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Adventitious Root Formation in Relation to Irradiance and Auxin Supply

B. C. JARVIS and A. I. SHAHEED

Department of Botany,
University of Sheffield,
Western Bank,
Sheffield S10 2TN, U.K.

Abstract. High irradiance during treatment of mung bean cuttings favours root formation in response to supplied auxin, whether the latter is IAA or IBA. On the other hand it is inhibitory towards root formation in the absence of supplied auxin. Light promotes the uptake of ^{14}C-IAA into cuttings and its upward movement into the leaves. When ^{14}C-IAA is applied to leaves of cuttings high irradiance favours movement of radioactivity into the epicotyl and hypocotyl. This movement is also enhanced by concomitant supply of IBA to the base of the cuttings. The irradiance under which stock plants are raised also affects the extent of root formation on cuttings. When cuttings are held in darkness without a supply of exogenous auxin they root best if prepared from seedlings raised under high irradiance. However, transport of ^{14}C-IAA out of leaves of cuttings is favoured when cuttings are prepared from seedlings grown under low irradiance. These observations are discussed in relation to auxin transport, photodestruction and, possibly, metabolism.

Additional index words: rooting, stem cuttings, transport.

Irradiance can have a marked effect on the extent to which stem cuttings produce adventitious roots. The irradiance under which stock plants are raised is important (*e.g.* BOROWSKI *et al.* 1981), as well as the conditions in which cuttings themselves are held (*e.g.* JARVIS and ALI 1984). Nevertheless, the precise conditions which favour root development vary from species to species. Moreover, the direct influence of irradiance on stem cuttings may depend on whether exogenous auxin is supplied or not (JARVIS and ALI 1984).

Nothwithstanding the possible complexities implied by the above observations there has been widespread speculation that irradiance influences root regeneration *via* effects on photosynthate and/or auxin availability (see ANDERSON (1986) and references cited therein). As yet it is not possible to assess the relative extents to which these two factors control root formation in any species. With respect to cuttings of mung bean, which are widely used in studies on root regeneration, previous work has suggested that carbohydrate content *per se* is not a limiting factor (MIDDLETON *et al.* 1980).

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Consequently, we are undertaking a wide reaching study of the relationship between irradiance and auxins in such cuttings. The current report confines itself to the effects of irradiance on exogenous auxin supplied to cuttings either *via* their cut stems or *via* their leaves. Irradiance has been varied during growth of stock plants and during supply of auxin to the cuttings themselves. Experiments have centred largely around the first 24 h after cuttings are made. This period coincides with the first cell divisions in phloem parenchyma cells which give rise to roots and has been defined as the inductive phase (JARVIS 1986).

MATERIALS AND METHODS

Growth of Stock Seedlings and Preparation of Cuttings

Seeds of *Phaseolus aureus* ROXB. cv. Berkin were surface-sterilized by immersion in 4 % (m/v, filtered) sodium hypochlorite solution for 20 min. They were rinsed, then sown in trays containing fine granular vermiculite, which was kept saturated with deionized water. Seedlings were raised at 25 °C under continuous irradiance supplied by fluorescent tubes (38 W m^{-2}, 400—700 nm) and in a relative humidity of 81 %.

Cuttings were prepared from 10 day old seedlings and consisted of apical bud, a pair of expanded primary leaves, epicotyl and 30 mm of hypocotyl. For rooting tests sixteen cuttings were used per treatment and were placed four per glass vial containing a 30 mm depth of the appropriate solution (14 cm^3). The depth of solution was maintained by daily addition of deionised water.

Treatment of Cuttings

Indol-3-ylbutyric acid (IBA) and indol-3-ylacetic acid (IAA) were dissolved initially in ethanol, which was present at a final concentration of 2 cm^3 dm^{-3}. Previous work (MIDDLETON *et al.* 1978a) has established that ethanol at these concentrations is without effect on rooting of mung bean cuttings. Rooting response was determined by treating cuttings in a 30 mm depth of 10^{-4} M IBA or 5×10^{-4} M IAA for 24 h then transferring them to boric acid (10 μg cm^{-3}), which is essential for formation of primordia, as well as their subsequent growth (MIDDLETON *et al.* 1978b, JARVIS *et al.* 1983). During the period of treatment with auxin and borate cuttings were held under the same conditions as those in which stock seedlings were raised. The only exceptions to this involved those experiments concerned with the effects of different irradiances during growth of stock plants or during treatment of cuttings. Root numbers were determined six days after transfer of cuttings to borate.

Basal Application of ^{14}C-IAA

Cuttings, or isolated hypocotyl segments (upper 30 mm), were placed in 14 cm^3 of IAA (5×10^{-4} M) containing sufficient indolyl (1-^{14}C) acetic acid to give a specific activity of 2558 kBq $mmol^{-1}$. (^{14}C-IAA was supplied by Amersham International plc, UK). The volume of auxin employed provided a 30 mm depth of solution such that the entire hypocotyl was submerged.

Cuttings were held under those conditions employed to raise seedlings, as described above. After the appropriate time cuttings were removed and hypocotyls thoroughly rinsed with water. They were then divided into hypocotyl, epicotyl, primary leaves and bud before estimation of radioactivity.

Foliar Application of ^{14}C-IAA

Indolyl (1-^{14}C) acetic acid (1.78 GBq mmol^{-1}) was applied to leaves in 0.2 % Tween 80. Each leaf was supplied with a 5 µl droplet of ^{14}C-IAA, the quantity of which is stated in the legends of appropriate tables. The droplet was contained in the centre of the leaf by lanolin, which was supplied to the upper surface of the leaf as a thin film in the form of a ring of diameter 3 mm. During transport of ^{14}C-IAA out of leaves some cuttings were placed with their hypocotyls in IBA (10^{-4} M). Control treatment in such experiments was to place hypocotyls in 2 cm^3 dm^{-3} ethanol. Where treatment with IBA was not employed cuttings were placed in deionised water.

Determination of Radioactivity

Plant tissue was routinely extracted with 80 % methanol at 5 °C. Three extractions were employed over a total period of 48 h, each involving 5 cm^3 of 80 % methanol for each two hypocotyls, epicotyls or pairs of leaves. Invariably some radioactivity remained in the tissue residue, usually less than 4 % of the total. This was readily solubilized by scintillation fluid. Radioactivity of both the extract and tissue residue was determined by scintillation spectrometry using the scintillation fluid described by TURNER (1968). As an alternative method tissue was placed directly into one-step scintillation fluid/digestant ('Fluorosol', National Diagnostics, N. J., USA) and heated to 50 °C for 3 h prior to assay for radioactivity. Leaves were bleached with 1.1 % benzoyl peroxide. Previous experiments had established that, after suitable correction for quenching by the Channels ratio method, both these methods produced very similar estimates of radioactivity.

Data presented for experiments involving ^{14}C-IAA are from single experiments. The major observations made here have been confirmed in several other studies, although the total amount of radioactivity recovered does vary from experiment to experiment.

Estimation of Transpiration

Stem cuttings were placed in 14 cm^3 deionized water in glass vials similar to those used for routine treatment of cuttings. The surface of the water was covered with a layer of purified linseed oil to minimize surface evaporation. Transpiration rate was estimated gravimetrically, with appropriate correction for what little surface evaporation was detected in a vial containing no cuttings. Sixteen cuttings, held in four vials, were used for each individual estimate of transpiration rate.

RESULTS

The rooting response of stem cuttings held under different irradiances is shown in Table 1. In the absence of exogenous auxin root formation is in-

TABLE 1
Root formation in relation to the irradiance under which cuttings are held

Irradiance [W m^{-2}] during treatment of cuttings	Mean number roots $cutting^{-1}$		
	Control	IBA	IAA
0	28.3 ± 4.3	40.1 ± 3.7	46.9 ± 4.1
16	16.7 ± 3.2	67.3 ± 7.4	54.5 ± 5.3
38	10.2 ± 1.7	75.5 ± 5.1	83.2 ± 5.3
70	11.0 ± 1.5	82.1 ± 5.8	76.8 ± 3.7

Cuttings were prepared from stock seedlings raised under an irradiance of 38 W m^{-2}. They were held under different irradiances during treatment with 10^{-4} M IBA or 5×10^{-4} M IAA for 24 h and after their subsequent transfer to borate. Data are cited ± 95 % confidence limits.

hibited by light. In darkness, 28.3 ± 4.3 roots developed per cutting compared with 16.7 ± 3.2 at an irradiance of 16 W m^{-2}. At 38 W m^{-2} rooting was significantly less than in cuttings held at 16 W m^{-2}. Increasing the irradiance from 38 to 70 W m^{-2} had no further influence on root formation. By way of contrast cuttings treated with exogenous auxin, whether it was IBA or IAA, produced fewer roots in darkness than in the light. Rooting was enhanced with increasing irradiance such that at 70 W m^{-2}, and in response to 10^{-4} M IBA, approximately 82 roots per cutting developed. This was more than double the number in darkness. At the same irradiance, and in response to 5×10^{-4} M IAA, approximately 77 roots per cutting formed. This was 63 % more than in darkness.

The irradiance under which stock plants were raised had a significant influence on the number of roots subsequently developing on cuttings derived

TABLE 2
Root formation on cuttings in relation to the irradiance under which stock plants are raised

Irradiance [W m^{-2}] during growth of stock seedlings	during treatment of cuttings	Mean number roots $cutting^{-1}$	
		Control	IBA
16	16	12.9 ± 2.3	69.1 ± 7.8
	darkness	17.6 ± 6.4	41.8 ± 5.6
38	38	9.0 ± 1.8	75.5 ± 5.1
	darkness	23.8 ± 6.7	40.1 ± 3.7
70	70	8.3 ± 1.8	97.4 ± 12.4
	darkness	28.5 ± 7.4	33.3 ± 7.8

Stem cuttings were prepared from seedlings grown under various irradiances. During treatment with, or without, IBA as well as after subsequent transfer to borate, they were either held at the same irradiance as that used to raise stock seedlings or, alternatively, in darkness. Treatment with IBA (10^{-4} M) was for 24 h. Data cited are ± 95 % confidence limits.

TABLE 3
Uptake of ^{14}C-IAA into cuttings and isolated hypocotyls

	whole cutting	Radioactivity [Bq] in hypocotyl	epicotyl	leaves	terminal bud
Intact cutting	332 ± 52	204 ± 15	79 ± 17	48 ± 22	1.8 ± 0.9
Leafless cutting	117 ± 19	110 ± 19	6 ± 3		0.5 ± 0.05
Intact cutting with epidermis sealed	236 ± 32	171 ± 18	47 ± 20	18 ± 4	0.9 ± 0.7
Intact cutting with basal end sealed	181 ± 84	133 ± 59	34 ± 19	12 ± 3	1.0 ± 0.4
Isolated hypocotyl		91 ± 19			
Isolated hypocotyl with both ends sealed		82 ± 14			

Cuttings or isolated hypocotyl segments (upper 30 mm) were placed vertically in ^{14}C-IAA (5×10^{-4} M, 2558.1 kBq mmol^{-1}) for 4 h such that only the hypocotyl of the cutting or the whole isolated hypocotyl segment was submerged. Five individual cuttings or segments were employed per treatment. Data presented are mean values ±95 % confidence limits. Lanolin was used to seal the epidermis, the basal cut end of the hypocotyl or both ends of isolated hypocotyl segments.

from them (Table 2). When cuttings themselves were held in darkness this was especially so in the absence of exogenous auxin. Cuttings prepared from seedlings raised under an irradiance of 16 W m^{-2} but held in darkness regenerated about 18 roots each, whereas those from stock plants grown at 70 W m^{-2} and similarly kept in darkness developed approximately 28. Cuttings from the same stock plants held in the light developed fewer roots irrespective of the irradiance level under which the stock seedlings were raised. Indeed, the higher the irradiance under which stock plants and cuttings were held the fewer roots formed per cutting. However, when cuttings were treated with IBA the situation was quite different. Irrespective of the irradiance under which stock plants were raised, root formation was always favoured by maintaining the cuttings themselves in the light. The higher the irradiance under which stock plants and cuttings were held the larger the number of roots formed per cutting. When 16 W m^{-2} was employed to raise stock plants and treat cuttings, 69.1 $\pm$ 7.8 roots per cutting arose whereas when 38 W m^{-2} was used, 75.5 $\pm$ 5.1 roots developed and at 70 W m^{-2}, about 97 roots per cutting were recorded. When the cuttings themselves were held in darkness there was little difference in the numbers of roots arising in cuttings from any of the stock seedlings. Cuttings from stock plants raised at 16 W m^{-2} but treated in darkness regenerated 41.8 $\pm$ 5.6 roots whereas those from seedlings grown under 70 W m^{-2} had 33.3 $\pm$ 7.8 roots.

The role of leaves in the uptake and distribution of ^{14}C-IAA into stem cuttings can be assessed from the data in Table 3. Approximately 332 Bq entered each intact cutting in 4 h. Of this 204 Bq was still within the hypocotyl, whereas approximately 79 and 48 Bq were found respectively in the epicotyl and primary leaves. There was evidently rapid transport of ^{14}C out of the hypocotyl. Relatively little radioactivity was found in the terminal bud — only 1.8 $\pm$ 0.9 Bq. Uptake was markedly reduced if the primary leaves had been removed from cuttings. Of the 117 $\pm$ 19 Bq entering the cutting, only 6 $\pm$ 3 Bq were located in the epicotyl and 0.5 $\pm$ 0.05 in the terminal bud. The latter two values constituted 5.6 % of the total radioactivity within the cutting whereas when leaves were left intact over 38 % of radioactivity taken up was transported out of the hypocotyl. When the epidermis of the hypocotyl of intact cuttings was sealed with lanolin uptake of ^{14}C-IAA was reduced by 29 % compared with the control, each cutting acquiring 236 $\pm$ 32 Bq. Sealing the cut end of intact cuttings resulted in a drop in ^{14}C-IAA uptake, such that 181 Bq was found per cutting. The proportion of this radioactivity transported out of the hypocotyl was similar to that found in intact cuttings whose epidermis was sealed. Isolated, excised hypocotyls acquired 91 Bq when supplied with ^{14}C-IAA, an amount not significantly changed when both cut ends were sealed with lanolin. Presumably, therefore, entry of ^{14}C-IAA was virtually entirely through the epidermis.

The influence of irradiance on the uptake and distribution of ^{14}C from ^{14}C-IAA supplied basally to cuttings and on transpiration rate is shown in Table 4. Uptake was markedly reduced when cuttings were kept in darkness. After 24 h in the light over 976 Bq had entered the cutting, whereas in the dark each cutting had taken up approximately 556 Bq. This is not surprising given the likely role of transpiration in the uptake of supplied

TABLE 4
The influence of irradiance on uptake and distribution of ^{14}C-IAA and transpiration rate

Condition	Uptake cutting^{-1} [Bq]	Percentage of total radioactivity found in				Transpiration [mg] water cutting^{-1}
		hypocotyl	epicotyl	leaves	terminal bud	
Light	976 ± 225	53.7	38.5	7.1	0.7	686 ± 54
Darkness	556 ± 179	70.2	26.0	3.3	0.5	298 ± 27

^{14}C-IAA (5×10^{-4} M, 2558.1 kBq mmol^{-1}) was supplied basally to the hypocotyl. Cuttings were held at 38 W m^{-2} or in darkness at 25 °C for 24 h, after which radioactivity in various parts of the cuttings was determined. Four individual cuttings were employed per treatment. For estimation of transpiration four cuttings per treatment were employed with 4 replicates for each treatment. Data for radioactivity [Bq] or transpiration [mg] are cited ±95 % confidence limits.

TABLE 5
The effect of irradiance on ^{14}C-IAA transport

Irradiance during *transport*	Radioactivity [Bq] recovered from				Radioactivity located in stem	
	leaves	*epicotyl*	*hypocotyl*	*entire cutting*	*Bq*	*as percentage* of that in entire cutting
Untreated cuttings						
Darkness	20 793	44	20	20 862	64	0.3
38 W m^{-2}	8 245	68	43	8 356	111	1.3
70 W m^{-2}	6 428	75	38	6 541	113	1.8
IBA-treated cuttings						
Darkness	21 730	69	13	21 812	82	0.4
38 W m^{-2}	9 565	139	31	9 735	170	2.2
70 W m^{-2}	7 808	168	45	8 021	213	2.7

Stem cuttings were taken from stock seedlings grown under 38 W m^{-2}. Basipetal transport of ^{14}C-IAA, applied to both primary leaves (14.8 kBq/leaf), was for 24 h in darkness or at 38 or 70 W m^{-2}. During transport cuttings were treated basally with, or without, IBA (10^{-4} M). Four cuttings per treatment were employed. Data are Bq per cutting. (Stem = epicotyl + hypocotyl).

TABLE 6

The influence of irradiance of stock plants on subsequent transport of ^{14}C-IAA from leaves of cuttings

Irradiance [W m^{-2}] during growth of stock plants	Radioactivity [Bq] recovered from			
	leaves and terminal bud	epicotyl	hypocotyl	whole cutting
Untreated cuttings				
16	8303	145	86	8534
70	5264	71	64	5399
IBA-treated cuttings				
16	9657	228	94	9979
70	3453	127	51	3631

Stem cuttings were taken from stock seedlings grown under 16 or 70 W m^{-2}. Basipetal transport of ^{14}C-IAA, applied to both primary leaves (18.5 kBq/leaf), was for 24 h at 38 W m^{-2}. During this time cuttings were treated basally with, or without, IBA (10^{-4} M). Four cuttings per treatment were employed. Data presented are mean values.

chemicals into stem cuttings, and the earlier observation that the leaves are responsible for 65 % of total uptake into cuttings (Table 3). After 24 h in the light transpiration loss was 686 $\pm$ 54 mg per cutting, whereas in the dark it was 298 $\pm$ 27 mg (Table 4). Despite the reduced uptake of ^{14}C-IAA which resulted from keeping cuttings in darkness, a diminished proportion of the radioactivity which did enter cuttings was transported out of the hypocotyl. The percentage of radioactivity located in the hypocotyl after 24 h in the light was approximately 54, whereas approximately 70 % of the radioactivity taken up was retained in the hypocotyl in darkness.

Table 5 is concerned with the effects of irradiance on the basipetal transport of ^{14}C-IAA out of leaves. Transport was assessed in the presence and absence of a basal supply of IBA to cuttings. Movement of radioactivity from leaves into stem is enhanced by light. When cuttings were held in darkness the percentage and total amount of radioactivity located in the stem were lower than when they were irradiated. Untreated cuttings accumulated over 64 Bq in the stem when held in darkness. This represented 0.3 % of the total radioactivity recovered from the cuttings. Similar cuttings held at 38 W m^{-2} accumulated more than 111 Bq in the stem *i.e.* approximately 1.3 % of the total radioactivity found in them. The radioactivity accumulated in stems of cuttings held under 70 W m^{-2} was virtually the same as that in stem tissues of cuttings maintained at 38 W m^{-2}, but at the higher irradiance a smaller proportion of the radioactivity was found in the hypocotyl. Nevertheless, increasing irradiance led to a decrease in the amount of radioactivity recovered from cuttings. Presumably this was due, at least partially, to photodestruction of the supplied ^{14}C-IAA. A basal supply of IBA during the 24 h period of transport enhanced the movement of radioactivity from leaves into stem at all levels of irradiance under which cuttings were maintained. As with untreated cuttings increasing irradiance favoured transport out of the leaves. Again this was evidenced by an increase in the percentage of radioactivity located in the stem as well as in the total amount of radioactivity located there. Basal supply of IBA to cuttings had two further effects. Firstly,

TABLE 7
The transport of ^{14}C-IAA out of leaves of cuttings held in light and darkness

Time of transport [h]	Radioactivity [Bq] located in		Percentage of radioactivity recovered which was located in stem
	epicotyl	hypocotyl	
In darkness			
16	14.0 ± 2.8	4.6 ± 1.9	0.9
24	17.1 ± 6.0	4.4 ± 1.6	1.0
40	16.1 ± 4.3	6.1 ± 1.7	1.2
64	15.2 ± 3.0	4.9 ± 1.4	1.0
At 70 W m^{-2}			
16	22.6 ± 3.7	4.2 ± 1.0	1.5
24	20.6 ± 3.6	8.3 ± 1.8	2.2
40	24.5 ± 2.1	9.5 ± 2.5	2.3
64	17.3 ± 3.3	6.2 ± 2.9	2.1

Stem cuttings were taken from seedlings grown at 70 W m^{-2}. Basipetal transport of ^{14}C-IAA applied to both primary leaves (3.7 kBq/leaf), was for up to 64 h in darkness or at 70 W m^{-2}. Five cuttings were employed per treatment. Data presented are mean values ± 95 % confidence limits.

it led to enhanced recovery of radioactivity whether cuttings were irradiated or not. Secondly, although it enhanced movement of ^{14}C from leaves to stem the proportion of radioactivity in the hypocotyl, relative to the epicotyl, was less than in untreated cuttings.

Transport of ^{14}C-IAA from leaves into the stems of cuttings, as influenced by the irradiance under which stock plants are raised, is shown in Table 6. In the absence of supplied IBA 231 Bq was recovered from the epicotyl and hypocotyl of cuttings taken from seedlings raised under an irradiance of 16 W m^{-2}. Less than 40 % of this was in the hypocotyl. Approximately 135 Bq was recovered from the stem of cuttings taken from seedlings raised at 70 W m^{-2}. Again less than half the radioactivity was located in the hypocotyl. Basal treatment of cuttings with IBA enhanced transport of ^{14}C out of leaves into stem tissues of cuttings taken from seedlings grown under both irradiances. Irrespective of whether they received a basal supply of IBA or not, cuttings taken from stock plants grown under the lower irradiance level accumulated more radioactivity outside the leaves than did those from seedlings raised at the higher level. This may reflect a long term effect of irradiance on auxin metabolism since the total radioactivity remaining in cuttings after 24 h was much greater when cuttings were from seedlings raised under the lower irradiance.

Table 7 shows the accumulation of radioactivity in epicotyl and hypocotyl when leaves of cuttings were supplied with ^{14}C-IAA and the cuttings maintained in darkness or at 70 W m^{-2}. These cuttings were prepared from seedlings raised under an irradiance of 70 W m^{-2}, a treatment which favours natural regeneration when derived cuttings are subsequently held in darkness (Table 2). Irradiance results in enhanced transport of radioactivity out of leaves with the greatest accumulation in the hypocotyl being evident after

40 h. In irradiated cuttings the radioactivity recovered from both hypocotyl and epicotyl was significantly less after 64 h than it was after 40 h. This may be taken as evidence that following formation of the root primordium, between 40 and 48 h, there is enhanced metabolic breakdown of auxin thereby allowing subsequent growth (see JARVIS 1986, and references cited therein). The rate at which radioactivity accumulates in stem tissues continually declined over the 64 h duration of the experiment irrespective of whether cuttings were in light or darkness.

DISCUSSION

Relatively high irradiance levels during treatment of mung bean cuttings favour rooting in response to supplied auxins whereas they are inhibitory towards root formation which arises due to natural endogenous factors (Table 1). Similar, though less extensive data has been reported previously (JARVIS and ALI 1984). When exogenous auxin is employed the beneficial effect of high irradiance on root formation is probably related in part to enhanced uptake of the auxin. The data in Table 4 show that cuttings held in the light take up more auxin than those held in darkness. Given that the uptake of auxin is essentially passive *via* the transpiration stream (Table 4 and JARVIS and SHAHEED (1986)), this is hardly surprising. The inhibitory effect of high irradiance on natural root regeneration might be explained by photodestruction and enhanced metabolic breakdown of endogenous auxin such that concentrations in the hypocotyl become limiting. Evidence of these possibilities is seen in the data of Table 5 which shows diminishing recovery of radioactivity from cuttings supplied with ^{14}C-IAA as irradiance increases. Further evidence is provided by the observation that rooting is enhanced in these cuttings if the hypocotyl is maintained in darkness but the rest of the cutting is exposed to irradiance (JARVIS and ALI 1985). Other work has established that accumulation of ^{14}C in the hypocotyl of cuttings held in the light, and supplied with ^{14}C-IAA *via* the leaves, is enhanced by localised shading of the hypocotyl. In addition chromatographic evidence indicates that ^{14}C-IAA is transported out of leaves without prior metabolism (ALI 1986).

Notwithstanding any effect it may have on auxin metabolism within the leaves incident irradiance can markedly enhance transport of auxin out of leaves (Tables 4 and 5), an observation previously made for other plant tissues (*e.g.* GUTTENBERG and ZETSCHE 1956, KOEVENIG and JACOBS 1972). The data presented here clearly indicate that irradiance enhances basipetal transport of ^{14}C-IAA from leaves to hypocotyl to a greater extent than is any ensuing photodestruction of auxin in the hypocotyl. Any such destruction of transported ^{14}C-IAA is seemingly also counteracted by supply of IBA to the base of the cuttings (Table 5). Previous work from this laboratory showed basal supply of IAA, up to 10^{-4} M to have a similar stimulatory effect on the amount of ^{14}C-IAA transported out of leaves (JARVIS and SHAHEED 1986). In that work it was reasoned that a basal supply of auxin at 10^{-4} M could result in enhanced basipetal transport 480 times greater than in untreated cuttings. IBA given basally is seemingly converted to IAA in leaves (MCGEE and JARVIS, unpublished data), as has been shown in other cuttings by EPSTEIN and LAVEE (1984). Therefore the amount of radioactivity transported out of leaves supplied with ^{14}C-IAA is likely to be an

underestimate of the amount of auxin moved out since non-radioactive IAA arising from metabolism of IBA could reduce the overall specific activity of IAA in the leaf. This could account for the lack of correlation between the number or roots regenerating and the amount of radioactivity accumulating in the hypocotyl (see Tables 1 and 5).

Cuttings prepared from seedlings raised under 70 W m^{-2} export less ^{14}C-IAA out of their leaves than do those taken from seedlings grown at 16 W m^{-2} (Table 6). There is evidently a long term effect of irradiance on subsequent auxin metabolism since less radioactivity was recovered when cuttings were prepared from seedlings raised at 70 W m^{-2} than when they were taken from seedlings raised at 16 W m^{-2} (Table 6). Whether, or not, this difference in metabolism is the sole reason for the differences in extents to which ^{14}C accumulates in hypocotyls is unclear.

It seems likely that leaves of cuttings act not only to control uptake of supplied auxin but also its subsequent loading into the appropriate basipetal transport system (JARVIS and SHAHEED 1986). Moreover, inhibitors of basipetal transport of auxin prevent rooting of mung bean cuttings whether it be induced by supplied auxins, vitamin D or other growth regulators (JARVIS and YASMIN 1987). In the light of results presented here the efficacy of supplied auxin is likely to be related to its uptake, acropetal movement, metabolism and re-mobilisation out of leaves, basipetal transport to the region of root regeneration as well as its subsequent metabolism in this region. The balance between these is likely to be of paramount importance in determining the extent of regeneration. The different effects of irradiance on rooting induced by exogenous auxin and that due to endogenous factors can thus be explained. This does not preclude the possibility that factors other than auxin have major roles in root development. However, it has been argued previously that auxin may be the only factor needed for initiating root formation which has to be transported to the site of re generation, other factors arising locally (JARVIS 1986). Whether this proves to be so, or not, there is clearly need of detailed studies on auxin metabolism in stem cuttings, particularly with respect to irradiance. Such work should include quantitative assessment of amounts of auxin transported into the hypocotyl and accumulating there.

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