ROOTING OF CUTTINGS OF *ACER RUBRUM* L. AND *EUCALYPTUS CAMALDULENSIS* DEHN.*

By E. P. Bachelard† and B. B. Stowe‡

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Summary

Rooting of cuttings of *A. rubrum* and *E. camaldulensis* taken from seedlings grown under controlled conditions was stimulated by auxin treatments. Indolylbutyric acid at a concentration of 1·0 mg/l was the optimal auxin treatment. Rooting of cuttings was affected by the light conditions under which seedlings were grown.

In addition to auxin, leaves were essential for optimal rooting. Nitrogen compounds alone or together with sucrose appeared to play some role in this leaf effect, at least with *A. rubrum* cuttings, but this did not appear to be a direct role.

Gibberellic acid and kinetin both inhibited rooting when applied at the base of *A. rubrum* cuttings. This inhibitory effect of gibberellic acid was noted even in the presence of added auxin. In contrast to its effect when applied at the base, kinetin stimulated rooting of *A. rubrum* cuttings when applied to the leaves. There was no evidence that this stimulatory effect was due to changes in the auxin–kinetin balance.

Variation in rooting of replicate cuttings was observed; this variation in rooting of *A. rubrum* cuttings was correlated positively with the amount of anthocyanin found in the leaves. A possible link between rooting and anthocyanin synthesis is discussed and a speculative scheme of reactions important in root initiation is presented.

I. Introduction

The rooting of cuttings is one method by which plants can be propagated vegetatively. It is the method of choice in cases where cuttings can be rooted readily. Unfortunately cuttings from many plants root only with difficulty or not at all. This is particularly true with cuttings from forest trees.

A number of factors may affect the rooting of cuttings from trees. These include (i) the age of the tree from which the cutting is taken (Thimann and Delisle 1939; Heitmuller 1952); (ii) the position of the cutting on the tree (Snow 1938; Edgerton 1944); (iii) the type of cutting used, i.e. greenwood (succulent) or "hardwood" cuttings (Nienstaedt *et al.* 1958); (iv) the time of year at which cuttings are taken (Snow 1938; Heitmuller 1952; Enright 1958); (v) the sex of the parent tree (Snow 1942; Edgerton 1944); (vi) the nutrient status of the cutting (Nienstaedt *et al.* 1958); (vii) the environmental conditions under which cuttings are rooted (Nienstaedt *et al.* 1958). It is likely that all of these factors have a physiological basis and that an understanding of the physiology of root initiation is the key to the successful rooting of cuttings.

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† Present address: Forests Commission of Victoria, Melbourne.

‡ Department of Biology, Yale University.

Auxins have been shown to stimulate rooting on cuttings from many species (Thimann and Bohnke-Rogers 1950). Auxins, however, are ineffective in overcoming the limitations in rooting imposed by the factors specified above. An important generalization is that auxins fail to stimulate rooting on cuttings which are never known to root without them (Avery and Johnson 1947). A number of studies (e.g. Went 1938; Thimann and Delisle 1939; van Overbeek and Gregory 1945) have shown that factors other than auxin are involved in the rooting of cuttings. The nature of these factors is, however, unknown.

This paper presents some results from a study designed to investigate the physiology of root initiation on cuttings of *Acer rubrum* L. and *Eucalyptus camaldulensis* Dehn.

II. Material and Methods

Cuttings were taken from *A. rubrum* and *E. camaldulensis* seedlings grown at 23°C in vermiculite and irrigated with a complete nutrient solution under continuous light and under 16 hr of light each day. Some *A. rubrum* cuttings were also taken from seedlings grown under 8 hr of light each day. Light was provided by a mixture of fluorescent and incandescent lamps; the intensity was approximately 1400 f.c. in all cases. *A. rubrum* seedlings were cut just above the cotyledons to provide single cuttings, whereas three equal segments were cut from *E. camaldulensis* seedlings to provide tip, median, and basal cuttings. *A. rubrum* cuttings carried four or six leaves unless otherwise specified. Two half-leaves only were retained on *E. camaldulensis* cuttings.

Test tubes (18 by 150 mm), filled with solutions under test, were placed in light-tight boxes. The exposed tops of these tubes were covered with caps made of aluminium foil. Cuttings were inserted through these caps into the darkened solutions. All tests were made in continuous light at 26°C.

Solutions were replenished as required. All treatments were completely randomized.

III. Experimental and Results

(*a*) *Effect of Auxins and other Growth Regulators on Rooting*

In addition to auxins, the growth regulators gibberellic acid and kinetin have been shown to affect the rooting of cuttings. Gibberellic acid generally inhibits rooting even in the presence of added auxin (Brian, Hemming, and Radley 1955; Brian 1957). Kinetin also may inhibit rooting (De Ropp 1956; Humphries and Maciejewska-Potapczyk 1960) but Allsopp and Sweykowska (1960) found that kinetin induced root formation on *Marsilea* (water fern) leaves under conditions where rooting had never been observed previously.

The effects of various concentrations of the auxins 3-indolylacetic acid (IAA), 3-indolylbutyric acid (IBA), α-naphthaleneacetic acid (NAA), β-naphthoxyacetic acid (NOA), and *p*-chlorophenoxyacetic acid (CPOA), of gibberellic acid, and of kinetin on the rooting of *A. rubrum* cuttings taken from seedlings which were 4–5 weeks old are shown in Tables 1 and 2. Some cuttings, especially the short cuttings taken from seedlings grown under 8 hr of light each day, died during treatment and were not included in the results.

TABLE 1

EFFECT OF IAA, NOA, GIBBERELLIC ACID, AND KINETIN ON ROOTING OF A. RUBRUM CUTTINGS

Cuttings taken from 4-weeks-old seedlings grown under either continuous light or 16 hr or 8 hr light. Mean number of roots per cutting 15 days after treatment shown

Rooting Solution	Concn. (mg/l)	Continuous Light		16 Hr Light		8 Hr Light	
		Mean No. of Roots (± S.E.)*	No. of Cuttings†	Mean No. of Roots (± S.E.)	No. of Cuttings	Mean No. of Roots (± S.E.)	No. of Cuttings
Distilled water (control)	—	7·8±1·0	18	11·3±1·4	18	3·6±0·5	10
IAA	0·3	15·1±2·7	18	19·1±2·8	20	6·2±1·1	13
	1·0	20·1±4·3	15	33·2±4·9	16	9·4±1·9	12
	3·0	20·7±5·7	16	27·7±8·7	16	9·8±2·1	15
	10·0	6·4±4·0	11	5·9±3·5	7	2·0±2·0	7
NOA	0·03	3·6±1·4	15	8·9±1·2	19	3·7±0·5	13
	0·1	8·2±1·5	14	13·2±1·2	19	6·1±1·3	13
	0·3	12·3±2·1	16	17·2±2·4	19	5·8±0·8	14
	1·0	11·1±2·8	15	25·8±2·6	19	6·9±0·9	15
Gibberellic acid	0·03	0·1±0·1	16	0·8±0·3	18	3·5±0·6	8
	0·1	0·3±0·2	15	0·5±0·3	15	2·6±0·5	17
	0·3	0·8±0·3	17	0	14	2·1±0·5	12
	1·0	0·9±0·5	14	0·3±0·1	18	1·3±0·4	8
Kinetin	0·1	0·2±0·1	18	0·8±0·5	18	0·3±0·2	9
	0·3	0·1±0·1	16	0	18	0·4±0·4	7
	1·0	0	12	0	18	0	12
	3·0	0	11	0	15	0	14

* Standard error of the mean.

† Number used to estimate mean; originally 20, difference indicates number which died during treatment.

TABLE 2

EFFECT OF IBA, NAA, AND CPOA ON ROOTING OF A. RUBRUM CUTTINGS

Cuttings taken from 4-weeks-old seedlings which were grown under either continuous light or 16 hr or 8 hr light. Mean number of roots per cutting 19 days after treatment shown

Rooting Solution	Concn. (mg/l)	Continuous Light		16 Hr Light		8 Hr Light	
		Mean No. of Roots (± S.E.)*	No. of Cuttings†	Mean No. of Roots (± S.E.)	No. of Cuttings	Mean No. of Roots (± S.E.)	No. of Cuttings
Distilled water (control)	—	0·3±0·2	18	0·5±0·3	16	4·0±2·7	3
IBA	0·3	12·5±3·8	18	15·2±3·2	20	8·3±3·5	10
	1·0	31·5±4·3	19	32·1±5·2	19	18·7±3·5	10
	3·0	19·3±6·1	15	28·7±7·1	15	10·2±3·4	11
	10·0	12·5±6·2	8	15·3±5·7	7	0	4
NAA	0·3	6·5±2·5	16	7·6±2·0	19	7·7±2·1	6
	1·0	7·1±2·3	16	5·2±2·3	19	3·0±1·9	7
	3·0	6·6±2·7	17	9·6±4·0	14	3·0±1·2	8
	10·0	2·2±1·6	5	3·3±1·9	12	0	2
CPOA	0·03	1·5±0·6	20	0·6±0·5	19	1·7±1·2	6
	0·1	0·7±0·3	18	1·7±0·6	20	0·2±0·2	5
	0·3	0·4±0·3	18	0·9±0·7	17	2·0±0·3	6
	1·0	3·3±1·2	19	1·8±0·8	18	0·3±0·3	6

* Standard error of the mean.

† Number used to estimate mean; originally 20, difference indicates number which died during treatment.

Although there was considerable variability in the rooting of replicate cuttings, these results show that IAA, NOA, IBA, and NAA all had some stimulatory effect on rooting. CPOA had no stimulatory effect. The optimal auxin treatment was IBA at a concentration of 1·0 mg/l. Comparisons (paired *t*-tests) of mean numbers of roots formed after treatments with IAA, NOA, IBA, and NAA showed that cuttings from seedlings grown under 16 hr of light formed most roots ($P < 0{\cdot}01$); cuttings from seedlings grown under 8 hr of light formed least roots ($P < 0{\cdot}001$).

Root formation was essentially complete 3 weeks after the cuttings were placed in the solutions. A progressive lag in the time of maximum rate of root formation on cuttings treated with increasing concentrations of IAA was interpreted as indicating that IAA was gradually broken down in solution. IBA, a synthetic auxin is more stable in solution and no lag in the time of maximum rate of root formation was observed with increasing concentrations of this auxin.

Data in Table 1 show that gibberellic acid and kinetin both inhibited rooting of *A. rubrum* cuttings. Higher concentrations of gibberellic acid, however, were required to inhibit rooting on cuttings taken from seedlings grown under 8 hr of light each day than on cuttings from seedlings grown under longer light periods.

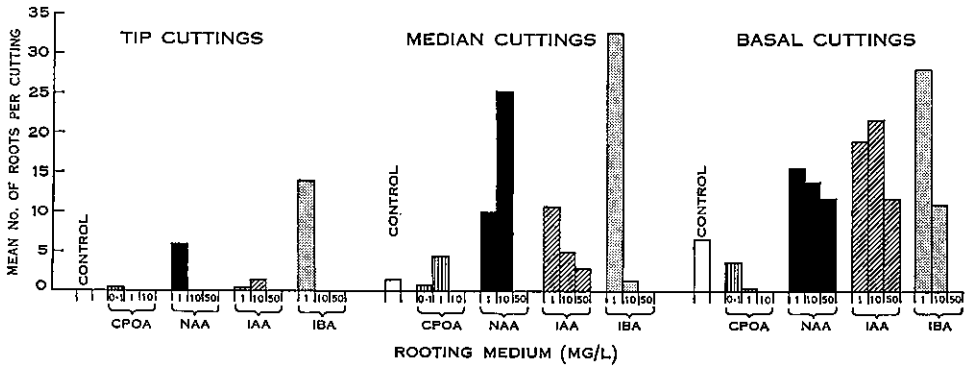

Fig. 1.—Rooting of *E. camaldulensis* cuttings treated with various concentrations of *p*-chlorophenoxyacetic acid (CPOA), naphthalene acetic acid (NAA), 3-indolylacetic acid (IAA), and 3-indolylbutyric acid (IBA). Distilled water used as controls.

In addition to the variability in rooting of replicate cuttings observed in these experiments, there was a marked difference in the rooting of cuttings in distilled water in the two experiments. Cuttings used in both experiments were taken from seedlings of the same age grown from seed under identical environmental conditions. The experiments were, however, carried out at different times.

In the case of *E. camaldulensis* the effect of various concentrations of auxins on the rooting of cuttings taken from 12-weeks-old seedlings is shown in Figure 1. Ten replicate cuttings, five from seedlings grown under continuous light and five from seedlings grown under 16 hr of light, were used. Analysis of variance of the data from this experiment showed that the type of cutting used and the rooting solutions each had a highly significant effect on rooting ($P < 0{\cdot}001$).

Basal cuttings were the only ones to produce a substantial number of roots in the absence of added auxin and these cuttings were far less sensitive to variations

in auxin concentration than were tip and median cuttings. Tip cuttings formed much fewer roots than either median or basal cuttings. With all types of cutting, however, IBA at a concentration of 1·0 mg/l was the most effective auxin treatment. NAA and IAA each had some effect whereas CPOA had no effect in stimulating rooting. The higher concentrations of auxin used in this experiment were frequently toxic. A feature of root formation on *E. camaldulensis* cuttings in this and in subsequent experiments was that roots formed fastest on basal cuttings and slowest on tip cuttings.

(*b*) *Effect of Leaves on Rooting*

(i) *Necessity for Leaves*

The necessity for leaves in rooting some cuttings has been reported (van Overbeek and Gregory 1945), and the requirement for leaves for optimal rooting of *A. rubrum* and *E. camaldulensis* cuttings is shown quite clearly in Figure 2 and Table 3.

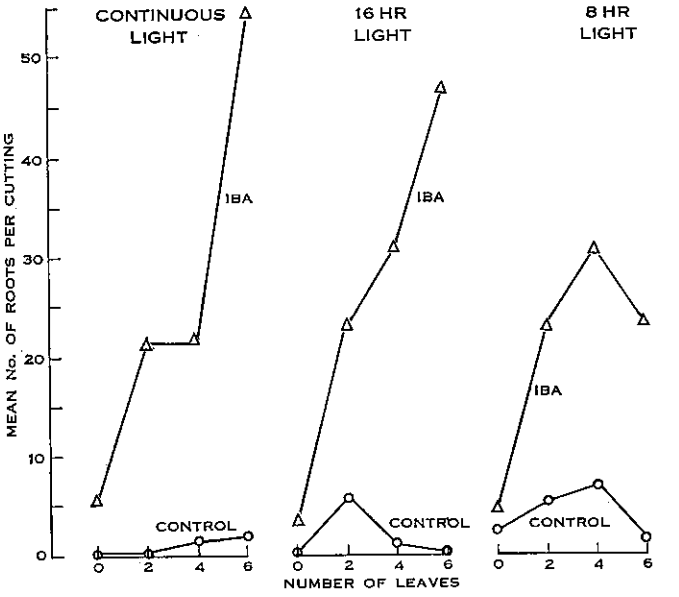

Fig. 2.—Effect of leaves on the rooting of *A. rubrum* cuttings taken from 12-weeks-old seedlings grown under either continuous light, or under 16 hr or 8 hr of light each day. Rooting solution IBA (1·0 mg/l); distilled water used as controls.

Ten replicate cuttings from 12-weeks-old seedlings were used in these experiments. With *A. rubrum* cuttings the oldest leaves were removed first; all leaves were removed from some *E. camaldulensis* cuttings.

It is frequently stated (e.g. Hartmann and Kester 1959) that the main requirement for leaves or buds in rooting cuttings is as a source of auxin. This was clearly not the case in these experiments as auxins were ineffective in promoting rooting in the absence of leaves and leaves had no stimulatory effect in the absence of added auxin.

TABLE 3

EFFECT OF PRESENCE OF LEAVES ON ROOTING OF E. CAMALDULENSIS CUTTINGS TREATED WITH IBA

Values are mean number of roots per cutting ± standard error of the mean after 18 days of treatment. Number of cuttings used in calculating mean given in parenthesis

IBA Concn. (mg/l)	Leaves Present			Leaves Absent		
	Tip Cuttings	Median Cuttings	Basal Cuttings	Tip Cuttings	Median Cuttings	Basal Cuttings
0	0·3±0·3 (10)	2·7±1·3 (10)	10·9±1·9 (10)	0·7±0·4 (10)	0 (9)	0·8±0·7 (9)
0·03	2·6±1·5 (9)	9·6±2·9 (10)	17·1±3·5 (10)	1·6±0·6 (9)	1·0±0·6 (10)	1·0±0·4 (10)
0·1	2·5±1·6 (10)	16·5±3·7 (10)	19·6±2·9 (10)	4·3±1·2 (9)	0·3±0·3 (6)	3·8±1·2 (10)
0·3	6·6±2·5 (9)	25·6±3·7 (10)	34·4±8·5 (10)	4·6±1·7 (8)	2·2±0·9 (9)	2·3±0·7 (8)
1·0	23·3±2·5 (6)	39·2±7·2 (10)	30·7±6·2 (9)	6·0±2·1 (9)	1·9±0·9 (8)	2·2±0·8 (10)
Mean	5·7	18·7	22·4	3·4	1·1	2·0

TABLE 4

EFFECT OF PRETREATMENT WITH VARIOUS CHEMICALS IN REPLACING THE REQUIREMENT FOR LEAVES IN THE ROOTING OF A. RUBRUM CUTTINGS

Cuttings taken from seedlings grown under either continuous or 16 hr of light. Rooting solution IBA (1·0 mg/l). Mean number of roots per cutting ± standard error of the mean after 23 days treatment shown

Pretreatment	Continuous Light			16 Hr Light		
	0 Leaves	2 Leaves	6 Leaves	0 Leaves	2 Leaves	6 Leaves
Distilled water	5·6±2·0	5·1±1·5	21·8±6·0	3·6±1·6	4·3±1·8	23·9±9·1
Ammonium sulphate (1 g/l)	6·3±2·1	10·8±2·4	16·9±7·1	7·0±2·5	14·0±4·6	29·0±9·2
Arginine (10 mg/l)	4·8±1·3	8·4±2·9	12·3±5·6	5·1±1·9	7·5±2·7	19·0±6·4
Sucrose (4%)	6·4±2·0	9·4±4·3	8·0±3·1	7·4±1·9	3·3±1·8	10·1±4·1
Sucrose (4%) + ammonium sulphate (1 g/l)	11·4±3·6	15·6±4·2	14·0±4·3	8·5±2·7	11·6±2·0	18·6±6·9
Sucrose (4%) + arginine (10 mg/l)	5·8±2·1	5·4±2·9	15·9±7·0	10·2±3·1	9·7±3·3	13·4±5·6

(ii) *Replacement of Requirement for Leaves by Chemicals*

Van Overbeek, Gordon, and Gregory (1946) found that the requirement for leaves in rooting some *Hibiscus* cuttings could be replaced completely by treatments with sucrose and nitrogen compounds. Such treatments were only partially successful in replacing the requirement for leaves in rooting other cuttings. The effect of basal pretreatments, for 24 hr, with solutions containing sucrose and nitrogen compounds on the rooting of *A. rubrum* cuttings carrying varying numbers of leaves is shown in Table 4. Ten replicate cuttings taken from 4–5-weeks-old seedlings were used; the oldest leaves were removed first.

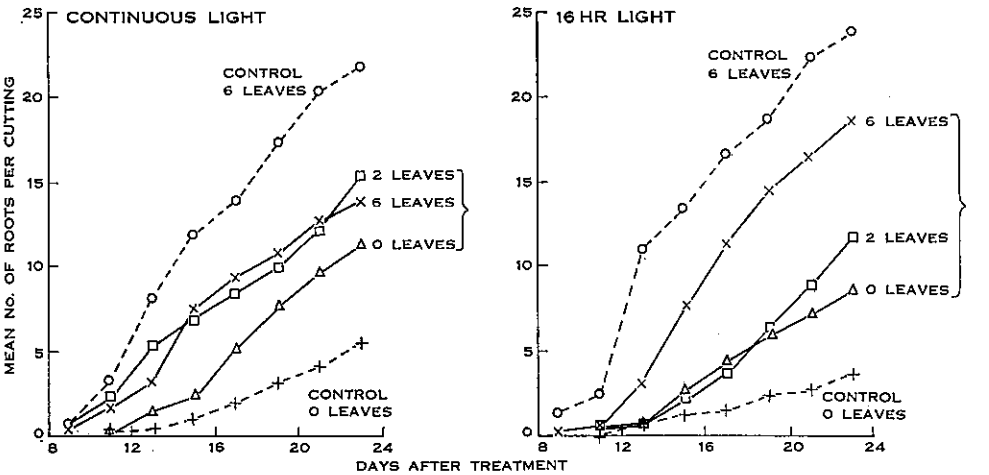

Fig. 3.—Time course of root formation on *A. rubrum* cuttings carrying different numbers of leaves and treated (bracketed curves) with sucrose plus ammonium sulphate or not treated (controls). Rooting solution IBA (1·0 mg/l).

The rooting response of replicate cuttings in this experiment was again very variable but treatment with sucrose plus ammonium sulphate significantly increased ($P < 0{\cdot}05$) rooting on two-leaved cuttings compared with two-leaved control cuttings. Treatment with sucrose plus ammonium sulphate also showed some signs of increasing rooting on leafless cuttings. However, the time course of root formation (see Fig. 3) indicated that treatment with sucrose plus ammonium sulphate did not increase rooting on leafless cuttings until after 13–15 days in the rooting solution. By this time new leaves had formed on "leafless" cuttings. Other treatments which showed some signs of increasing rooting on "leafless" and two-leaved cuttings were ammonium sulphate alone and sucrose plus arginine. Treatment with sucrose alone appeared to depress rooting on cuttings carrying a full complement of leaves.

In a subsequent experiment, basal pretreatments with ammonium sulphate or potassium nitrate significantly stimulated ($P < 0{\cdot}01$) rooting on *A. rubrum* cuttings taken from 7-weeks-old seedlings and carrying one of the oldest leaves (Table 5).

Treatment with arginine had no effect. The inhibition of rooting of cuttings in IBA (1·0 mg/l) by gibberellic acid at a concentration of 0·03 mg/l was highly significant ($P < 0·01$).

Basal pretreatments with sucrose and ammonium sulphate were completely ineffective in replacing the requirement for leaves in rooting *E. camaldulensis* cuttings (Fig. 4). This experiment differed from that with *A. rubrum* cuttings in that all new leaves were removed as they formed.

TABLE 5

EFFECT OF BASAL PRETREATMENT WITH NITROGEN-CONTAINING COMPOUNDS ON ROOTING OF A. RUBRUM CUTTINGS ROOTED IN EITHER IBA SOLUTION ALONE OR TOGETHER WITH GIBBERELLIC ACID

Cuttings were taken from 7-weeks-old seedlings and carried one of the oldest leaves. Mean number of roots per cutting after 22 days in the rooting solutions given

Pretreatment	Rooting Solutions	
	IBA (1·0 mg/l)	IBA (1·0 mg/l) + Gibberellic Acid (0·03 mg/l)
Distilled water	7·7	2·2
Arginine (10 mg/l)	4·9	2·8
Ammonium sulphate (1 g/l)	18·5	6·9
Potassium nitrate (1·4 mg/l)	21·2	8·9

(c) *Effect of Leaf Treatments with Kinetin on Rooting*

Results described above showed that leaves were essential for optimal rooting of *A. rubrum* and *E. camaldulensis* cuttings. Nitrogen compounds alone or together with sucrose appeared to play some role in this leaf effect, at least with *A. rubrum* cuttings. Richmond and Lang (1957) and Mothes and Engelbrecht (1961) have shown that kinetin may have a marked effect on the movement of nitrogen compounds in leaves. It was of interest to determine whether applications of kinetin to the leaves affected rooting of cuttings.

In a preliminary experiment, an unexpected result was that kinetin (10 mg/l) when applied to the leaves of *A. rubrum* cuttings increased rooting. This increase in rooting was observed only in the absence of basal pretreatments of the cuttings with solutions containing casamino acids (0·01%) or ammonium sulphate (1 g/l). Basal pretreatments for 24 hr with casamino acids significantly inhibited ($P < 0·01$) rooting of kinetin-treated cuttings.

This stimulation of rooting by kinetin contrasted completely with the inhibitory effect of kinetin when applied at the base of cuttings (see Table 1) and was of particular interest in view of current ideas (cf. Skoog and Miller 1957; Phinney and West

1960) that growth and differentiation in plants depends more on the balance existing between a number of growth substances such as auxins, gibberellins, and kinins rather than on the presence or absence of specific growth substances. The effect of various concentrations of kinetin, applied to the leaves, on the rooting of *A. rubrum* cuttings in several auxin concentrations was examined.

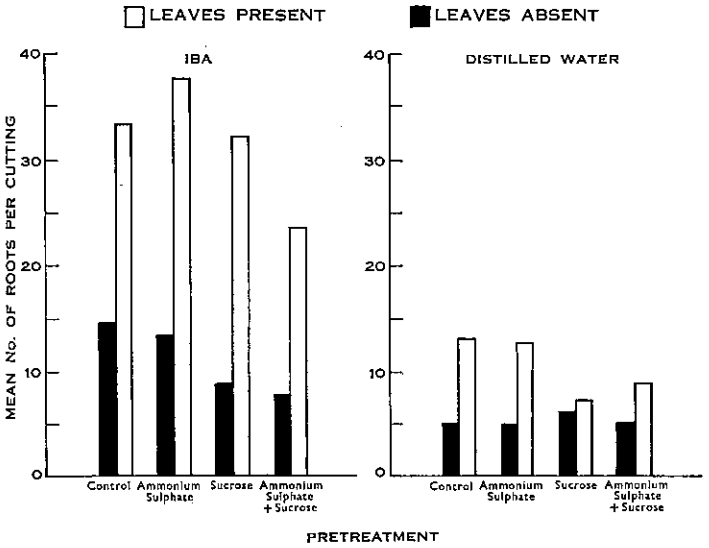

Fig. 4.—Effect of pretreatments with ammonium sulphate and sucrose in replacing the requirement for leaves in rooting *E. camaldulensis* cuttings. Rooting solutions: IBA (1·0 mg/l) and distilled water.

Kinetin was sprayed on the leaves of cuttings immediately before the cuttings were placed in auxin solutions. The kinetin solutions contained a detergent ("Pluronic F68" or "Tween 80") to wet the leaves. "Pluronic F68" is a mixed polyoxyethylene and polyoxypropylene polymer; "Tween 80" is polyoxyethylene sorbitan monooleate. Stowe (1960) showed a synergistic response in growth of excised pea segments to auxin and lipides, and "Tween 80" was used to determine whether lipides had any effect on the rooting of cuttings in auxin solutions. Some cuttings received basal pretreatments for 24 hr with solutions containing 0·01% casamino acids.

Results from this experiment are shown in Table 6. Analysis of these results (Table 7) confirmed that kinetin, applied to the leaves, stimulated rooting of *A. rubrum* cuttings but, in this experiment, basal pretreatments with amino acids did not inhibit rooting of kinetin-treated cuttings. There was no evidence that the balance between auxin and kinetin affected rooting of *A. rubrum* cuttings. Kinetin at a concentration of 0·1 mg/l was equally as effective as higher concentrations in stimulating rooting at all auxin concentrations. Rooting of cuttings treated with "Tween 80" alone was significantly less than rooting of cuttings treated with "Pluronic F68" alone when these cuttings were rooted in IBA (1·0 mg/l).

(d) *Relation between Rooting and Anthocyanin Formation*

In all experiments described above, considerable variation in the rooting of replicate cuttings and in the rooting of cuttings taken at different times was observed. It was obvious that there were marked differences between replicate cuttings, and that these differences were of fundamental importance in root initiation. None of the treatments so far applied was effective in overcoming these differences.

TABLE 6

EFFECT OF KINETIN SOLUTIONS, WHICH WERE APPLIED TO THE LEAVES OF CUTTINGS IN TWO TYPES OF DETERGENT, ON THE ROOTING OF A. RUBRUM CUTTINGS IN IBA SOLUTIONS

Basal pretreatment with 0·01% casamino acids received by some cuttings. Mean number of roots per cutting after 20 days in rooting solutions shown. Number of cuttings used in estimating mean given in parenthesis

Detergent Used	Pretreatment with Casamino Acids	IBA Concn. (mg/l)	Kinetin Concentration (mg/l)				Mean
			0	0·1	1·0	10·0	
"Pluronic F68" (0·05%)	+	0	0·8 (10)	0·5 (11)	3·2 (12)	2·7 (9)	1·8
		0·1	6·5 (13)	7·1 (12)	10·2 (10)	7·6 (13)	7·7
		1·0	9·6 (7)	27·7 (9)	36·3 (11)	28·9 (11)	27·2
		Mean	5·3	10·6	16·3	13·4	
"Tween 80" (0·05%)	+	0	0·5 (11)	4·4 (13)	2·3 (12)	2·3 (13)	2·4
		0·1	3·9 (12)	11·1 (12)	5·5 (12)	13·9 (11)	8·5
		1·0	18·2 (13)	14·2 (11)	29·9 (12)	16·8 (13)	19·8
		Mean	8·0	9·6	12·6	10·9	
"Pluronic F68" (0·05%)	−	0	2·8 (12)	0·9 (14)	1·0 (9)	1·4 (13)	1·5
		0·1	4·6 (13)	15·4 (12)	9·8 (14)	14·9 (12)	11·0
		1·0	26·4 (9)	26·9 (11)	26·4 (12)	24·6 (11)	26·1
		Mean	9·7	13·3	13·2	13·0	
"Tween 80" (0·05%)	−	0	1·6 (13)	4·1 (14)	3·3 (12)	3·6 (14)	3·2
		0·1	10·0 (12)	14·5 (14)	14·0 (10)	13·1 (14)	12·9
		1·0	16·4 (9)	23·8 (13)	11·5 (11)	16·6 (9)	17·4
		Mean	8·5	13·9	9·2	10·3	

One observation which was noted consistently in all experiments with *A. rubrum* cuttings was that heaviness of rooting was correlated positively with the amount of anthocyanin formed in the leaves during the experimental treatment. This relationship has been described previously (Bachelard and Stowe 1962) when the effects of riboflavin and sucrose, chemicals involved in anthocyanin biosynthesis in some systems (Thimann and Edmondson 1949; Thimann and Radner 1958), on the rooting of *E. camaldulensis* cuttings was also reported. Subsequent to that report

it has been shown (Bachelard 1962) that basal pretreatment of *A. rubrum* cuttings with solutions of 8-aza-adenine (0·1–10·0 mg/l) significantly increased ($P < 0·01$) both the percentage of cuttings forming roots and the proportion of cuttings forming anthocyanin in the leaves. Although they by no means prove it these data are consistent with the idea that a link between anthocyanin synthesis and root formation on cuttings may exist.

TABLE 7

ANALYSIS OF VARIANCE OF THE EFFECT OF KINETIN, PRETREATMENT WITH CASAMINO ACIDS, AND THE DETERGENTS "PLURONIC F68" AND "TWEEN 80" ON ROOTING OF A. RUBRUM CUTTINGS IN SEVERAL CONCENTRATIONS OF IBA

Logarithmic transformation of data according to Quenouille (1950)

Source of Variation	Degrees of Freedom	Mean Square	*F*
Detergent	1	0·0197	<1
Kinetin	3	1·2151	3·79**
Casamino acids	1	0·7549	2·36†
Auxin	2	22·8313	71·28***
Interactions			
Detergent × kinetin	3	0·3535	
Detergent × auxin	2	2·0500	6·40**
Kinetin × casamino acids	3	0·0257	
Kinetin × auxin	6	0·2853	
Error	538	0·3203	
Total	559		

** $P < 0·01$. *** $P < 0·001$. † Not significant.

IV. DISCUSSION

Both auxin and leaves were essential for optimal rooting of *A. rubrum* and *E. camaldulensis* cuttings. Nitrogen compounds alone or together with sucrose appeared to play some role in this "leaf effect" in *A. rubrum* cuttings, a finding consistent with that of previous investigators (Stuart 1938; Doak 1940, 1941; Thimann and Poutasse 1941; van Overbeek and Gregory 1945). Nitrogen compounds and sucrose, however, did not appear to replace the requirement for leaves completely or directly. Some leaves, but too few for maximum rooting, were required before nitrogen compounds alone or with sucrose stimulated rooting, and, in no case, did basal treatments with these chemicals speed the rate of root formation above that of cuttings carrying a full complement of leaves.

Rooting of *A. rubrum* cuttings was influenced by the light conditions under which seedlings were grown. Cuttings from seedlings grown under long days rooted better than those from seedlings grown under either continuous light or under short days.

Basal cuttings of *E. camaldulensis* formed roots faster and were less dependent on variations in auxin concentration for rooting than either median or tip cuttings.

These differences in rooting were not due to the different types of leaf carried by the cuttings (Bachelard 1962) and it is possible that substances, including auxin, important in root initiation are most concentrated in the basal segments of *E. camaldulensis* seedlings and least concentrated in the tip segments. Alternatively, it is possible that natural gibberellins which inhibit rooting are produced at the tip (cf. Lockhart 1957).

Both gibberellic acid and kinetin, when applied at the base of *A. rubrum* cuttings, inhibited rooting, but kinetin, when applied to the leaves, stimulated rooting. Allsopp and Sweykowska (1960) reported another instance where kinetin stimulated rooting when applied to leaves. There was no evidence that kinetin stimulated rooting of *A. rubrum* cuttings by altering the auxin–kinetin balance in the cuttings. It is possible that kinetin stimulated rooting through some effect on the nitrogen metabolism in cuttings. The interaction between casamino acids and kinetin observed in one experiment with *A. rubrum* cuttings offers some support for this view. However, this interaction was not observed in a subsequent experiment. Klein and Hagen (1961) found that kinetin stimulated anthocyanin synthesis in *Impatiens balsamina* petals and it is possible that the effect of kinetin in stimulating rooting of *A. rubrum* cuttings is associated with the relation between rooting and anthocyanin formation in these cuttings.

Our brief previous report that variations in rooting of *A. rubrum* cuttings were correlated positively with the amount of anthocyanin formed in the leaves during the experimental treatment can be discussed more completely in the context of the results of this paper. The suggestion is that a link between anthocyanin biosynthesis and root initiation may exist. A number of observations in the literature support the idea of such a link.

In some tree species, particularly broad-leaved species, the only cuttings that can be induced to root are succulent cuttings taken from the current year's growth (Stoutemyer, Jester, and O'Rourke 1940; Nienstaedt *et al.* 1958; Hartmann and Kester 1959). Hartmann and Kester suggested that there may be a higher concentration of some root-promoting hormone in these terminal sections. Price and Sturgess (1938) noted that coloration in young leaves, due to anthocyanins and disappearing with maturity, was a phenomenon much more generally observed than autumnal anthocyanin development.

Both in apple (Stoutemyer 1937) and in ivy (Robbins 1957; Stoutemyer, Britt, and Goodwin 1961) cuttings from juvenile plant material root more readily than cuttings from mature parents. Characteristics of the juvenile form of both these plants include an abundance of anthocyanin. Robbins and Maneval (1924) observed an association between anthocyanin formation and lateral root development on excised corn roots grown in culture in the light. Some factor essential for rooting *Hibiscus* cuttings was present in the leaves of a red-flowered variety but was not present, or was present in much smaller quantities, in leaves of a white-flowered variety (van Overbeek and Gregory 1945). *Spirodela* and *Lemna*, the only anthocyanin-forming genera in the Lemnaceae, are the only genera in this family with roots (Hillman 1961).

There is evidence that anthocyanin-like materials may play a role in the growth of plant tissues. Growth-promoting activity due to leucoanthocyanins has been observed in extracts from *Aesculus woerlitzensis* fruits and in coconut milk (Steward and Shantz 1959). Hillis (1955) found that all the anthocyanin and most of the leucoanthocyanin disappeared from young eucalypt leaves with increasing age. Considerable amounts of leucoanthocyanin were found in the cambial region of *E. regnans* F. Muell. at times of active cambial growth but they were difficult to detect in the cambium after the spring flush of growth had passed.

In addition to these observations, a number of parallels between factors affecting anthocyanin biosynthesis and root initiation may be noted. Factors affecting both processes include:

(1) Carbohydrates and nitrogen compounds.
(2) Light.
(3) Genetic factors.
(4) Physiological age of the tissues.
(5) Seasonal variations.
(6) Position of the tissues on trees.

These observations, together with the results from this study, suggest that some substance involved in anthocyanin biosynthesis may also be involved in root formation. There is evidence that the C_6(B)–C_3 portion of flavonoids is formed from carbohydrates via shikimic acid and that the C_6(A) ring is formed from acetate units (Bogorad 1958; Neish 1960). It is possible that some C_6–C_3 compound is involved in both anthocyanin synthesis and root formation. There was some evidence that "Tween 80", applied to the leaves of *A. rubrum* cuttings, may depress rooting of these cuttings in the optimal auxin concentration of 1·0 mg/l (see Tables 6 and 7). If this is a real depression of rooting a possible explanation would be that acetate units, formed in the leaves by degradation of the fatty acid part of "Tween 80", combine to form C_6 rings which competed for a C_6–C_3 compound important in root initiation. Torrey (1959) found that some substance of a phenolic nature inhibited the formation of lateral roots on isolated pea roots grown in culture. This substance may have competitively inhibited, or combined with, some C_6–C_3 compound.

A speculative scheme of reactions to explain the effects of light, carbohydrates, and nitrogen compounds on the formation of some C_6–C_3 compound important in both anthocyanin synthesis and root formation is shown in Figure 5.

This scheme is based on the assumption that some C_6–C_3 compound, formed from shikimic acid, is important in anthocyanin synthesis and in root formation. It should be noted that shikimic acid had no effect on the growth of *E. camaldulensis* roots in vitro (Bachelard and Stowe 1963); the effect of shikimic acid on the rooting of cuttings was not tested. The scheme involves the participation of both the glycolytic and pentose phosphate pathways of respiration, as pyruvic acid and D-erythrose-4-phosphate, the precursors of shikimic acid, are intermediates of these two pathways.

In addition to its obvious effect on the formation of carbohydrates, light may play a role in this series of reactions by blocking respiration. It has been suggested

that light may block respiration via the glycolytic pathway by maintaining triose phosphate dehydrogenase DPN in the reduced form (Bidwell, Krotkov, and Reed 1955) or by blocking pyruvate oxidation (see Gibbs 1959).

Ammonium nitrogen and nitrate nitrogen have been shown to have differential effects on rooting in some cases (Thimann and Poutasse 1941) indicating that these two sources of nitrogen may exert their effect on rooting in different ways. Bidwell, Krotkov, and Reed (1955) found that ammonium nitrate overcame the inhibition of respiration by light in wheat leaves, a result they attributed to the action of ammonia

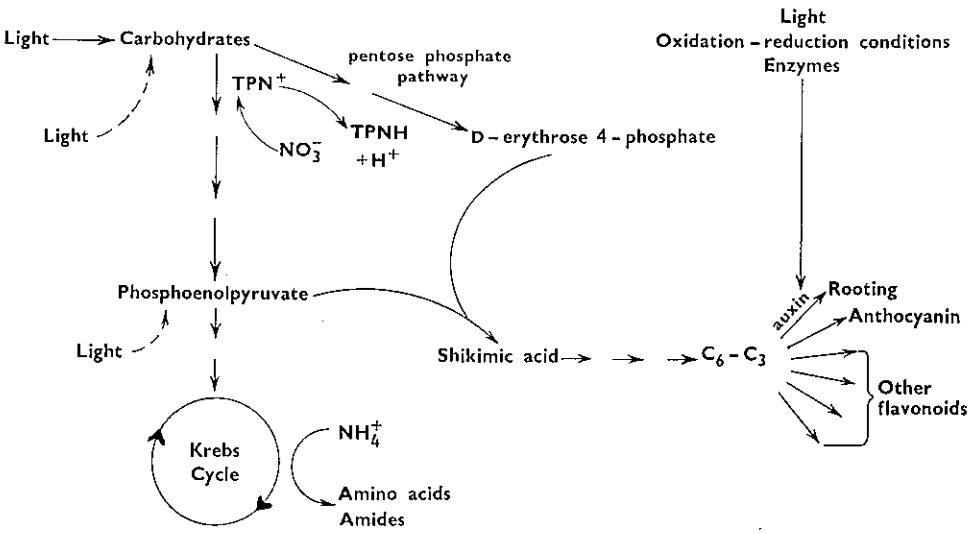

Fig. 5.—Speculative scheme of reactions important in root initiation.

causing a "vacuum" of intermediates in the Krebs cycle. Nitrate reduction is coupled with the oxidation of pyridine nucleotides (Yemm and Folkes 1954; Steward and Pollard 1957; Burris 1959) and, as TPN is reduced during the pentose phosphate pathway of respiration, oxidation of reduced TPN in nitrate reduction could stimulate activity of this pathway. Flavoproteins could also be involved here, thus accounting for the effect of riboflavin in stimulating anthocyanin biosynthesis (Thimann and Radner 1958) and rooting (Bachelard and Stowe 1962).

The ultimate fate of the C_6–C_3 compound may be determined by its exposure to light (cf. Alston 1958), by the oxidation and reduction conditions within the tissues, or by the presence or absence of specific enzymes.

Although this series of reactions is completely speculative with respect to its importance in root formation it would appear to warrant further study.

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