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THE EFFECT OF INDOLEACETIC ACID ON THE DISTRIBUTION OF ^{14}C-ASSIMILATES IN *DAHLIA VARIABILIS*

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Abstract

Dahlia variabilis plants were fed at the four mature leaf—pair stage with $^{14}CO_2$ for 30 min. With plants fed at one time, intact plants had predominant distribution of ^{14}C to the root at 5 hours. Hormone-directed transport from fed leaf to decapitated internode can be detected in the cut internode within three hours. It is dependent on the presence of hormone in the cut internode. This short term accumulation is not associated with the formation of ethanol-insoluble materials. Instead, it appears that sucrose is unloaded from the phloem in the region of the node which bears the fed leaf. Decapitation alone gave greater retention in the lower stem. Distally-applied indoleacetic acid has increased the activity in the ethanol-soluble fraction in the cut internodes 6-15 hours from decapitation.

Introduction

In early studies involving the substitution of the apical bud by auxin it was found that the application of auxin to the cut surface of decapitated bean plants caused the accumulation of carbohydrates, nitrogenous substances and ions in the cut stump (Mitchell and Martin, 1937; Stuart, 1938; Brunsetter, Myers, Mitchell, Stewart and Kaufman, 1948). These experiments took over periods of several days.

Hew, Nelson and Krotkov (1967) showed that indoleacetic acid can affect the distribution of labelled ^{14}C-metabolites in soybean within one hour from its application. It has been well established that metabolite mobilization is enhanced when hormone or certain plant growth substances are applied to stems of decapitated stump (Davies and Wareing 1965; Mullins, 1970; Bowen and Wareing 1971; and Patrick and Wareing 1973; 1976 and 1978).

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The pattern of distribution of assimilates can be regarded as being determined by the distribution of growth activity which, in turn, is determined by hormone.

The present study describes the effect of indoelacetic acid on the location of ^{14}C-activity in *Dahlia variabilis*. Plants for analysis, were separated into different parts in order to locate more clearly the region at which hormone may have an effect (or at which unloading may take place).

Materials and Methods

Plant Material

All plants were raised from seeds of *Dahlia variabilis* (wild) Desf cv. Coltness Yellow purchased from Asmer Seeds Ltd., Leicester.

Seeds were germinated on paper towelling moistened with distilled water in seed trays, and kept for one week at 65/80 W warm white fluorescent light at 20±2°C. After seven days seedlings (3.5–4.5 cm high) were potted in John Innes No. 1 compost in 65 mm diameter plastic pots. Before and during experiments plants were maintained in a growth room under a bank of 65/80 W warm white fluorescent tubes with an irradiance level at bench height of 800 lux for 16 hours light per day. Temperature was maintained at 20±2°C and relative humidity at about 80%. Plants of five weeks old were selected for uniformity. At this time they had 4 pairs of fully-expanded leaves and were 15–20 cm in height. In all experiments the oldest pair of leaves was designated number 1 and the youngest pair number 4.

Methods for Studying the Distribution of ^{14}C-Assimilates Preparation and use of agar blocks containing hormones

Indoelacetic acid (IAA) were prepared in 1.25% w/v agar. Hormones were first dissolved in 0.2–0.5 ml of 80% ethanol and diluted to 100 ml in distilled water. The final concentration of ethanol in the solution was below 0.2%.

Cylinders of agar were cut with a cork borer of 3 mm diameter to give agar blocks with a volume of approximately 21 mm^3. For controls, agar blocks were prepared which contained an equivalent amount of ethanol.

For decapitation experiments, blocks of agar with or without IAA were applied to the freshly-cut surfaces. when a block had been in position for 3 h it was replaced by a new block.

Feeding with $^{14}CO_2$

A feeding chamber of 26×9×5 cm in size was been used to supply single leaf of plant with $^{14}CO_2$ simultaneously. The fourth leaf of each plant

was sealed in the box with masking tape and paraffin grease. A planchette containing 20 μCi of $Ba^{14}CO_3$ was placed in the base of the chamber and $^{14}CO_2$ released by adding a few drops of 50% v/v orthophosphoric acid from a syringe inserted through a rubber plug in the lid. Holes made by the syringe needle were immediately sealed with paraffin grease. The total concentration of CO_2 in the box was less than 0.06% and the atomosphere was circulated continuously by means of two fans mounted at one end of the feeding chamber. After 30 min about 10 ml of 20% w/v sodium hydroxide was added to strips of filter paper in the box to absorb residual $^{14}CO_2$. The lid of the box was then removed and each plant was allowed to photosynthesis for a predotermined chase period in a normal atmosphere containing $^{12}CO_2$. All $^{14}CO_2$ Feeding experiments were carried out between first and sixth hour light period.

Extraction of labelled assimilates

Samples were extracted three times under reflux in boiling 80% ethanol. The pooled extracts were then dried under reduced pressure at 50°C.

The residues remaining after extraction were dried at 70°C, weighed and ground to a fine powder using a ball mill.

Counting

(a) *Soluble fraction*

The dried ethanol extracts were redissolved in a selected volume of 80% ethanol. Aliquots of ethanol-soluble extracts were dried on aluminum planchettes using an infra-red lamp. Planchettes were counted in triplicate with a gas-flow planchette counter (Nuclear Chicago decade scaler and printer automatic sample changer). All the counts were corrected for the background.

(b) *Insoluble fraction*

The insoluble fraction was counted as dry powder on planchettes using the gas-flow counter. Preliminary experiments showed that losses due to self absorption occurred in samples over 12 mg, to avoid such losses all samples were of less than 10 mg.

Separation of ethanol extracts of ^{14}C-assimilates into acidic, basic and netural fractions

The ethanol soluble fraction was fractionated by ion-exchange resins into three components, namely netural compounds, basic and acidic compounds (Romberger 1960).

Chromatography

Carbohydrates were tentatively identified by conventional descending paper chromatography using ethyl acetate-acetic acid-water (E. A. W.) in proportion 14:3:3 (Smith 1960). The carbohydrates were defected with silver nitrate-sodium ethoxide (Trevelyam; proctor and Harrison, 1950), and identified from their R_g values (Smith, 1960). Further identification and quantitative analyses were made with gas-liquid chromatography (G. L. C.) using the methods of Holligan and Drew (1971). A pye series 104 Column Analytical Gas Chromatograph, was used and samples were trimethylsilylated according to the method of Sweeley *et al.* (1963).

Radioassay of chromatograms

Scanning

Paper chromatograms were cut into strips 1.5 inches wide and the distribution of radioactivity was determined by scanning each strip using a Nuclear Chicago Actigraph III.

Results

The effect of IAA concentration on the Accumulation of ^{14}C-Assimilates in the Fifth Internode

The aim of this experiment was to determine the optimum IAA concentration for the mobilization of assimilates to the decapituted internode. A range of concentrations of IAA in agar from 0.1 to 100 mg/l was used (Fig. 1). The plants was then fed with $^{14}CO_2$ for 30 minutes and sampled after a further 5 h in a normal atmosphere. Radioactivity in the ethanol-soluble fraction was determined for extracts of the treated internode only.

The results are as follows. ^{14}C-activity in the fifth internode increased with increasing concentration of IAA to a high value and declined thereafter (Fig. 1). A concentration of 10 mg/l IAA led to an accumulation of ^{14}C-activity approximately three times higher than that in the control. Seth and Wareing (1967) found that radioactivity of ^{32}P increased in the cut internodes of decapitated *Phaselus vulgaris* seedlings with increasing concentration of IAA up to 10^3 ppm with a decrease at 10^4 ppm. These results are very comparable with those of the present study, except that the IAA concentration used by Seth and Wareing was 100-fold higher. This difference probably reflects the use of lanolin instead of agar as a means of applying auxin.

Long-term effect of IAA on the distribution of ^{14}C-assimilates

In this experiment an identical constant period between $^{14}CO_2$ application and harvest was used. A comparison was made between decapitated plants

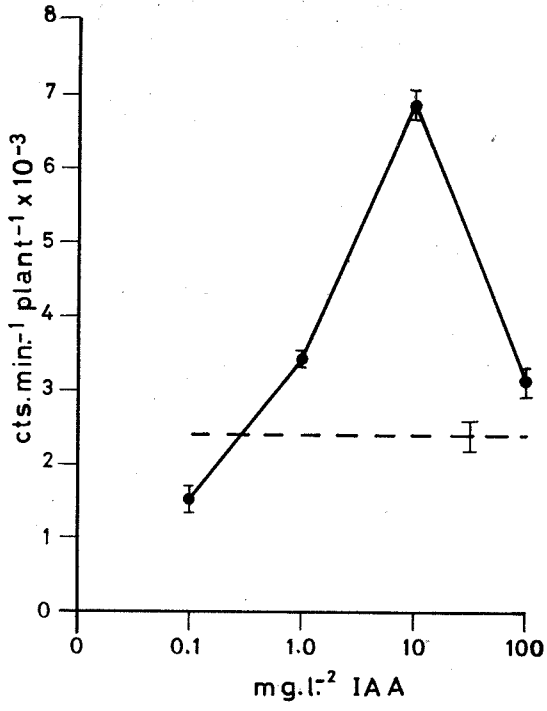

Fig. 1. The effect of concentration of IAA in agar blocks on the accumulation of ^{14}C-activity in the ethanol-soluble fraction of the fifth internode. Values are counts per minute per plant and each value is the mean of three replicates.
Key: —— IAA, ------ Control.

with and without application of IAA to the decapitated surface. The agar application technique was used and three-hourly harvests were made up to 15 h. The distribution of radioactivity in the ethanol-soluble fraction was determined at each harvest, the plants being divided into five component parts. Nine plants per treatment were used at each time of sampling. The extracts produced were used to follow the distribution of radioactivity in carbohydrates present in the fifth internode.

The distribution of activity in the ethanol-soluble fraction is shown as a percentage of the translocated activity (Fig. 2). The results indicate a constant increase in the fifth internode but a falling proportion in the stem.

In comparison with untreated plants those treated with IAA show a similar pattern of change with time for the stem and for the roots, with proportions relatively higher in the stem and lower in the roots (Fig. 2). The percentage of activity present in the fifth internode treated with IAA increased steadily with time, and the divergence of the curves suggests that IAA begins to affect distribution between 3 and 6 h from decapitation. There

was an indication that the steady increase in the proportion of activity in the IAA-treated cut stem with time accelerated between 12–15 h.

Figure 2 also illustrated that insignificant activity was present in the mature leaves other than the fed leaf. This was characteristic of all experiments concerned with assimilate distribution.

A comparison of the patterns of distribution of ^{14}C-assimilates in intact plants, decapitated and decapitated plants to which IAA was applied

Intact plants and decapitated plants to which plain agar blocks or blocks containing 10 mg/l IAA were affixed and supplied with $^{14}CO_2$ for 30 min from time 0. Plants were harvested after 1, 2, 3, 4 and 5 h.

Comparison of the time-course of assimilated distribution between treatments will be based on the results presented as a percentage of total activity

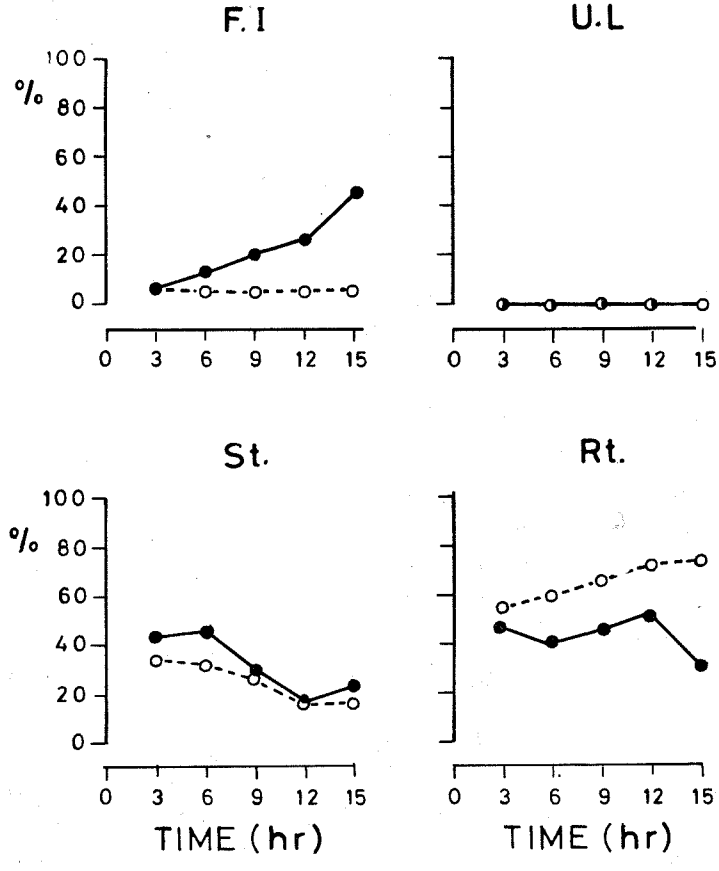

Fig. 2. The distribution of ^{14}C-assimilates as a percentage of translocated activity of the ethanol-soluble fraction
F.I.=Fifth internode, U.L.=Unfed leaves, St.=Stem, Rt.=Root.
Key: —— IAA, ------ Control.

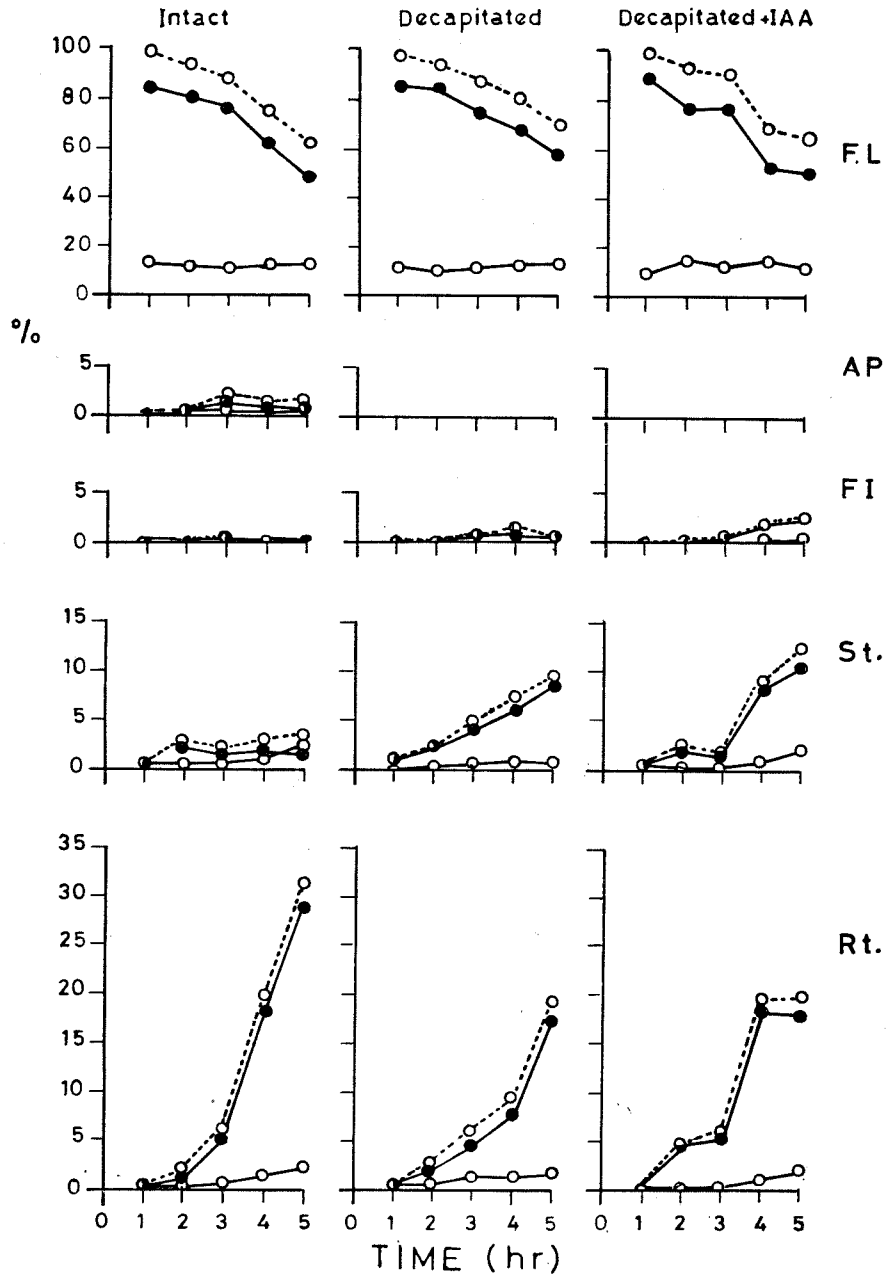

Fig. 3. The distribution of ^{14}C-assimilates as a percentage of total counts.
F.L.=Fed leaf, AP=Apical region, F.I.=Fifth internode, St.=Stem, Rt.=Root.
Key: ------ Total activity, —— Soluble fraction, —— Insoluble fraction.

(Fig. 3). In each treatment, the proportion of assimilate exported from the fed leaves increased with time, reaching about 30–40% at 5 h. This change reflected a decreased activity in the ethanol-soluble fraction, the ethanol-insoluble fraction remaining remarkably constant in the region of 15–18% throughout the five hour period. At five hours the ratio of ethanol-soluble: ethanol-insoluble activity (ES: EI ratio) is approximately 4:1. There are no marked differences between the intact plants and decapitated plants with and without IAA.

A relatively small proportion of activity was present above the fed leaf and the appropriate parts of Fig. 3 are shown as an extended scale in Fig. 4.

In intact plants activity in the main shoot apex rose rapidly from the first hour, reaching a peak at 3 h and fluctuating in a downwards trend thereafter. Activity in the fifth internode (between the apex and the fed leaf) mirrored that in the apex there was only 10–40% of that in the apex. Most of the activity in the internode was in the ethanol-soluble fraction, presumably sugars in transit to the apical region. In the apical region a much

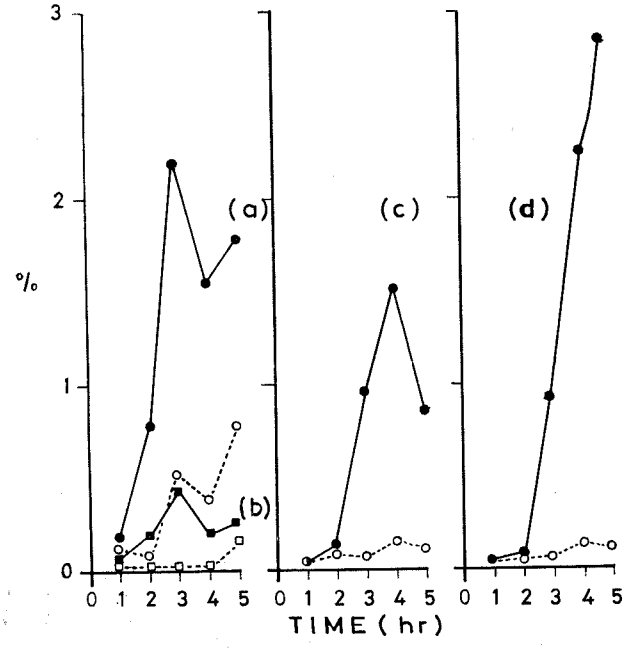

Fig. 4. The ^{14}C-activity in the apical region and in the fifth internode as a percentage of the total counts (Expanded scale).
(a) Apical region of intact plant, (b) Fifth internode of intact plant, (c) Decapitated fifth internode without IAA, (d) Decapitated fifth internode with IAA.
Key: —— Soluble fraction, ------ Insoluble fraction.

greater proportion of activity was ethanol-insoluble with an ES: EI ratio of 1.8:1 at 5 h. This probably indicates incorporation of ^{14}C into protoplasm and walls of the cells of the actively-growing apical region.

In the decapitation treamtments the fifth internode forms the only sample above the fed leaf. The increase in activity with time in both decapitation treatments was of a similar order to that of the apical regions of intact plants but both showed a lag of one hour. In decapitated plants both with and without IAA the proportion of total activity increased in a similar fashion up to 3 h. However, IAA application resulted in a continued rise at 4 and 5 h. The value at 5 h exceeded that of the combined apical region and fifth internode of intact plants. In both decapitation treatments, the ES : EI ratio was higher than that for the fifth internode of intact plants but lower than that of the main shoot apex. At 5 h, decapitation alone gave an ES : EI ratio of 7:1, with IAA the ratio was 24:1.

In all treatments the major proportion of the distributed activity was present in the parts of the plant below the fed leaf. However, there was a clear difference between intact plants and decapitated plants, with respect to the proportions present in the stem and in the roots (Fig. 3).

In intact plants the percentage of total activity in the stem rose between 1 and 2 h and then remained at a fairly constant level between 2% and 4% up to 5 h. In contrast, values for the roots continued to rise throughout the five hours, reaching more than 30% at 5 h. Both decapitation treatments showed patterns of distribution which in the earlier samples were similar to that of the intact plants. However, by 5 h the stems of decapitated plants had 2–3 times the ^{14}C-activity in the stem and about two-thirds of the activity in the root of that in the intact plants. Treatment with IAA led to a slightly higher value in the stem at 5 h and little increase in the roots between 4 and 5 h.

In the stem of intact plants a relatively high proportion of activity was ethanol-insoluble, with an ES : EI ratio of 1:1 at 4 and 5 h. This is comparable with that in the apical region. In the root the ratio was 12:1. Decapitation alone gave a ratio of 9:1 for both stem and root. With decapitation plus IAA the ratios were stem, 5:1 and root, 9:1.

Carbohydrate Analysis

Quantitative determinations of the carbohydrates present in the stem (including the fifth internode), leaves and in the root were made by reference to calibration curves for peak heights and are shown in Table 1. Sugar levels were the lowest in the leaves and the highest in the roots. Expressed as hexose, the leaves had a higher proportion of sugars as sucrose (45%) than

Table 1. *Quantitative determiantion ofcarbohydrates present in the stem (including the fifth internode), leaves and root*

Part of the Plant	Sugars Concentration mg/g dry weight			
	Fructose	$\alpha+\beta$ Glucose	Inositol	Sucrose
Leaves	7.17	7.99	3.27	14.37
Stem	15.83	21.5	1.37	20.84
Root	34.83	39.46	2.00	46.5

the stem (36%) and the roots (39%). The ratio of glucose to fructose was higher in the stem (1.36:1) compared with the leaves and roots (1.11:1 and 1.14:1, respectively).

The effect of IAA on sugar mobilization in the fifth internode of decapitated plants

The following study was undertaken to determine the nature of the carbohydrates present in the fifth internode and the effect of IAA on their levels. Two groups of nine plants each were decapitated at the top of the fifth internode. Blocks of agar with IAA were applied to the cut surfaces of one group, blocks without IAA to the other. Six hours later the fifth internodes only were harvested and the ethanol-soluble and insoluble carbohydrates were extracted for GLC analysis. Quantitative determinations of 80% ethanol-soluble sugars and insoluble carbohydrates as sugars after hydrolysis, were made by GLC analysis.

Samples of GLC traces of the netural fraction for the ethanol-soluble extracts are shown in Fig. 5a and b.

In both treatments there were five major peaks, from left to right representing fructose, $\alpha+\beta$ glucose, inositol and sucrose. All peaks were higher when IAA was present.

Samples of GLC traces of the ethanol-insoluble carbohydrates after hydrolysis are shown in Fig. 6a and b. In this fraction, there were three peaks; the first one was relatively high and larger in both treated and untreated tissues and represents fructose, the others represent $\alpha+\beta$ glucose. As in the neutral soluble fraction, application of 10 mg/l IAA led to increased height of the peaks.

Table 2 shows the effect of IAA on the actual amounts of sugars present in the fifth internode.

Application of IAA to decapitated fifth internodes increased the concentration of all carbohydrates in comparison with untreated controls with the most marked increases occurring in fructose (72% increase), sucrose (46%)

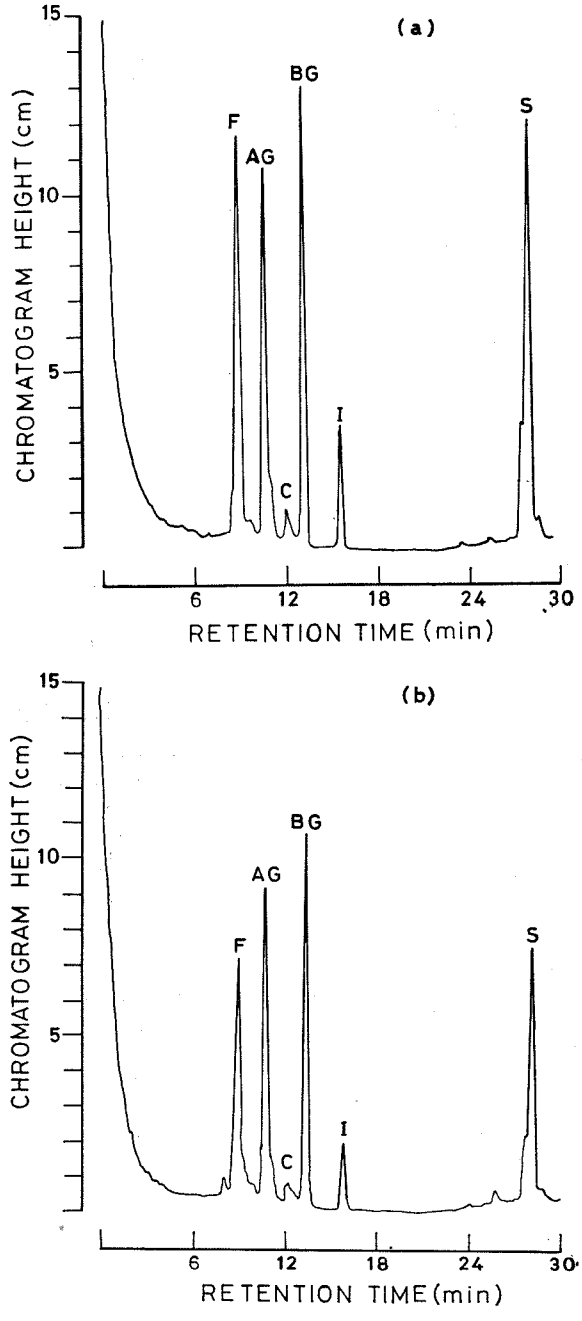

Fig. 5. Representative chromatograms of TMS derivatives of the netural soluble fraction of treated and untreated fifth internodes. Temperature programme 150°-290°C at 40°/min, attenuation 1×10^4.
(a) Treated fifth internode, (b) Untreated fifth internode.
Key to Peaks: F=Fructose, AG=α-Glucose, BG=β-Glucose, I=Inositol, S=Sucrose, C=Unknown.

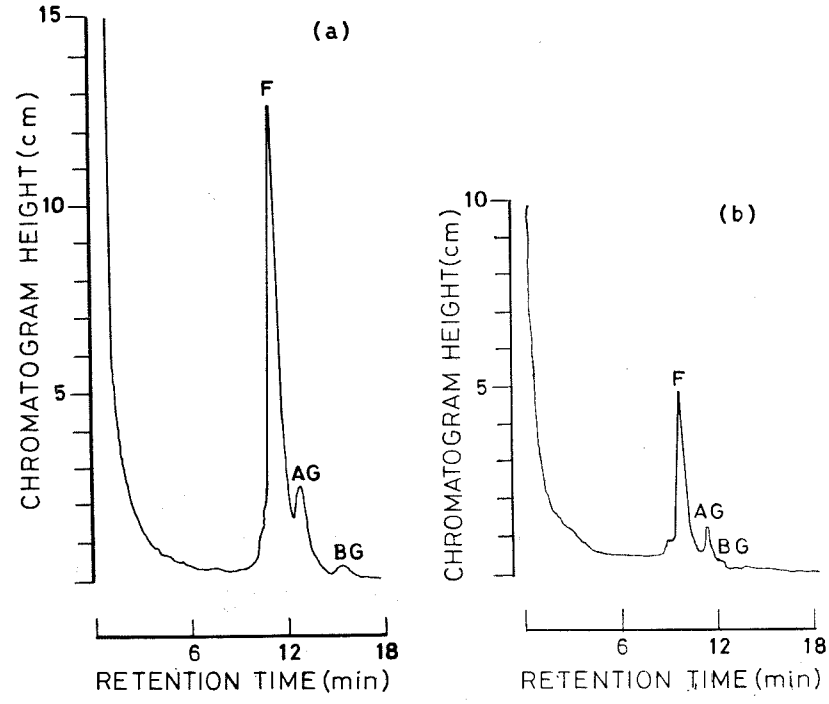

Fig. 6. Representative chromatograms of TMS derivatives of the ethanol-insoluble fraction of treated and untreated fifth internodes after hydrolysis. Temperature programme 150°-290°C at 4°/min, attenuation 1×10^4.
(a) Treated fifth internode, (b) Untreated fifth internode.
Key: AG=α-Glucose, BG=β-Glucose, F=Fructose.

Table 2. *Effect of 10 mg/l of IAA on the dry weight of carbohydrates accumulated in the fifth internode. Values are the means of three replicates and standard errors are given*

	Carbohydrate Concentration mg/g dry weight	
	Control	IAA
	Mean S.E.	Mean S.E.
Fructose	10.83±0.57	18.61±0.19
Glucose	21.28±0.40	25.63±0.46
Inositol	1.50±0.049	2.82±0.029
Sucrose	24.35±1.93	35.45±3.15
Hydrolysed Carbohydrate (as hexose)	11.61±0.397	17.85±0.18

and in the hydrolysed carbohydrate (54%). The ratio of fructose : glucose in the hydrolysed carbohydrate was 11:1 (IAA treatment) and 9:1 (control) indicating that oligosaccharides of ten or more hexose units, as expected in an ethanol-insoluble extract.

Comparison of the actual amounts of glucose, fructose and sucrose in the fifth internode with those in the stem (Table 1), indicates almost identical values for glucose. However, the amount of fructose was about 30% lower and sucrose 20% higher in the untreated fifth internode.

Fractionation of plant extracts by ion-exchange resins

The purpose of this experiment was to determine the relative amounts of the ^{14}C-activity in the basic, acidic and neutral compounds of the ethanol-soluble fraction after feeding with $^{14}CO_2$. Six intact five-week old plants were selected for uniformity and fed with $^{14}CO_2$ for 30 min. The plants were divided into two groups of three plants each. The first group was harvested after a chase period of 3 h, and the second after a chase period of 6 h.

In a preliminary experiment only parts of the shoot above the fed leaves were used (fifth internodes) for fractionation, but because counts of radio-activity in these parts were so low after fractionation, in the present experiment whole plants (less the fed leaves) were used. Ethanol-soluble substances were extracted as previously described, and crude extracts were separated into basic, acidic and neutral components using ion-exchange resins.

The results, Table 3, indicate that at 3 h almost 90% of the activity recovered after fractionation was in the neutral (sugar) fraction with most of the remainder in the acidic fraction. At 6 h the amount of ethanol-soluble

Table 3. *Accumulation of ^{14}C-assimilate in neutral, acidic and basic compounds*

Chase Period of 3 h				Chase Period of 6 h			
Before Fractionation	After Fractionation			Before Fractionation	After Fractionation		
cpm/plant	cpm/plant			cpm/plant	cpm/plant		
	Neutral	Acidic	Basic		Neutral	Acidic	Basic
20.050	15.690	1.865	157	63.546	37.012	1.994	277
	% of total rctivity recovered				% of total activity recovered		
	62.6	7.4	0.6		58.24	3.06	0.43
	% of total activity (assuming 100% recovery)				% of total activity (assuming 100% recovery)		
	88.6	10.5	0.9		94.2	5.1	0.7
	% of recovery				% of recovery		
	70.7				61.8		

^{14}C in the plant had more than doubled and an even larger proportion was recovered as the neutral fraction.

The effect of IAA on the distribution of ^{14}C-activity between sugars extracted from the fifth internode of decapitated plants

This experiment was designed to determine the effect of IAA on the ^{14}C-assimilates transported to the fifth internode with respect to the amounts of label in the fructose, glucose, sucrose and fructosans present in the ethanol-soluble fraction. The neutral soluble fraction was separated into the various sugars using paper chromatography as described. Sucrose, glucose, fructose and 'fructosans' were eluted from the paper chromatogram by refluxing for 20 min in hot 80% ethanol three times. For each sample, replicate spots of 0.04 ml were chromatogrammed. The bulked extracts were reduced to dryness and taken up in 0.2 ml of 80% ethanol and radioassay techniques were carried out as previously described.

Representative scans of paper chromatograms of the neutral compounds extracted from treated and untreated fifth internodes are shown in Fig. 7.

Accumulation of ^{14}C-activity was shown to be largely in sucrose with

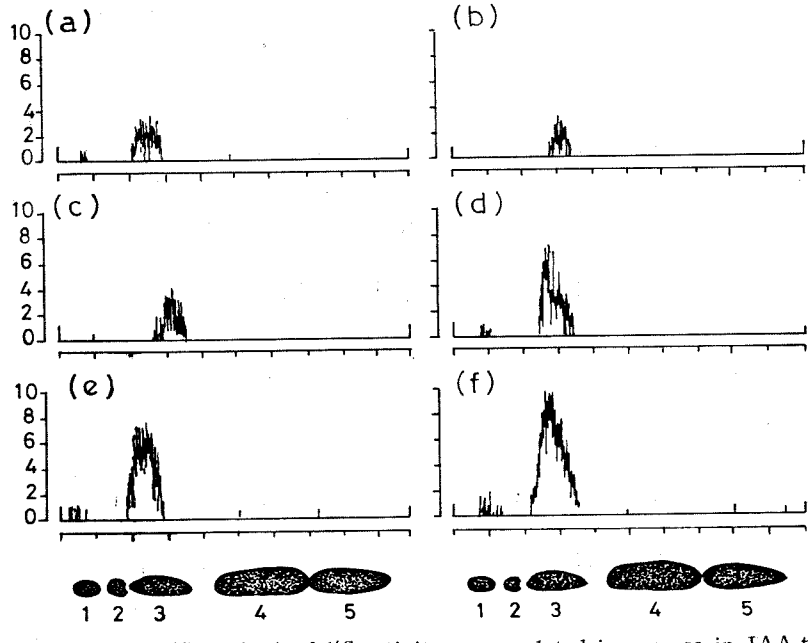

Fig. 7. Time course (Scanning) of ^{14}C-activity accumulated in sucrose in IAA-treated and control fifth internodes.
(a) Control (identical for all times), (b) IAA treatment 3 h, (c) IAA treatment 6 h, (d) IAA treatment 9 h, (e) IAA treatment 12 h, (f) IAA treatment 15 h.
Spots developed with silver nitrate reagent (identical for all treatments) are: 1 and 2-fructosans, 3-sucrose, 4-glucose, 5-fructose.

some activity in the fructosan oligosaccharides which also increased with time.

Figure 7 shows the quantitative effect of IAA on the distribution of ^{14}C-activity between the sugars. These data show clearly that the increased mobilization to the fifth internode in response to the application of IAA resulted from an increased activity in sucrose. Glucose activity was also increased in the intermediate samples and increased activity in the fructosans occurred in the later samples.

Discussion

Assimilate distribution in the intact plant

Approximately 40% of the ^{14}C assimilated by the fourth leaf of the intact plant was exported after 5 h.

Increasing export within the period of 1–5 h was associated with decreased activity in the ethanol-soluble fraction. The ethanol-insoluble fraction remained remarkably constant in the range of 15–18%.

Although translocated activity was transiently higher in the stem, a clear indication of predominant transport to the roots soon became obvious (Fig. 3). More than 80% of the activity was recovered from the roots at 5 h, with 10% in the lower stem and less than 10% in the shoot apical region and fifth internode combined. In the intact plant little ^{14}C moved out of the fed leaf in the first hour. Export increased with time and was a function of loss of ethanol-soluble activity. The main sink for ^{14}C was clearly the roots. Sinks are usually regarded as growing or storage regions in which the translocated assimilate is converted into an insoluble form. In *Solanum tuberosum* the roots have been found to have as much as 95% of ^{14}C-activity in an ethanol-insoluble form (Lovell and Booth, 1972). In contrast, activity was largely in an ethanol-soluble form in *Dahlia*. This may be due to differences in storage products, starch and inulin, respectively, and to the shorter time between feeding and harvest, 5 h in *Dahlia* compared with 24 hours in *Solanum*. Inulin itself is ethanol-insoluble but its oligosaccharide precursors are soluble (Wain, Rutherford, Weston and Griffiths, 1964). In *Dahlia* the highest proportions of insoluble activity were found in the stem. Apart from the fed leaf, the highest proportion of ethanol-insoluble activity at 5 h was found in the lower stem. In the plant as a whole (excluding the fed leaf) 86% of the activity was ethanol-soluble. Separation of ethanol-soluble material on resins indicated that the bulk of this activity was neutral-soluble (carbohydrates), with less than one tenth in the acidic fraction and practically none in the basic fraction. In the major sink for assimilates, the root system

has more than 92% of the activity recovered was ethanol-soluble. Thus, in *Dahlia*, sink activity is not directly associated with the conversion of assimilates arriving in the phloem into structural and storage products of negligible osmotic activity.

The effects of decapitation on assimilate distribution

In decapitation treatments almost the same proportion of activity was exported from the fed leaves as in the intact (Fig. 3). This decapitation alone had no discernible effect on the transport of ^{14}C-assimilates out of the fed leaf. This suggest two conclusions; firstly that damage to the phloem resulting from decapitation must be restricted to the fifth internode; secondly that removal of a major source of auxin does not reduce the amount of assimilate transported from the leaf in the phloem. If auxin is required for the function of sieve tubes, as has been proposed, then adequate supplies of auxin must be produced in mature leaves, at least for translocation into the stem.

The apical region is not a major sink for assimilate and in this respect the effect of its removal might be expected to be slight in terms of a conventional source-sink interpretation. On this basis compensatory increases in the activity in the roots would be expected whereas less activity was found in the roots after 5 h, more remaining in the lower stem. This retention in the stem was not associated with increases in the ethanol-insoluble fraction.

One effect of decapitation is the outgrowth of lateral buds. In the *Dahlia* plants used in experiments these are very samll in size. It has been shown in pea that growth of lateral buds commences after a lag of 6–8 h after decapitation (Nagao and Rubenstein, 1976). Assuming that a similar lag is shown in *Dahlia*, the enhanced accumulation of assimilate in the stem cannot be accounted for by incorporation into lateral buds. This is supported by the finding of a similar enhancement of activity in the stem of decapitated plus IAA-treated plants in which lateral bud growth is suppressed.

The changed distribution which results from decapitation could arise by indirect effect of decapitation on the levels of hormones within a given region of the plant. The intact apical region may serve both as a producer and a consumer of hormones.

The fifth internode of decapitated plants does gain some assimilate. The rise in activity in the cut internode is initially closely similar to that in plants supplied with IAA, (Fig. 4), divergence between these treatments occurring after about 3 h from the start of feeding. Such movement into the untreated cut internodes could be ascribed to the effects of residual

endogenous auxin. Depletion of auxin by basipetal translocation coupled with respiratory losses could lead to a levelling-off or a fall in the activity in the fifth internode. Doubt is cast on this interpretation by the fact that in the experiment with a constant period of 3 h between feeding and harvest the proportion of activity in the cut internode remains more or less constant between plants with intervals of 0–12 h between decapitation and feeding. Depletion of auxin should have been complete in the later harvests. A further possibility is that hormones are produced in response to wound reactions at the cut surface.

The effects of the application of IAA to decapitated plants

Application of 10 mg/l IAA resulted in the accumulation of ^{14}C-assimilates in the cut fifth internode, with a level of activity approximately equal to that found in the apical region of the intact plant. However, it is important to note that resemblance to the intact plant is superficial. The presence of IAA does not restore the overall pattern of assimilate distribution to that of the intact plant. Distribution remains essentially that of the decapitated plant with enhanced accumulation in the stem compared with intact plants. In the latter, assimilate transport upwards into the fifth internode and the shoot apical region is detected within one hour of feeding. In decapitated plants there is a lag of approximately one hour before similar increases in ^{14}C-activity are detected (Fig. 3).

It has been noted earlier that enhanced accumulation of activity in the lower stem with decapitation could result from auxin deficiency giving a decreased capacity of the phloem for translocation. If this is so then the application of IAA to the decapitated internode might be expected to restore phloem function with the result that the stem would be cleared and more activity would be present in the roots. However, the general trend is for higher activity to be recovered from the lower stem when IAA is present. Proponents of the auxin-induced phloem transport hypothesis argue that phloem transport occurs along an auxin gradient. At the node bearing the fed leaf, the auxin gradient in the decapitated plus IAA-treated plant must always be such that translocation should take place via an upwards direction.

There is some evidence that after about 4 h from the start of feeding activity in the stem rises more sharply and that this occurs at the expense of activity in the roots. Estimates of the velocity of IAA transport in stems give values in the range of 5–20 mm/h (Goldsmith, 1969) although Patrick and Woolley (1973) estimated velocity of 28 mm/h for cut internodes of *Phaseolus vulgaris*. Thus the time at which this rise in activity in the lower

stem occurs coincides approximately with the time IAA from the cut surface may be expected to reach the lower stem. This supports the view that IAA affects unloading of the phloem at the site where it is present.

Features of IAA-induced accumulation in the cut stump will now be considered. It is of interest to note that the effect of IAA concentration on mobilization is similar to that of other responses regulated by IAA, with increased activity up to a concentration of the order of 10 mg/l, with 100 mg/l acting as a supra-optimal concentration. Seth and Wareing (1967) found a comparable effect in *Phaseoulus vulgaris* although they used applications in lanolin and their optimum concentration was one hundred times higher than that recorded for *Dahlia* supplied via agar gel. Patrick and Wooley (1973) found the optimum concentration was 20 mg/l with IAA supplied as a solution.

Comparison of unlabelled carbohydrates in the cut stump (on the basis of a unit dry weight of cut internode) revealed that IAA led to increases in all the compounds analysed. The percentage increases varied, with fructose showing the largest rise. This variation suggest that IAA leads to metabolic changes in the cut stump in addition to enhancing the uptake of sucrose. Hatch and Glaszion (1963) proposed an unloading mechanism for sugar cane which involved invertases. The respiration of the glucose component of sucrose could lead to relatively large increases in frucose. However, although the basis for the changes in *Dahlia* is unclear it appears that the increased levels of mono- and disaccharides do not arise from the breakdown of the polysaccharide fraction.

The tentative conclusion which can be made is that assimilates which are taken up into the cut internode are unloaded from the phloem (in which they are undoubtedly transported from the fed leaf) at the node. Movement upwards from the node could then take place in or between cells other than phloem sieve tubes.

Unloading could take place into cells of the cambium and immature vascular elements, into companion cells or into ray parenchyma cells. Pate (1975) has drawn attention to the fact that nodes appear to possess a greater potential for exchange not only within specific element of the vascular network but also between quite different vascular pathways. Transfer cells have been characterized at the nodes in many species of plant (Gunning *et al.*, 1970) and they are commonly associated with the phloem at the margin of the leaf-trace gaps. It is possible that transfer cells facilitate unloading of the conducting elements at the nodes and that their function is hormone-regulated.

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吲哚乙酸對大利花的碳同化作用之影響

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經由葉部供應碳十四二氧化碳給已具有四對成熟葉的 *Dahlia variabilis* 三十分鐘。只供給一次時，五小時後大部份碳十四累積在根部。在切斷的莖節塗上激素時，三小時後可在莖節偵測到碳十四。此種短程累積與乙醇不溶物質無關。在供應碳十四葉部的莖節附近蔗糖自靱皮部游離出來。去除頂芽可使低莖部保持更多放射性。在末梢塗吲哚乙酸六至十五小時後可提高去除頂芽的莖節的乙醇可溶分劃之放射性。