts from stem tissue of 'English Roseum', and only cofactor 4 was found in stem tissue of 'Dr. H. C. Dresselhuys' in significant amounts (Fig. 1). A rooting inhibitor was found in significant quantities at the region of R_f 0.03 in stem extracts of 'Cunningham's White' and 'English Roseum'. 'Dr. H. C. Dresselhuys' had the inhibitor present but not in a significant amount.

Leaf tissue of 'Cunningham's White' contained significant amounts of two cofactors, 2 and 4, and leaf tissue of 'English Roseum' had three cofactors; 2, 3, and 4. Only cofactor 2 was present in a significant amount in leaf tissue of 'Dr. H. C. Dresselhuys'. No significant inhibiting effect was found in extract from leaf tissue of 'Cunningham's White' or 'English Roseum', but 'Dr. H. C. Dresselhuys' contained the inhibitor in a significant amount.

September experiment. A considerable increase in rooting cofactor levels, and a decrease in inhibiting activity, as measured by root initiation, was found in each clone as compared to the July extracts (Fig. 2). Extracts from stems of 'Cunningham's White' and 'English Roseum' contained 4 cofactors with no significant amount of inhibitor. Extracts from 'Dr. H. C. Dresselhuys' stems had significant amounts of two rooting cofactors, 2 and 4, and no significant inhibiting activity was found. Extracts from leaf tissue exhibited similar trends in amounts of rooting cofactors and inhibitor with those in the extracts of stem tissues.

November experiment. The decrease of rooting cofactor levels and reappearance of the inhibitor in November extracts is remarkable. 'Cunningham's White' and 'English Roseum' stems still contained all 4 cofactors with no significant amounts

of inhibitor, but 'Dr. H. C. Dresselhuys' stem tissue had only cofactor 4 and the inhibitor in significant amounts.

Leaf tissue of 'Cunningham's White' and 'English Roseum' had all 4 rooting cofactors, whereas 'Dr. H. C. Dresselhuys' contained only two cofactors, 2 and 4, in significant amounts. The inhibitor was not found in significant amounts in the leaf tissue of any clone.

These results generally showed that in every season the levels of endogenous rooting cofactors were greatest in 'Cunningham's White', followed in order by 'English Roseum' and 'Dr. H. C. Dresselhuys'. 'Cunningham's White' roots very easily with 100% rooting, 'English Roseum' shows intermediate rooting response of 71% rooting, and 'Dr. H. C. Dresselhuys' has 29% rooting (Table 1). These rooting

Table 1. Effect of different scions on size of root ball and per cent of cuttings rooted for 3 *Rhododendron* clones.

Group	Stock	Scion	Diameter of root ball (inches)	% rooted
I.....	C	None	2.88	100
	C	E	1.95	92
	C	D	1.56	88
II.....	E	None	1.36	71
	E	C	1.68	92
	E	D	1.39	84
III.....	D	None	0.22	29
	D	C	1.36	96
	D	E	0.87	51

C = *Rhododendron* 'Cunningham's White', E = *Rhododendron* 'English Roseum', D = *Rhododendron* 'Dr. H. C. Dresselhuys'.
L.S.D. at 5% level for root ball diameter: Group I = 0.96, Group II = N.S., Group III = 0.86.
L.S.D. at 5% level for % rooted: Group I = 7.60, Group II = N.S., Group III = 42.00.

percentages are similar to the number of rooting cofactors and it seems likely that differences between clones of *Rhododendron* in rooting response may be related to the level of rooting cofactors contained in the cuttings. Other investigators (1, 3, 6) have reported a relationship between rooting ease and amount of endogenous root-promoting substances.

The effect of substances inhibitory to root initiation has been studied in many plants (1, 3, 6). From these experiments, although a root-inhibiting area could also be found, it would seem that the inhibitor was not a factor in the clonal variation of rooting response. In September extracts neither stem nor leaf tissue of any clone contained the inhibitor in significant amounts and in November extracts only 'Dr. H. C. Dresselhuys' stem tissue showed inhibiting activity.

EXPERIMENT II

Table 1 shows the effect of different scions on size of root ball and number of cuttings rooted for 3 *Rhododendron clones.* A leaf and bud scion of 'Cunningham's White' significantly improved both rooting percentages and root ball size of cuttings of 'Dr. H. C. Dresselhuys'. Similar scions did not significantly increase rooting of intermediate-to-root clone of 'English Roseum' cuttings, but the tendency for increased rooting capacity could be found.

Scions of 'English Roseum' did not decrease rooting on 'Cunningham's White' cuttings nor did they significantly increase rooting percentage or root ball diameter of cuttings of 'Dr. H. C. Dresselhuys'.

Scions of 'Dr. H. C. Dresselhuys' reduced rooting response of 'Cunningham's White' cuttings. These scions did not influence the rooting capacity of 'English Roseum' cuttings. Other investigators (6, 7) have found that rooting of some difficult-to-root cuttings could be increased by grafting onto the cutting a leaf and bud scion from an easy-to-root cultivar.

Results in this experiment indicate the presence of endogenous root-promoting substances in scions and it shows a clonal variation in amount of these substances. It seems likely too, that improving rooting capacity of 'Dr. H. C. Dresselhuys' may be due primarily to the increase of unidentified rooting cofactors in the leaf and bud of the scion which was a rich source of the promotive substances.

No significant differences were found between these clones of rhododendron in the amount of inhibition of rooting when extracts from cuttings taken in September were used in the mung bean bioassay (Fig. 2). These facts indicate that rooting cofactors may be more responsible for the rooting response of *Rhododendron* cuttings than is the inhibitor. They also indicate that auxins are not the only root-inducing factor, and rooting response is effected by the amount of endogenous root-promoting substances present in the cuttings.

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