SHORT COMMUNICATIONS

THE INTERRELATIONS BETWEEN ROOT FORMATION AND ANTHOCYANIN SYNTHESIS IN RED MAPLE CUTTINGS: EFFECTS OF GIBBERELLIC ACID, CCC, and 8-AZAGUANINE*

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A relationship between anthocyanin content and root formation in red maple cuttings has been described earlier (Bachelard and Stowe 1962, 1963). Gibberellic acid inhibits rooting of cuttings, including those of red maple (Bachelard and Stowe 1963), and Furuya and Thimann (1964) have recently shown that anthocyanin synthesis in two species of *Spirodela* is inhibited by gibberellic acid, which in one of the species is active at extremely low concentrations. It was, therefore, of interest to study the effect of gibberellic acid on anthocyanin formation in the leaves of red maple cuttings.

Although the so called "antigibberellins", e.g. Amo 1618, Phosfon, and CCC (2-chloroethyltrimethylammonium chloride) have received considerable attention recently there have been few reports of their effects on root formation. Cathey and Stuart (1961) found Amo 1618 to have little or no effect on the rooting of *Chrysanthemum* cuttings. More recently, however, Libbert and Urban (1964) reported a moderate stimulation of rooting in *Convolvulus sepium* cuttings following treatment with CCC. Some experiments in this laboratory (Bachelard, unpublished data) have shown that although CCC may, at times, stimulate rooting of Alaska pea cuttings and that pretreatments with CCC may overcome the inhibitory effect of gibberellic acid, it is not always possible to repeat these results.

In the experiments described below, the effects of gibberellic acid and CCC on root formation and anthocyanin synthesis in red maple cuttings were examined. For comparison, the purine analogue 8-azaguanine, which inhibits anthocyanin synthesis in *Spirodela* (Thimann and Radner 1962), was examined in the same system.

Materials and Methods

Red maple seedlings were grown in a controlled-environment room at 27°C. They received 16 hr of light daily at an intensity of *c.* 800 f.c. Cuttings were made from seedlings 4–5 weeks old (*c.* 6–7 cm tall) by severing them immediately above the cotyledons. The cuttings were supported directly in the solutions under test in the manner described previously (Bachelard and Stowe 1963). They were placed in either the 16-hr-day room described above or under continuous light (intensity *c.* 800 f.c.) at 17°C. The bases of the cuttings, with their root initials, were always tightly screened from the light by covering the test tube with aluminium foil. Basal pretreatments were given by standing the cuttings in the solutions for 24 hr in continuous light (intensity *c.* 300 f.c.) at 25°C. The bases of the cuttings were not protected from light during the pretreatments.

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Clearly visible roots were counted at the end of the rooting period. The anthocyanin in the leaves of each cutting was extracted by cutting the leaves into strips and covering them with 5 ml cold 0·1N hydrochloric acid for 24 hr. The amount of anthocyanin present was estimated spectrophotometrically by measuring the absorption of the extract at 510 mμ which was ascertained to be close to the peak absorption of this pigment. Chromatographic behaviour of this pigment indicated that it is a cyanidin glycoside (Bachelard and Stowe 1962).

TABLE 1

EFFECT OF GIBBERELLIC ACID ALONE OR IN COMBINATION WITH CCC ON ROOTING AND ANTHOCYANIN FORMATION IN RED MAPLE CUTTINGS

Concentration of gibberellic acid in rooting solution was 0·05 mg/l, and of CCC 10^{-4}M. Concentration of indolylbutyric acid in pretreatment solutions was 10 mg/l, of CCC 10^{-2}M, and of gibberellic acid 5 mg/l. Each value is the mean of 6–10 observations. The standard error of the mean is also given for each value

Pretreatment Solution (24 hr)	Rooting Solution	Room 1* (after 4 weeks)		Room 2† (after 2 weeks)	
		Antho-cyanin Content	Roots per Cutting	Antho-cyanin Content	Roots per Cutting
Distilled water	Distilled water	2·48±0·34	7·7±1·55		
Indolylbutyric acid	Distilled water			1·18±0·30	18·5±4·58
Distilled water	Gibberellic acid	1·00±0·11	5·3±0·73		
Indolylbutyric acid	Gibberellic acid			0·63±0·13	5·3±0·97
CCC	Gibberellic acid	1·00±0·30	1·4±0·91		
CCC+indolylbutyric acid	Gibberellic acid			0·77±0·18	1·5±0·72
Distilled water	Gibberellic acid+CCC	0·84±0·14	2·6±0·82		
Indolylbutyric acid	Gibberellic acid+CCC			0·75±0·21	8·5±1·20
Distilled water	CCC	1·65±0·20	9·4±1·75		
Indolylbutyric acid	CCC			1·15±0·17	11·1±1·98
Gibberellic acid	CCC	0·84±0·12	1·4±0·67		

* 16-hr day. † Continuous light.

Results and Discussion

Table 1 shows that gibberellic acid at a concentration of 0·05 mg/l ($1·44 \times 10^{-7}$M) inhibits anthocyanin synthesis in the leaves of red maple by some 50%. This system, then, is as sensitive as the most sensitive system described by Furuya and Thimann (1964). Gibberellic acid also had a strong inhibitory effect on the rooting of cuttings pretreated with 10·0 mg/l 3-indolylbutyric acid. CCC, whether given alone, prior to, following, or in mixture with gibberellic acid had little or no effect on either anthocyanin or root formation. A broad correspondence between root formation and anthocyanin content is thus shown in the results, as indeed was shown in those presented earlier (Bachelard and Stowe 1962).

In their studies on anthocyanin biosynthesis in *Spirodela oligorrhiza*, Thimann and Radner (1962) observed that antagonists of purines and pyrimidines were powerful

inhibitors, 8-azaguanine being one of the most potent. Results from earlier work (Bachelard and Stowe 1963) indicated that pretreatment with 8-aza-adenine may increase both rooting and anthocyanin formation in red maple cuttings. It was of interest, therefore, to determine the effect of 8-azaguanine on root formation and anthocyanin synthesis in red maple cuttings. The concentration of 8-azaguanine used is one which would have caused at least 50% inhibition of anthocyanin synthesis in *Spirodela* (Thimann and Radner 1962). Cuttings in this experiment were rooted in the continuous light room.

Results from this experiment (Table 2) show that gibberellic acid again markedly inhibited the formation both of anthocyanin and of roots. The same concentration of 8-azaguanine also lowered anthocyanin content and, possibly, rooting, but the inhibition caused by 8-azaguanine was very much less than that caused by gibberellic acid. In all instances, the general correspondence between anthocyanin formation and rooting held.

TABLE 2

EFFECTS OF 8-AZAGUANINE AND GIBBERELLIC ACID ON ROOTING AND ANTHOCYANIN FORMATION IN RED MAPLE CUTTINGS

Each value is the mean of 7–9 observations. The standard error of the mean is also given for each value

Rooting Solution	Anthocyanin Content	Inhibition (%)	Roots per Cutting	Inhibition (%)
Distilled water	$1 \cdot 58 \pm 0 \cdot 37$	—	$7 \cdot 7 \pm 4 \cdot 21$	—
Gibberellic acid (5×10^{-7}M)	$0 \cdot 34 \pm 0 \cdot 06$	79	$2 \cdot 1 \pm 0 \cdot 95$	73
8-Azaguanine (5×10^{-7}M)	$0 \cdot 91 \pm 0 \cdot 26$	42	$5 \cdot 7 \pm 2 \cdot 36$	26

The results of these experiments show that gibberellic acid is a remarkably strong inhibitor of both rooting and anthocyanin formation in red maple cuttings—much stronger than an equivalent concentration of the purine analogue, 8-azaguanine. Rooting and anthocyanin formation are the only two processes in plants which gibberellic acid has been found to inhibit, and the results of these experiments are consistent with the earlier hypothesis that root initiation and anthocyanin synthesis are in some way linked (Bachelard and Stowe 1962). It will be important to determine whether such a linkage holds also for other plants.

Treatment of red maple cuttings with phenolic substances which would be expected to be intermediates of anthocyanin synthesis, e.g. cinnamic, phenylpyruvic, shikimic, caffeic, and *p*-coumaric acids at concentrations of 10^{-6}M had no effect on either rooting or anthocyanin formation. This finding is consistent with that observed by Thimann, Edmondson, and Radner (1951) with *Spirodela*.

An interesting and characteristic feature of anthocyanin synthesis in red maple leaves is that the process in this material is reversible. The anthocyanin formed in the leaves of red maple cuttings in the controlled growth rooms was found

to disappear, almost completely, in less than 4 weeks following removal of the cuttings to normal room lighting conditions. The leaves became green, or only weakly reddish, but were perfectly healthy in appearance. The anthocyanin reappeared within 3 days following replacement of the cuttings in the controlled growth rooms, and the amount formed was apparently more or less proportional to the amount present before the fading. Portions of some leaves, shaded by adjacent leaves, did not redden on returning the cuttings to the controlled growth rooms, and the distinction between red and green areas on some leaves was most marked. In view of the more usual great stability of anthocyanin, once formed, in herbaceous plants, this ready reversal warrants further study.

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