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Translocation of Indole-3-acetic Acid-1′-^{14}C and Tryptophan-1-^{14}C in Seedlings of Phaseolus coccineus L. and Zea mays L.[1]

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Summary. Indole-3-acetic acid-1′-^{14}C (IAA-^{14}C) and tryptophan-1-^{14}C injected in small amounts into cotyledons of *Phaseolus coccineus* L. seedlings were found to be translocated acropetally into the epicotyls and young shoots. Similarly IAA-^{14}C was translocated acropetally into coleoptiles of *Zea mays* following injection into the endosperms. Labeled metabolites of the injected compounds were also extractable from shoot tissue. However, evidence that IAA-^{14}C itself was translocated acropetally was obtained by collection in agar blocks applied to cut surfaces of coleoptiles of injected seedlings. The acropetal translocation in *Phaseolus* was shown not to occur in the transpiration stream but in living tissue. Cotyledons of *Phaseolus coccineus* and *Phaseolus vulgaris* contain extensive vascular tissue.

Tryptophan-^{14}C was not actively translocated through excised segments of *Phaseolus coccineus* epicotyl and *Zea mays* coleoptile when supplied from donor agar blocks in concentrations as high as 100 μM. The small amount of tryptophan-^{14}C which did reach receiver blocks when high concentrations were used may be accounted for by passive diffusion through the fluid-filled xylem vessels. Translocation of a non-toxic dye, Light Green SF Yellowish, through xylem vessels was found to occur when supplied from donor blocks placed acropetally or basipetally. Metabolism of the supplied tryptophan-^{14}C by the tissue segments was shown to occur during the 3 to 6 hour translocation experiments. IAA-^{14}C was transported in a strictly basipetal manner in both tissues. Only 1 labeled compound with an R_F value of IAA was found in receiver blocks. Composition of a simple green safelight suitable for work in plant physiology is described.

Fletcher and Zalik (5) found that when IAA-^{14}C was injected in small amounts into cotyledons of *Phaseolus vulgaris* L., label was translocated upwards into the epicotyl and young leaves as well as downwards. In view of the basipetal polarity of auxin transport in most young shoots, this upward translocation was somewhat surprising. The phenomenon was further investigated to see whether it was IAA-^{14}C itself or a labeled metabolite of it that was translocated acropetally.

Another species of *Phaseolus, P. coccineus* L. (Scarlet Runner beans) was chosen for this work as this species is hypogeal in its germination and therefore more convenient because the epicotyl elongates rather than the hypocotyl. The upward translocation was found to occur in this species also.

Since etiolated seedling shoots must derive all of their organic nutrients from the storage material of the seed, IAA in the shoot tip must be derived from the seed. Either a) IAA itself or b) a compound from which IAA may be derived must be translocated to the shoot. The first alternative, that IAA as such is translocated acropetally, is doubtful in view of the now well established basipetal polarity of young seedling shoots, unless it could be translocated in a tissue or in such a way that it is not subject to the polarity mechanism. However, acropetal IAA diffusion from cut surfaces has not been demonstrated (21, 25). Gordon (9, 10) states that it is clear that the auxin as such which increases in the seed upon germination does not move to the apex for redistribution as a correlative agent. For alternative b) i.e. translocation from the storage material of the seed of a compound from which IAA may be formed in the tip, 2 possibilities exist: 1) the precursor may be a complex formed from IAA in the seed (14), 2) a true precursor rather than an IAA-complex may be translocated. Although the exact pathway for IAA production in higher plants has not been elucidated, tryptophan appears to be an intermediate which is commonly occurring in plant tissue (10). That the shoot tip is able to produce IAA from tryptophan (e.g. 26) indicates that tryptophan or a precursor of it might be translocated. Skoog (21) and Went and Thimann (25) suggested in fact that tryptophan might be the translocated precursor. They were able to collect a substance or substances

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diffusing from apical cut surfaces of coleoptiles which would produce curvature in the *Avena* curvature test only several hours after application, similar to that which occurs when tryptophan is tested in the curvature test. In studies on indole compounds which could be collected from cut surfaces of epicotyls of *Phaseolus vulgaris* and *Phaseolus coccineus*, Whitehouse (28) found tryptophan to be predominant. Therefore, in these studies, attention was focused on tryptophan as a possible precursor which is translocated to the tip.

Schrank and Murrie (20) showed that tryptophan-^{14}C was not actively translocated in *Avena* coleoptile sections, either acropetally or basipetally, although it was taken up by the tissue slowly and for a limited distance from the source. Concentrations as high as 1 mM applied to the basal ends of 17.5 mm coleoptile tips did not increase elongation over controls during a 20 hour growth period, and no radioactivity could be extracted from the apical 5 mm.

In addition to the investigation of the upward translocation of label following injection into cotyledons of *P. coccineus* seedlings and *Z. mays* endosperm this work also investigates the translocation of tryptophan-^{14}C and IAA-^{14}C through excised segments of the tissues.

Materials and Methods

Seedlings of *Phaseolus coccineus* L. (Scarlet Runner bean) were grown in the dark in a potting mixture of fine sand and peat moss, 1:1 at room temperature (23 ± 2°). Seedlings were used between 5 and 7 days after sowing the seeds. Seedlings with epicotyls 3 to 5 cm in length from the cotyledons to the hook were selected for the injection experiments. For the translocation studies seedlings with epicotyls 10 cm in length were selected and segments 6 mm in length were cut from 1 cm below the hook with a guillotine.

Corn seeds (*Zea mays* var. Morden) were treated with a 10 % (v/v) solution of commercial sodium hypochlorite (Perfex) for 10 minutes at room temperature in the dark and then rinsed for 3 hours in running tap water (6). They were then sown at a depth of about 0.5 cm in previously moistened potting mixture in covered Pyrex dishes. On the second day after planting, when the coleoptiles were beginning to emerge, they were given a red light treatment (15 watt bulb with a sheet of red Plexiglas at a distance of about 30 cm) for 3 hours to repress elongation of the first internodes and to enhance elongation of the coleoptiles (2). Seedlings 3.5 to 4.0 cm tall were used on the third day after sowing. Segments of coleoptiles, 5 mm long, from which the first leaves had been removed, were excised 2 mm below the tip with a guillotine. For the injection experiments seedlings 1.0 to 1.5 cm tall were selected.

Both *P. coccineus* and *Z. mays* seedlings were kept in darkness but manipulations were carried out using a green safelight. The safelight was constructed from a 15 watt Sylvania green fluorescent tube and a filter composed of 3 layers of green Plexiglas No. 2092 and 1 of amber Lucite. Transmission of the filter was checked using a Beckman DK-1 spectrophotometer and was found to be zero at 500 and 560 nm with a peak at 525 nm. This is similar to the range recommended and used by other workers (e.g. 27).

Injections were made with a 50 µl or a 100 µl graduated hypodermic syringe fitted with a fine needle. Solutions containing more than 20 % (v/v) alcohol caused tissue damage. Consequently concentrations lower than this were used for injections.

(Indole-3)-acetic acid-1′-^{14}C was purchased from California Corporation for Biochemical Research and had a specific activity of 13.3 mc/mmole. DL-tryptophan-1-^{14}C (alanine-3-^{14}C) purchased from the same company had a specific activity of 32.5 mc/mmole.

The studies on polarity of transport through tissue segments were similar to those described by other workers, (e.g. 1, 8, 15, 17) except that cylindrical agar blocks were used instead of the usual agar blocks which are square in cross-section. Bacto-Agar was dissolved in distilled water by boiling. The final agar concentration for all blocks was 1.5 %. For blocks containing additives the measured solution and agar solution were mixed in a glass vial kept in a water bath at 45 to 55°. After thorough mixing the labeled agar was transferred with a syringe to glass tubing 15 cm in length and 3 or 4 mm internal diameter which was stoppered at the lower end and kept in the water bath to maintain the agar molten. The tube with agar was removed from the water bath and allowed to solidify. The rod of agar was extruded onto a glass plate and sliced into 2.0 mm blocks with a guillotine. Glass tubing of 4 mm internal diameter was used initially for both coleoptiles and epicotyls, but later 3 mm blocks were used for coleoptiles as

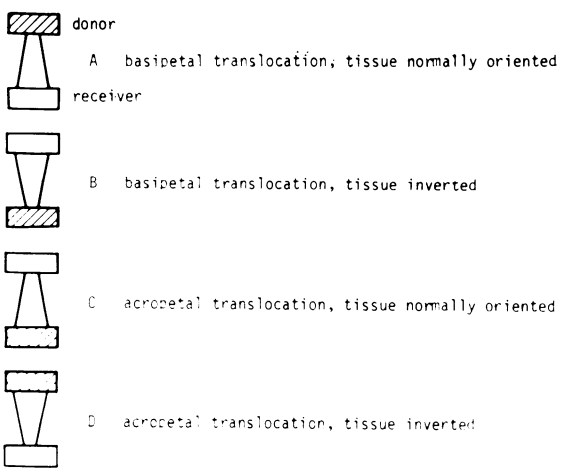

FIG. 1. Arrangement of agar blocks and tissue segments for experiments on transport polarity. (See tables II and III.)

the 4 mm blocks were unnecessarily large. The volumes of the 4 mm diameter blocks were 25 mm^3 and of the 3 mm blocks, 14 mm^3.

Ten replicates were used for each treatment. The treatments (arrangements of agar blocks and tissue segments) are depicted in figure 1. The agar blocks and tissue segments were arranged in covered Petri dishes to prevent dehydration. These were then placed in a humidified cabinet at 20 ± 1° for the duration of the experiment.

After treatment times of 3 or 6 hours the agar blocks were removed and placed in the centres of aluminum planchets to which small amounts of Haupt's adhesive had been added. These were dried in the air and counted using a Gas Flow Counter. All samples were treated and counted in the same manner and no allowance was made for self absorption.

In some cases the agar blocks and tissue segments were extracted with methanol after the translocation experiment. These extracts were chromatographed in isopropanol:ammonium hydroxide (28 %):water, 8:1:1 (23) and the chromatograms scanned using a Nuclear Chicago Actigraph II. Radioautograms of some of the chromatograms were made using Ansco Non-Screen *X*-ray film.

Translocation experiments were also conducted incorporating the non-toxic vital stain, Light Green SF Yellowish into donor blocks. Only treatments A and D were used (fig 1), i.e. basipetal translocation with tissue normally oriented with respect to gravity, and acropetal translocation with tissue inverted, respectively.

Results

Injection of Phaseolus coccineus *with IAA-^{14}C.* Seedlings of *P. coccineus* were injected with IAA-^{14}C in 10 % (v/v) ethanol. Ten µl were administered to each cotyledon. The dose used was 0.1 µc, equivalent to 1.3 µg, IAA-^{14}C/plant. Duplicate shoots were extracted with methanol after various time intervals and the radioactivity determined in these extracts. The results indicated in table I show that considerable radioactivity is translocated to the shoots and that the amount of activity increases with time. Chromatography of the shoot extracts showed that a labeled compound with an R_F value of IAA was extractable from the shoots but most of the activity chromatographed at low R_F values (0, 0.05, 0.07).

Injection of Zea mays *Seedlings with IAA-^{14}C.* Ten µl of 10 % (v/v) ethanol containing 0.65 µg IAA-^{14}C were injected into the endosperms of 3-day old seedlings of corn. After 8 hours the seedlings were cut below the nodes, agar blocks applied to the cut surfaces and the assembly kept in a humid atmosphere. The agar blocks were removed after 2.5 hours and transferred to methanol. The internodes were re-cut and fresh agar blocks applied for a further period of 3 hours. These were pooled with the previous blocks. The blocks were extracted overnight at 3°, the methanol decanted and the blocks re-extracted twice with fresh methanol for periods of approximately 3 hours. The extracts were pooled.

The apical 6 to 10 mm of the coleoptiles were excised and placed on agar blocks in covered Petri dishes for 5.5 hours. The agar blocks were extracted with methanol. The coleoptile tips, and the pieces of shoots removed between the internodes and the coleoptile tips (i.e. remains of the internodes, nodes, remains of the coleoptiles and also the first leaves) were crushed and extracted with methanol. The remains of the seeds were also extracted.

Extracts were chromatographed and radioautograms made of the chromatograms. In all 4 extracts distinct bands on the radioautograms corresponding to IAA-^{14}C were detected. Its presence in the agar blocks showed that it was diffusing both acropetally and basipetally out of the cut surfaces. In the extracts of the tissues, traces of compounds with low R_F values were also detected. Chromatograms of methanol extracts of seeds which had been injected with IAA-^{14}C revealed major peaks at R_F values corresponding to IAA (0.37–0.46) with minor peaks at 0.09 to 0.13.

Injection of Phaseolus coccineus *Seedlings with Tryptophan-^{14}C.* Five µl of a solution containing 0.003 µmole (0.61 µg) of tryptophan-^{14}C were injected into cotyledons of *P. coccineus* seedlings. Growing tips, epicotyls and cotyledons of 4 plants were extracted separately with methanol after 20 to 24 hours and the extracts chromatographed in 2 solvent systems, 1) isopropanol:ammonium hydroxide (28 %):water, 8:1:1, and 2) isopropanol:acetic acid:water, 4:1:1. In cotyledons the major methanol extractable labeled compound was tryptophan but another compound (X) present in a smaller amount was also extracted. This compound had an R_F value of 0.13 in solvent 1 and 0.72 in solvent 2. In epicotyls X was again present but tryptophan was present in a greater amount. In the extract of the growing tips again both tryptophan and X were present with compound X being present in a slightly larger amount than tryptophan.

Table I. *Radioactivity Extracted at Various Time Intervals from Shoots of* Phaseolus coccineus *Seedlings Following Injection of a Solution of IAA-^{14}C into the Cotyledons*

The dose was 0.1 µc/plant.

Time after injection	Radioactivity
hr	cpm/shoot
1	54
3	220
6	130
9	520
24	480
72	2840

Thus it appears that both IAA and tryptophan, injected into the storage material of the seed, can be translocated acropetally into the shoot.

Anatomical Study of Cotyledons and Endosperms. Thimann (24) and Leopold (16) point out that if IAA unnaturally enters the transpiration stream it will be carried upwards with the stream. Thus the question arose whether the substances were being administered in such a way as to gain artificial entrance into the transpiration stream. Little information has been reported (3, 4, 7, 19) on the presence and extent of vascular tissue in cotyledons. It was therefore necessary to examine these structures microscopically.

Cotyledons of *P. coccineus* and *P. vulgaris* and endosperms of *Z. mays* were fixed, embedded and sectioned according to standard procedures (13). Satisfactory sections of cotyledon tissue, 15 to 20 μ thick, were obtained, but considerable difficulty was encountered in preparing sections of corn endosperms. All sections were stained with safranin and fast green.

Microscopic examination of cotyledons revealed an extensive vascular system. Bundles containing both phloem and xylem branched throughout the cotyledons running mainly longitudinally from the region of attachment to the stem (figs 2–5). Cells containing many inclusions were localized around the vascular bundles (figs 2 and 3). The inclusions were mainly starch grains as evidenced from staining with iodine. Cells further from the vascular bundles were generally devoid of these inclusions.

Examination of corn endosperm indicated that it was devoid of vascular tissue but that a definite arrangement of vascular tissue was present in the scutellum.

Vital staining (11, 13) was also used to demonstrate the transpiration stream. Roots of seedlings were placed in the vital stain, Light Green SF Yellowish, some of the roots being cut to allow easier penetration of the dye. After uptake of the dye, the vascular system in cotyledons of *P. coccineus* could be clearly seen (figs 6 and 7). With corn seedlings no dye entered the endosperm but a distinct cylinder of vascular tissue was observed passing through the scutellum. This branched to enter the interface between the scutellum and endosperm. (In the coleoptile the 2 vascular bundles became clearly stained. Sometimes a third column of stain was observed in the coleoptile but on sectioning such stained coleoptiles it was found that this third column was associated with the thin region in older coleoptiles where the coleoptile later splits to allow emergence of the leaves).

Because of the presence of extensive vascular tissue in cotyledons it seemed possible that IAA-^{14}C could have gained artificial entrance into the xylem through injection. Twenty cotyledons of *P. coccineus* seedlings were injected with 10 μl of the dye per cotyledon and 20 cotyledons of *P. vulgaris* with 5 μl per cotyledon. Immediately the dye diffused through a limited region around the zone of injection. After 48 hours the cotyledons were sliced and examined. No evidence of any staining of the vascular tissue was found. Dye injected into endosperms of corn seedlings did not reach the xylem in the scutellum, but it did enter between the endosperm and scutellum.

Another experiment designed to illustrate whether IAA was translocated in the xylem to the shoots made use of the technique used by Snow (22). Epicotyls of young seedlings of *P. coccineus* were treated with a hot glass rod to destroy living tissue in a region about 6 to 7 mm long and approximately 3 cm above the cotyledons. The treated (branded) shoots were then supported by fastening loosely to glass rods with thin strips of Parafilm. Control shoots were similarly fastened to glass rods. They were then left in the dark for 24 hours for the heated tissue to die. The procedure, if done carefully, kills the living tissue in the region treated but does not affect the xylem. The following treatments were given: A) Branded plants injected with IAA-^{14}C and kept in dark. B) Branded plants injected with IAA-^{14}C and kept in light. C) Branded plants, roots cut and placed in vital stain and kept in dark. D) Branded plants, roots cut and placed in vital stain and kept in light. E) Unbranded plant injected with IAA-^{14}C and kept in dark. F) Unbranded plant injected with IAA-^{14}C and kept in light. G) Unbranded plants, roots cut and placed in vital stain and kept in dark. H) Unbranded plants, roots cut and placed in vital stain and kept in light.

A fan was placed in the dark room and directed towards the plants to increase transpiration. Those kept in the light were placed near a north facing window.

After 24 hours shoots of the plants of which the cotyledons were injected with IAA-^{14}C were cut at about 5 mm above the brand and at a corresponding height on the unbranded shoots. These shoots were ground and extracted with methanol for 3 hours. The extracts were plated on planchets and counted in a Gas Flow counter. Figure 8 shows the marked difference in the amounts of label translocated through the branded and unbranded shoots. In the branded shoots the amount was only just above background.

Examination of the dye-treated plants revealed that the dye had been translocated into the shoots of all plants (fig 8), indicating that the xylem strands remained intact in the branded plants. The branded shoots in all cases had grown very little whereas the unbranded shoots had grown considerably during the 24 hour period. These experiments were repeated with similar results. The results indicate that the label was not translocated into the shoot by the xylem.

Translocation in Excised Segments. As seen from table II very little or no tryptophan was translocated through receiver blocks in any of the treatments with both *P. coccineus* epicotyls and *Z. mays* coleoptiles. Even in treatments using a concentration of 100 μM tryptophan, which gave counts in the original donor blocks of 16,800 and 14,380 cpm

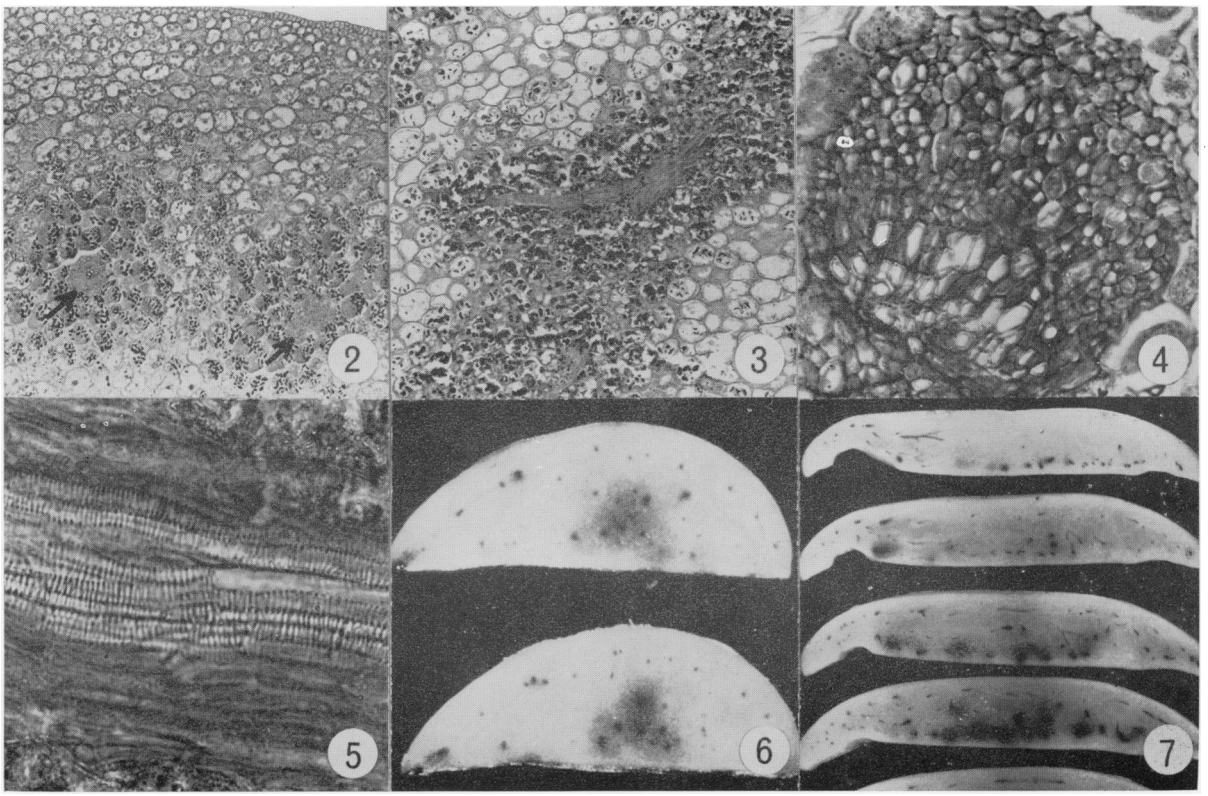

FIG. 2. Transverse section of cotyledon of *Phaseolus coccineus* showing vascular bundles (arrow) and cells containing inclusions distributed around the vascular bundles. (× 90).

FIG. 3. Longitudinal section of cotyledon of *Phaseolus coccineus* showing vascular bundle and cells containing inclusions distributed around the vascular bundle. (× 90).

FIG. 4. Transverse section of cotyledon of *Phaseolus coccineus* showing vascular bundle. (× 625).

FIG. 5. Longitudinal section of cotyledon of *Phaseolus coccineus* showing vascular bundle. (×625).

FIG. 6. Transverse sections of a cotyledon of *Phaseolus coccineus* after vital staining with Light Green SF Yellowish, showing the distribution of vascular tissue. (× 6).

FIG. 7. Longitudinal sections of a cotyledon of *Phaseolus coccineus* after vital staining with Light Green SF Yellowish, showing the distribution of vascular tissue. (× 3).

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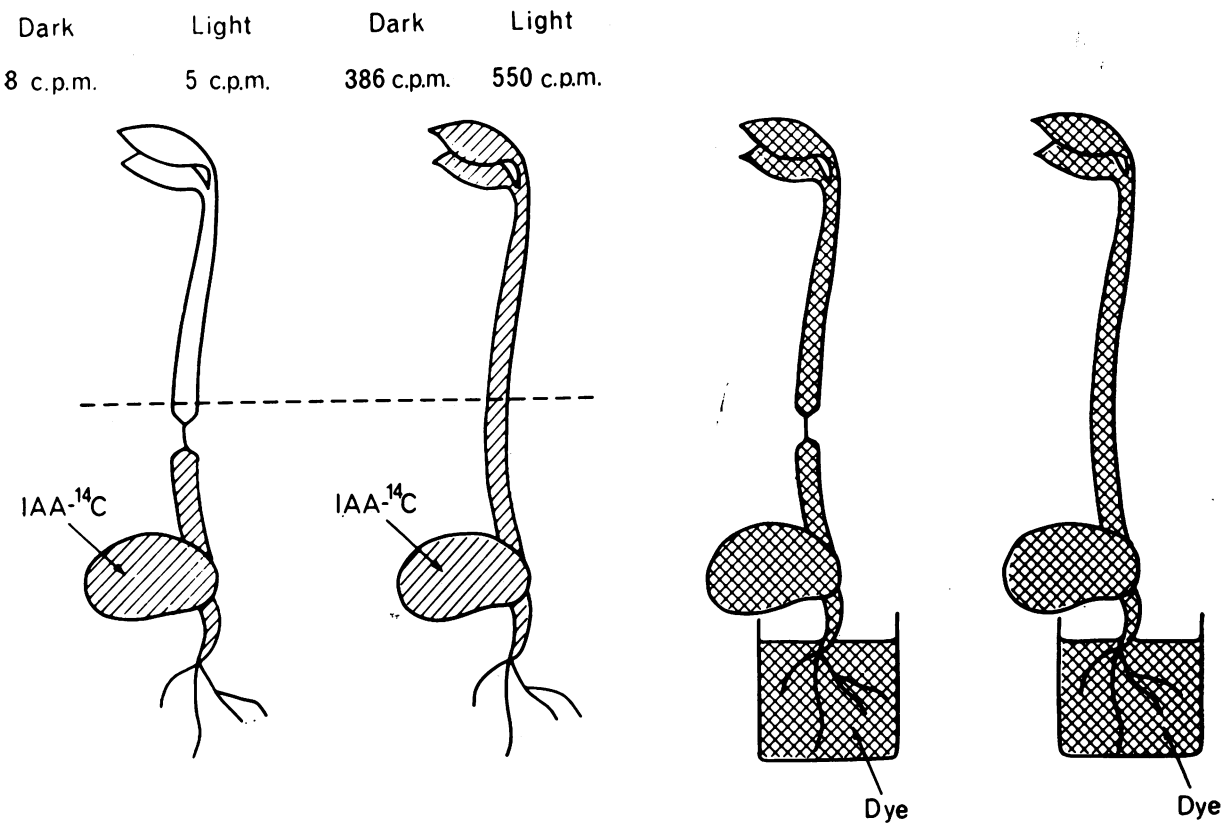

FIG. 8. The effect of branding on the translocation of label and dye into the shoots of *Phaseolus coccineus*. The label was administered by injecting IAA-^{14}C into the cotyledons and the dye, Light Green SF Yellowish, was allowed to be taken up by the roots.

Table II. *Translocation of Tryptophan-^{14}C in Epicotyl Segments of* Phaseolus coccineus *and Coleoptile Segments of* Zea mays

Results are averages of 10 replicates.

Phascolus coccineus epicotyls			*Zea mays* coleoptiles		
Orientation of tissue	Time (hr)	% Supplied activity in receiver blocks	Orientation of tissue	Time (hr)	% Supplied activity in receiver blocks
Expt 1					
Donor concn 10 μM					
(2510 ± 9* cpm)					
A	3	0.12			
B	3	0			
C	3	0.16			
D	3	0			
Expt 2					
Donor concn ca. 10 μM					
(1960 ± 8 cpm)					
A	3	0			
B	3	0.10			
C	3	0.15			
D	3	0.20			
Expt 3			Expt 4		
Donor concn 100 μM			Donor concn 100 μM		
(16,800 ± 30 cpm)			(14,380 ± 21 cpm)		
A	3	0.07	A	3	0.08
D	3	0.05	B	3	0.20
A	6	0.23	C	3	0.04
D	6	0.08	D	3	0.05

* Standard deviations of counting rates.

for *P. coccineus* and *Z. mays* respectively, very little was detected in the receiver blocks. After counting, the agar blocks and tissue segments used in experiment 3, treatment A (table II) were extracted with methanol and chromatographed. Scanning of the chromatograms of the receiver blocks for radioactivity showed the presence of a single labeled compound with an R_F value of tryptophan. This compound was barely detectable in the 3 hour treatment but was distinct in the 6 hour treatment. Chromatograms of the tissue extracts showed large peaks at R_F regions corresponding to tryptophan-^{14}C and minor peaks at R_F values of 0 and 0.08 to 0.14 for the 3 hour treatment. These peaks were considerably larger in the 6 hour treatment and also 2 more minor peaks with R_F values 0.66 to 0.69 and 0.80 to 0.85 were present. The compounds at R_F values 0 and 0.08 were also present in the donor blocks, being in larger amounts after the 6 hour treatment than after the 3 hour treatment.

The results presented in table III show that there is a strict basipetal polarity of IAA-^{14}C translocation in both *P. coccineus* epicotyls and *Z. mays* coleoptiles at the concentrations used. Little or no radioactivity was translocated in an acropetal direction. With both 10 μM and 100 μM IAA-^{14}C approximately the same proportion of the radioactivity supplied was translocated basipetally through to receiver blocks using *P. coccineus* epicotyl segments. With *Z. mays* coleoptiles doubling the time approximately doubled the proportion of radioactivity in the receiver blocks.

Only 1 labeled compound, corresponding in R_F value to IAA, was detected in receiver blocks after treatment of both *Z. mays* and *P. coccineus* tissue with IAA-^{14}C.

When dye was incorporated into donor blocks and applied to coleoptile segments it was observed that dye diffused into the segments to about 1 mm and then moved in 2 distinct columns only, which corresponded to the vascular bundles. This occurred whether the donor blocks were placed on the apical or basal ends of the segments. Occasionally when a segment from an older coleoptile was used, a third track of dye was observed but on sectioning this was found to be associated with the thin part of the coleoptile between the vascular bundles which later splits to allow the emergence of the leaves. Examination of the coleoptiles revealed that after a few hours treatment (up to 3 hr) the dye was present only in the xylem. In sections treated for a longer period the dye was present in the rest of the vascular bundles though it was restricted to these structures. The dye was translocated through the total length of the coleoptile segments in a few hours.

In *P. coccineus* epicotyl segments also the dye was translocated mainly in the vascular tissue. At the end of the tissue segment distal to the donor block the dye was present almost exclusively in the xylem. Nearer to the donor the dye was also present in the phloem and possibly in other tissue.

Discussion

A labeled compound with an R_F value of IAA was extracted from shoots of both *P. coccineus* and *Z. mays* following injection of IAA-^{14}C into the storage material of the seed. It is possible that a derivative of IAA could have been formed and then IAA itself released from it in the shoot, but at least in the case of *Z. mays*, IAA-^{14}C itself was found to be translocated acropetally and diffused into receiver agar blocks. Tryptophan-^{14}C was also found in the shoots of *P. coccineus* following injection into the cotyledons. Therefore it appears that IAA-^{14}C and tryptophan-^{14}C injected in small amounts into the

Table III. *Translocation of IAA-^{14}C in Epicotyl Segments of* Phaseolus coccineus *and Coleoptile Segments of* Zea mays

Results are averages of 10 replicates.

Phaseolus coccineus epicotyls			*Zea mays* coleoptiles		
Orientation of tissue	Time (hr)	% Supplied activity in receiver blocks	Orientation of tissue	Time (hr)	% Supplied activity in receiver blocks
Expt 5					
Donor concn 10 μM					
(610 ± 6* cpm)					
A	3	4.3			
B	3	3.3			
C	3	0			
D	3	0			
Expt 6			Expt 7		
Donor concn 100 μM			Donor concn 100 μM		
(5790 ± 15 cpm)			(3950 ± 11 cpm)		
A	3	4.3	A	3	6.7
B	3	2.7	D	3	0.02
C	3	0.05	A	6	16.5
D	3	0.05	D	6	0

* Standard deviations of counting rates.

seed storage material of the seedlings studied was translocated acropetally into the shoots. This is surprising in the case of IAA because of the strong basipetal polarity of young shoots.

From the vital staining experiments it was demonstrated that xylem is continuous from the roots to the cotyledons, i.e. that the xylem in the cotyledons conducts away from the stem to the distal end of the cotyledons. However, whether a xylem system also exists which conducts from the cotyledons up into the epicotyl has not been shown. Such a system, in fact, seems unlikely unless it plays a relatively large part in conducting organic nutrients from the storage tissue to the shoot.

The translocation studies with tryptophan-^{14}C using excised tissue segments indicate that unlike IAA, tryptophan is not actively transported through tissue segments. These findings are in keeping with those of Schrank and Murrie (20) who found that tryptophan-^{14}C was not translocated to any great extent in oat coleoptiles.

One might expect that in freshly excised segments as were used for these studies, dye should be translocated by diffusion through the xylem vessels as they are filled with fluid. Consequently, in experiments of long duration using high concentrations of soluble substances, traces of these substances in the receiver blocks might be due to diffusion through the liquid filled xylem vessels. This could have occurred in the experiments in which traces of tryptophan-^{14}C were detected in the receiver blocks when high concentrations of tryptophan-^{14}C were used in the donor blocks. In similar experiments in which IAA-^{3}H was supplied from donor blocks, the highest concentration of label was found by autoradiography in the vascular tissue (29).

The polarity experiments using IAA-^{14}C provides evidence that the epicotyl of *P. coccineus* seedlings is yet another structure in which a strict basipetal polarity of IAA transport occurs. Inverting the tissue segments with respect to gravity decreased the proportion of label translocated basipetally as was also observed by Hertel and Leopold (12) and Naqvi and Gordon (18).

An interesting observation is that in all experiments (i.e. using both types of tissue and both labeled compounds) the treatment in which least label was taken up from the donor blocks was treatment C, with tissue in normal orientation arranged to test acropetal translocation.

Although tryptophan was found not to be actively translocated in the type of experiment normally used to test transport polarity, the negative results do not indicate that it is not the IAA precursor which is translocated to the tip. They merely indicate that it is not actively transported in a manner similar to that in which IAA is actively transported and which can be tested with excised segments. The excised segment is after all in an unnatural environment and lacks various pressures which regulate over-all translocation in the intact plant.

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Literature Cited

1. Black, M. K. and D. J. Osborne. 1965. Polarity of transport of benzyladenine, adenine and indole-3-acetic acid in petiole segments of *Phaseolus vulgaris*. Plant Physiol. 40: 676–80.
2. Briggs, W. R. 1963. Mediation of phototropic responses of corn coleoptiles by lateral transport of auxin. Plant Physiol. 38: 237–47.
3. Eames, A. J. and L. H. MacDaniels. 1947. An introduction to Plant Anatomy. McGraw-Hill Book Company Inc., New York. 427 p.
4. Esau, K. 1960. Anatomy of Seed Plants. John Wiley and Sons, Inc., New York. 376 p.
5. Fletcher, R. A. and S. Zalik. 1965. Effect of light of several spectral bands on the metabolism of radioactive IAA in bean seedlings. Plant Physiol. 40: 549–52.
6. Gillespie, B. and K. V. Thimann. 1963. Transport and distribution of auxin during tropistic response. I. The lateral migration of auxin in geotropism. Plant Physiol. 38: 214–25.
7. Godfrin, M. J. 1884. Recherches sur l'anatomie comparée des cotylédons et de l'albumen. Ann. Sci. Nat. Botan. Biol. Vegetale. 6th Ser. 19: 5–158.
8. Goldsmith, M. H. M. and K. V. Thimann. 1962. Some characteristics of movement of indoleacetic acid in coleoptiles of *Avena*. I. Uptake, destruction, immobilization and distribution of IAA during basipetal translocation. Plant Physiol. 37: 492–505.
9. Gordon, S. A. 1954. Occurrence, formation and inactivation of auxins. Ann. Rev. Plant Physiol. 5: 341–78.
10. Gordon, S. A. 1961. The biogenesis of auxin. In: Encyclopedia of Plant Physiology. W. Ruhland, ed. Springer-Verlag, Berlin. XIV: 620–46.
11. Harvey, R. B. 1930. Tracing the transpiration stream with dyes. Am. J. Botany 17: 657–61.
12. Hertel, R. and A. C. Leopold. 1963. Versuche zur Analyse der Auxintransports in der Koleoptile von *Zea mays* L. Planta. 59: 535–62.
13. Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill Book Company, New York and London. 523 p.
14. Larsen, P. 1951. Formation, occurrence and inactivation of growth substances. Ann. Rev. Plant Physiol. 2: 169–98.
15. Leopold, A. C. 1955. Auxins and plant growth. University of California Press, Berkeley and Los Angeles. 354 p.
16. Leopold, A. C. 1961. The transport of auxin. In: Encyclopedia of Plant Physiology. W. Ruhland, ed. Springer-Verlag, Berlin. XIV: 671–82.
17. McCready, C. C. and W. P. Jacobs. 1963. Movement of growth regulators in plants. II. Polar transport of radioactivity from indoleacetic acid-(^{14}C) and 2,4-dichlorophenoxyacetic acid-(^{14}C) in petioles of *Phaseolus vulgaris*. New Phytologist 62: 19–34.

18. NAQVI, S. M. AND S. A. GORDON. 1966. Auxin transport in *Zea mays* L. coleoptiles. I. Influence of gravity on the transport of indoleacetic acid-2-^{14}C. Plant Physiol. 41: 1113–18.

19. PAMMEL, L. H. 1899. Anatomical characters of the seeds of *Leguminosae*, chiefly genera of Gray's Manual. Trans. of the Academy of Sciences of St. Louis. IX: 91–274.

20. SCHRANK, A. R. AND D. G. MURRIE. 1962. The absorption and utilization of tryptophan by *Avena* coleoptiles. Physiol. Plantarum 15: 683–92.

21. SKOOG, F. 1937. A deseeded *Avena* test method for small amounts of auxin and auxin precursors. J. Gen. Physiol. 20: 311–34.

22. SNOW, R. 1929. The transmission of inhibition through dead stretches of stem. Ann. Botany 43: 261–67.

23. STOWE, B. B. AND K. V. THIMANN. 1954. The paper chromatography of indole compounds and some indole containing auxins of plant tissues. Arch. Biochem. Biophys. 51: 499–516.

24. THIMANN, K. V. 1948. Plant growth hormones. In: The Hormones. G. Pincus and K. V. Thimann, eds. Academic Press, Inc., New York. v. I, 886 p.

25. WENT, F. W. AND K. V. THIMANN. 1937. Phytohormones. The Macmillan Company, New York. 294 p.

26. WILDMAN, S. G. AND J. BONNER. 1948. Observations on the chemical nature and formation of auxin in the *Avena* coleoptile. Am. J. Botany 35: 740–46.

27. WITHROW, R. B. AND L. PRICE. 1957. A darkroom safelight for research in plant physiology. Plant Physiol. 32: 244–48.

28. WHITEHOUSE, R. L. 1965. Studies on metabolism and translocation of tryptophan and indole acetic acid in seedlings of several species. Ph.D. thesis. University of Alberta, Edmonton, Alberta.

29. WHITEHOUSE, R. L. AND S. ZALIK. 1967. Autoradiographic evidence of tritiated indolyl-3-acetic acid in epicotyl tissue of *Phaseolus coccineus*. Experientia 23: 387–88.