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Translocation of Zeatin Riboside and Zeatin in Soybean Explants

Larry D. Noodén[1] and D. S. Letham[2]

[1]Botany Department, University of Michigan, Ann Arbor, Michigan 48109 USA
[2]Department of Developmental Biology, Research School of Biological Sciences, Australian National University, Canberra, ACT, 2601, Australia

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Abstract. Soybean explants consisting of a leaf, one or more young pods, and a subtending piece of stem were given a 1-h pulse of ^3H (ring-labeled)-zeatin riboside (ZR) or -zeatin (Z), via the base of the stem, followed by a 24-h incubation. At the end of the pulse, about 55% of the soluble ^3H was in the leaf blades, 11% in the petiole, 30% in the stem, 2% in the carpels, 0.1% in the seed coats, and 0.08% in the embryos. After 24 h, the percentages were 58, 7, 26, 6, 2, and 0.3, respectively. During this period, the total soluble ^3H decreased by 84%, the remainder being bound to "insoluble" material. The ^3H-cytokinin was rapidly converted to diverse metabolites including adenosine and adenine. At the end of the 1-h pulse, appreciable percentages (1–16%) of the total soluble ^3H in the seed coats chromatographed with ZR (or dihydro ZR) and with the 5′-phosphate of ZR, but these percentages declined markedly at 24 h. No distinct peaks of ^3H corresponded to known metabolites in the soluble extracts of embryos, and at 24 h, the ^3H equivalent to ZR must have been less than 0.0006% of the ^3H-ZR supplied. The bound ^3H corresponded to adenine and guanine in DNA and RNA. In contrast to cytokinin, ^3H-adenosine given as a pulse was readily translocated into the seed coats and embryos. Thus, cytokinin (ZR and Z) flowing up through the xylem from the root system does not readily enter the embryo (though metabolites such as adenosine could), and the seeds clearly do not compete with the leaves for this supply of cytokinin.

A knowledge of the translocation pattern of a hormone is essential for understanding its function. Considerable evidence indicates that the root apices synthesize cytokinins which are translocated up through the xylem into the leaves in the transpiration stream (Kulaeva 1962, Kende 1965, Letham 1978, van

Staden and Davey 1979). The root-produced cytokinins have long been suspected to prevent the senescence of leaves (Kulaeva 1962, Noodén 1980b), and cytokinins from the roots appear to be essential for maintaining the foliage of soybeans during pod development (Neumann et al. 1983, Garrison et al. 1984). The cytokinins from the roots also delay pod development and increase seed size in soybeans (Neumann et al. 1983).

The developing seeds of soybean cause senescence of the whole plant (monocarpic senescence) through induction of senescence in the foliage (Noodén 1980a). One variation of the "nutrient" diversion explanation for the induction of monocarpic senescence is that the seeds can monopolize the supply of cytokinin coming up from the roots, thereby creating a deficiency in the leaves (Noodén 1980 a,b). Implicit in the cytokinin diversion theory is the idea that the cytokinins in the seeds come from the roots. Although these theories can be tested directly, little effort has been made to do this. Hence, in this paper we have studied the translocation of ^{3}H-labeled zeatin riboside (ZR) and zeatin (Z) supplied to soybean cuttings (explants) via the transpiration stream and have endeavored to detect in the developing seeds known ZR and Z metabolites, namely, dihydrozeatin (DZ), dihydrozeatin riboside (DZR), adenine (A), adenosine (AR), O-β-D-glucopyranosylzeatin (OGZ) and its 9-riboside (OGZR), O-β-D-glucopyranosyldihydrozeatin (OGDZ) and its 9-riboside (OGDZR), 7- and 9-β-D-glucopyranosylzeatin (7GZ and 9GZ), lupinic acid (LA), dihydrolupinic acid (DLA), zeatin riboside 5'-monophosphate (ZMP) and its dihydro derivative (DZMP), and also isopentenyladenine (IPA) and isopentenyladenosine (IPAR).

Materials and Methods

Plant Material

Soybeans (*Glycine max* L. Merrill cv. Anoka) were grown in Ann Arbor, Michigan, as previously described (Lindoo and Noodén 1976); plants potted in soil were grown on a glasshouse bench for about 25 days and thereafter in environmental control chambers with 10-h days, 27°C day temperature, and 21°C night temperature. Soybeans, cv. Bragg, were started in glasshouses in Canberra, Australia (temperature about 25°C) in late April and were used in early July. These plants were placed in environmental control chambers set as above for 24 h before excision of the explants.

Application of the ^{3}H-labeled Compounds

Explants consisting of cuttings with a leaf, one or more pods, and a subtending stem segment (Neumann et al. 1983) were excised about 2 h after the start of the light period and kept in environmental control chambers as described above. In experiments with ^{3}H-zeatin, -zeatin riboside, and -adenosine, the explants each had two pods with seeds in early podfill (about 4 mm long). For one experiment with ^{3}H-zeatin riboside, the pods were still younger (prepodfill), with seeds 2 mm or less and carpels still extending.

The [8-^{3}H]-zeatin riboside and [2,8-^{3}H]-zeatin used were at the specific activity of 260 and 135 mCi/mmol, respectively, and were prepared as described elsewhere (Letham and Young 1971, Summons et al. 1980). Each explant was allowed to take up 1.0 ml of 10 μM ^{3}H-cytokinin (which required about 1 h). After this, the base of each explant was rinsed with water and the explant either transferred to water or harvested. During the harvest, each explant was quickly subdivided, weighed, and dropped into cold extraction medium #1, one organ at a time. This medium consists of methanol/$CHCl_3$/formic acid/H_2O, 12:2:1:2, v/v (modified from Bieleski 1964); about 10 ml were used per g of tissue or at least 5 ml for the smaller organs such as the seed coats. Each sample was stored at −20°C for 18 h and then ground thoroughly using a mortar and pestle with a small amount of sand. This mixture was centrifuged, and the pellet was resuspended in cold extraction medium #2, methanol/formic acid/H_2O, 6:1:4, v/v, left for 8–10 h in the cold, and then centrifuged. The two supernatants were combined (soluble ^{3}H); this and the pellet (insoluble ^{3}H) were saved for further analyses.

After the supernatants were dried on a rotary evaporator at 35°C, the soluble ^{3}H was redissolved in 50% (v/v) methanol using a volume about equal to the original tissue volume, but at least 0.50 ml per sample. Samples of this solution were then used for determination of the total soluble ^{3}H or for thin layer chromatography (TLC). Similar experiments were performed with [2,8−^{3}H]-adenosine (32 Ci/mmole, purchased from ICN, Irvine, California, USA).

All treatments and all determinations were, at the least, duplicated.

Analysis of the ^{3}H-labeled Soluble Cytokinin Metabolites

Solvents for TLC were: A, *n*-butanol/acetic acid/water (12:3:5, v/v); B, n-butanol/conc. NH_4OH/water (6:1:2, v/v; upper phase); C, isopropanol/conc. NH_4OH/water (12:1:7, v/v); D, methanol/water (3:7, v/v). The following dye markers were used: A, meldola blue (Gurr-Searle Diagnostic, High Wycombe, Bucks., England); B, drimarene brilliant blue K-BL (Polysciences, Warrington, Pennsylvania, USA); C, rhodamine B; D, toluidine blue (G. T. Gurr); E, fast green FCF (E. Gurr, London, England); F, bromocresol green (Aldrich, Milwaukee, Wisconsin, USA; G, orange G (E. Gurr).

The soluble ^{3}H derived from ^{3}H-cytokinins was fractionated initially by one-dimensional TLC (solvent A) using Merck silica gel 60 PF_{254}. Dye markers A, B, C, and D were added to the extract before TLC. Each chromatogram was cut into zones approximately 12 mm in length. Dye A was located in the upper third of zone 4 (cf. Fig. 1), but the other dye-containing zones were centered on the dye therein. The TLC zones contained cytokinin metabolites as follows: Zone 3: AMP, ZMP, DZMP; Zone 4: LA, DLA; Zone 5: OGZ, OGZR, OGDZ, OGDZR, 7GZ; Zones 6, 7: 9GZ, A, AR; Zones 8, 9: Z, DZ, ZR, DZR. This cluster is designated (D)Z/ZR.

To further characterize the metabolites of ^{3}H-ZR in extracts of leaves, carpels, seed coats, and embryos, the extracts were chromatographed on silica gel in two dimensions (first: solvent A; second: solvent B) with the following added markers: Z, ZR, A, AR, and dyes A and D. Before developing in the

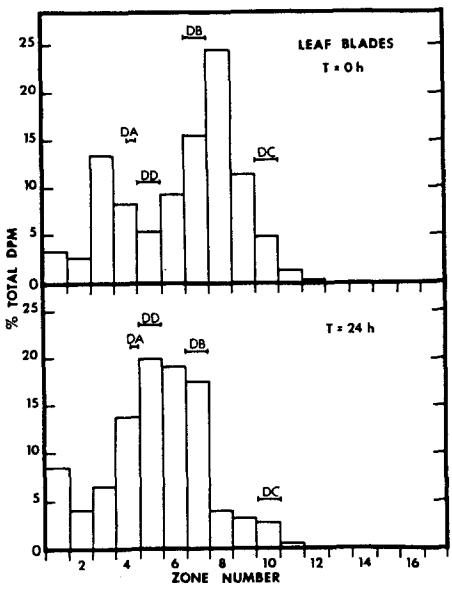


Fig. 1. Thin-layer chromatogram profiles of the soluble ^3H from the leaf blades of early podfill soybean explants (cv. Bragg) fed a pulse of ^3H-zeatin riboside via the cut base. Silica gel plates developed with *n*-butanol/acetic acid/H_2O (12:3:5, v/v). The zones occupied by the marker dyes are represented by horizontal bars.

second dimension, dyes F and G were added to the dye D zone, while dyes E and G were added to the dye A zone. In the second dimension, OGZ, OGZR, OGDZ, OGDZR, and 7GZ run as a band (the "glucoside zone") between dye G (R_f 0.09) and dye F (R_f 0.21), while LA and DLA run together beween dye G and E (R_r values 0.09 and 0.16). The above zones and marker spots were eluted for determination of ^3H (Gordon et al. 1974) and in some cases for further chromatography. Methanol/water/acetic acid (20:20:1, v/v) was used for the latter elution.

A ZMP zone was located on the basis of its mobility (R_A 0.64, relative to dye A in solvent A; R_f in solvent B, 0.01), but this would contain about half of any AMP present. To separate the ZMP from AMP, authentic ZMP was used as an additional marker, and after chromatography with solvent B, the lower region of the plate (below dyes E and G) was redeveloped in the direction of the second dimension with solvent C (R_f ZMP, 0.51; R_f AMP, 0.42).

Since the above two-dimensional TLC did not resolve the cytokinins with unsaturated side chains from the corresponding dihydro forms, these mixtures were designated (D)Z, (D)ZR, (D)ZMP, and (D)LA. ZR and DZR or Z and DZ were separated after elution by reverse-phase TLC (solvent D) on paraffin-impregnated Merck silica gel 60 HF_{254} (15 μm particle size). To impregnate the layers, a 6% (w/v) solution of liquid paraffin (0.87–0.89 g/ml, Ajax Chemicals Ltd, Sydney, Australia) in petroleum ether (60–80°) was allowed to flow to the top of the plate. To confirm the identity of ^3H-(D)ZMP, the appropriate two-dimensional TLC zone was eluted and hydrolyzed with alkaline phosphatase (Gordon et al. 1974). The hydrolyzate was co-chromatographed with ZR on silica gel (solvent B) and with ZR and DZR on paraffin-impregnated silica gel (solvent D).

Analysis of ^{3}H-labeled Solvent-insoluble Metabolites

The insoluble, ^{3}H-labeled material was either (a) resuspended in 0.1% (w/v) sodium dodecylsulfate and incubated overnight at room temperature before taking 1.0 ml for a rough estimation of the ^{3}H, or (b) washed and hydrolyzed to release the nucleic acid purine bases. In preparation for the hydrolysis (b), the ^{3}H-labeled, insoluble material was washed with cold 5% (w/v) trichloroacetic acid (2×), 80% (v/v) ethanol (3×), 100% ethanol (1×), and 3/1 (v/v) ethanol/$CHCl_3$ (1×).

To extract the purine bases from the total nucleic acid, this washed pellet was suspended in 1 M $HClO_4$, heated at 90°C for 1 h, brought to room temperature, adjusted to about pH 5 with 3.0 M KOH, cooled to 4°C, and centrifuged to remove the $KClO_4$, leaving the purine bases in the supernatant. This procedure, tested on AR, A, ZR, and Z, was found to free the purine bases without changing their TLC behavior. Alternatively, separate RNA and DNA extracts were prepared by a modification of the Schmidt-Thannhauser procedure (DeDeken-Grenson and DeDeken 1959), and these fractions were heated (90°C for 15 min) with 1 M $HClO_4$ to release the purine bases. The neutralized (KOH) base fractions from RNA and DNA were analyzed by TLC on fluorescent silica gel plates sprayed with 0.3 m sodium borate solution and developed in solvent B.

Results

Distribution of ^{3}H from ^{3}H-Cytokinins

When ^{3}H-ZR was fed into early podfill explants through the cut base of their stems, most of the soluble (extractable) ^{3}H appeared in the leaf blades after 1 h, with large amounts also in the stem and petiole (Table 1). After 24 h, the total soluble ^{3}H in the explant decreased from 2,371,000 dpm to 380,000 dpm; this could be expected to result from breakdown of the cytokinin and its incorporation into the insoluble material (discussed below). The soluble ^{3}H in the stem and petiole also decreased, but this decrease was proportionately greater than that in the leaves. The total and relative activity in the pods was quite small (about 2.1% of the total soluble ^{3}H) at the end of the pulse. Given the differing functions of the tissues within the pods and the differences in the vascular connections between the stem and these tissues (Thorne 1980, 1981), it seemed important to subdivide the pods, at least, into their major components. Most of the soluble ^{3}H in the pods occurred in the carpels, with very little in the seed coats and even less in the embryos. The total soluble ^{3}H in the seed coats and embryos after 24 h constituted only 0.14 and 0.034%, respectively, of the ^{3}H-ZR applied.

Because the organs represented in Table 1 differed greatly in their mass, it seemed useful to view the distribution of soluble ^{3}H on a g fresh weight basis. This also gave a crude average concentration for each organ. On a g fresh weight basis, the ^{3}H levels in petiole, stem, and seed coats were elevated (Table 2). This increase also applied to a lesser extent to the embryos, but the ^{3}H

Table 1. Distribution of ^3H from ^3H (ring-labeled)-zeatin riboside given as a 1-hour pulse via the base of soybean explants (cv. Bragg) cut at early podfill.

	Soluble ^3H			
	At end of pulse		After 24 h	
Explant part	DPM	% of soluble DPM	DPM	% of soluble DPM
Blades	1,218,500	51.4	236,500	62.3
Petiole	263,500	11.1	22,800	6.0
Stem	839,800	35.4	82,900	21.8
Carpels	45,800	1.9	27,500	7.2
Seed coats	1,750	0.1	8,100	2.1
Embryos	2,120	0.1	1,950	0.5

Table 2. Distribution (on a fresh weight basis) of ^3H (ring-labeled)-zeatin riboside given as a 1-hour pulse via the base of soybean explants (cv. Bragg) cut at early podfill.

	Soluble ^3H as DPM/g fresh weight[a]	
Explant part	At end of pulse	After 24 h
Blades	1,324,000	202,000
Petioles	1,198,000	142,000
Stem	2,210,000	244,000
Carpels	69,400	32,000
Seed coats	26,500	83,500
Embryos	10,300	5,800

[a] Calculated from Table 1.

level in embryos is still much lower than those in the other organs analyzed. The highest "concentration" was in the stem, followed by the leaf blades, then the petioles.

The flux of ^3H-ZR into the seeds, particulary into the embryos seemed minimal; thus it was decided to determine: (a) if a greater flux of ^3H-ZR into the seeds could be obtained with explants taken at an earlier stage, and (b) if ^3H-Z entered the seeds more readily.

Table 3 shows the translocation of ^3H-ZR and its metabolites in younger explants (the pod extension phase, before podfill). The distribution of soluble ^3H at the end of the pulse and after 24 h was basically the same as in early podfill (Table 1). Again, most of the ^3H entered the leaves, and rather little entered the seeds, especially the embryos. Only 0.15 and 0.019% of the ^3H applied ended up in the soluble fractions from the seed coats and embryos at 24 h.

^3H-Z fed to explants taken at early podfill showed the same distribution (Table 4) as the experiments with ^3H-ZR (Tables 1 and 3). In the seed coats and embryos, only 0.41 and 0.049%, respectively, of the ^3H applied occurred at 24 h.

In general, a rather small proportion of the ^3H from ^3H-ZR or -Z entered the

Table 3. Distribution of ^3H from ^3H (ring-labeled)-zeatin riboside given as a 1-hour pulse via the base of soybean explants (cv. Bragg) cut just before podfill.

	Soluble ^3H			
	At end of pulse		After 24 h	
Explant part	DPM	% of soluble DPM	DPM	% of soluble DPM
Blades	1,872,000	51.3	704,000	43.8
Petiole	619,400	17.0	150,800	9.4
Stem	1,050,600	28.8	626,530	39.0
Carpels	98,200	2.7	115,170	7.2
Seed coats	7,420	0.2	8,400	0.5
Embryos	4,850	0.1	1,060	0.07

Table 4. Distribution of ^3H from ^3H (ring-labeled)-zeatin given as a 1-hour pulse via the base of soybean explants (cv. Bragg) cut at early podfill.

	Soluble ^3H			
	At end of pulse		After 24 h	
Explant part	DPM	% of soluble DPM	DPM	% of soluble DPM
Blades	1,249,400	62.0	401,000	69.5
Petiole	182,150	5.6	32,400	5.6
Stem	535,550	26.6	103,850	18.0
Carpels	47,300	2.3	26,400	4.6
Seed coats	1,520	0.1	12,160	2.1
Embryos	630	0.03	1,480	0.2

embryos, but it might be questioned to what extent even this small amount of ^3H represented ^3H-cytokinin as opposed to metabolites.

Metabolism of the ^3H-Cytokinin

In all three types of experiments (Tables 1, 3, and 4), the soluble ^3H in the leaf blades, petioles, and stems declined after the end of the pulse from 32, 7.1, and 17%, respectively (averages from Tables 1, 3, and 4) of the ^3H applied to 9.7, 1.4, and 5.3%, respectively, at 24 h. This, of course, suggests metabolism of the cytokinin and incorporation into insoluble material. Indeed, crude preliminary analyses of the insoluble fractions corresponding to Table 1 indicated that most, if not all, of the rest of ^3H occurred in these fractions. Data on the nature of this insoluble ^3H will be presented below, after the analysis of the soluble fraction.

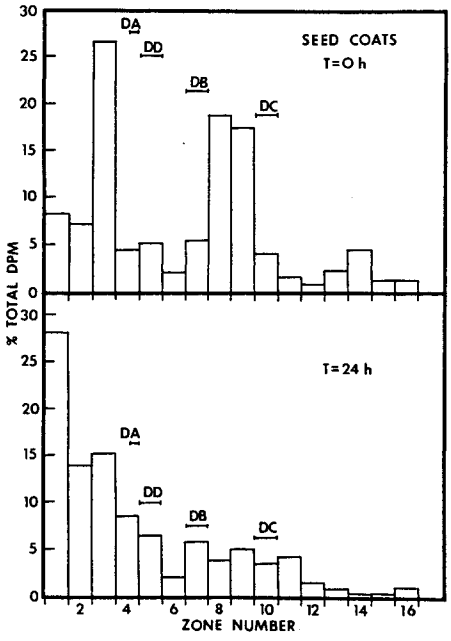
One-dimensional (and later two-dimensional) TLC analyses were made for the soluble ^3H in the leaf blade (Fig. 1), seed coat (Fig. 2,), and embryo extracts

(Fig. 3) as well as other parts (not shown). The profiles for ^3H distribution on the *n*-butanol-acetic acid-water (solvent A) chromatograms differed for each of the tissues shown in Figs. 1–3, and they changed with time. In these chromatograms, (D)Z and (D)ZR ran between dyes B and C (zones 8 and 9). Even at the end of the pulse (T = O), this fraction contained no more than 35% of the total soluble ^3H of blades and seed coats, and this decreased to 8% after 24 h. With leaf blades at T = 0, a high level of ^3H occurred in the zones (6 and 7) corresponding to A and AR, the expected breakdown products of Z and ZR (Letham 1978).

Complete analyses of these ^3H-labeled materials in extracts with significant levels of ^3H is a very involved project that will be discussed elsewhere. Nonetheless, some minimal characterization of these ^3H-compounds, especially in the seed coats and embryos, seems necessary here. Two-dimensional TLC indicated that ^3H-A and -AR derived from ^3H-ZR were present in the leaf blades, carpels, and seed coats (Table 5); however, the percentage of the soluble ^3H chromatographing as (D)ZR and (D)Z at T = 0 was 22.4 and 8.6%, respectively, for leaf blades and 12.8 and 5.0%, respectively, for seed coats at early podfill (Table 5). Preliminary analyses on the paraffin-coated TLC plates suggested that 30–60% of the ^3H chromatographing in two-dimensional TLC with Z or ZR in the leaf blade extracts (T = 0) was DZ or DZR, and this result was confirmed by reverse-phase (C_8) high pressure liquid chromatography. H^3-labeled material that chromatographed as (D)ZMP on two-dimensional TLC occurred in seed coat extracts at T = 0. Phosphatase hydrolysis and TLC of the resulting nucleoside(s) established that the ^3H was associated with ZMP but not with DZMP. At T = 24, the peaks in the seed coat extracts (Fig. 2) corresponding to (D)Z and (D)ZR (zones 8 and 9) and to (D)ZMP and AMP (zone 3) had become indistinct. At 24 h, two-dimensional TLC of the extracts showed that if ^3H-(D)Z and -(D)ZR were present at all in the seed coats, their contribution to total radioactivity was negligible (Table 5). Some ^3H in the seed coat extracts appeared to be attributable to O-glucosides (see Table 5); however, this was insufficient to permit further characterization. In work to be detailed elsewhere, O-glucoside metabolites have been identified in leaf blades.

The soluble fraction from the embryos contained very little ^3H with no peak corresponding to (D)Z/ZR on one-dimensional chromatograms (Fig. 3). The peak of ^3H that ran with dye C in extracts from embryos at T = 0 (Fig. 3) did not run with IPA or IPAR when eluted and chromatographed with solvent B. At T = 0, no more than 7% of the soluble ^3H was present in the (D)Z/ZR zone, and this was about the same after 24 h. Chromatography of these extracts on the two-dimensional system established that even this low level of ^3H did not represent (D)Z/ZR (Table 5). No clear peaks of activity corresponding to known metabolites were obtained by two-dimensional TLC; however, these data can still be used to set upper limits. The maximum from the (D)Z/ZR zone in the embryos was 0.0006% of the total ^3H applied.

All of the tissues from which soluble extracts had been obtained also contained insoluble ^3H, which was estimated roughly. In some cases, particularly in the embryos, the amount of bound ^3H (rough estimations of the sodium dodecylsulfate-solubilized material) could be relatively close, 5–20 times that



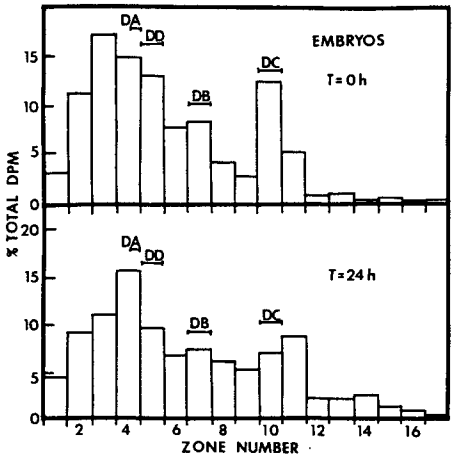


Fig. 2. (Left panels) Thin-layer chromatogram profiles of the soluble ^3H from seed coats of early podfill soybean explants (cv. Bragg) fed a pulse of ^3H-zeatin riboside via the cut base. Silica gel plates developed with *n*-butanol/acetic acid/H_2O (12:3:5, v/v). **Fig. 3.** (Right panels) Thin-layer chromatogram profiles of the soluble ^3H from the embryos of early podfill soybean explants (cv. Bragg) fed a pulse of ^3H-zeatin riboside via the cut base. Silica gel plates developed with *n*-butanol/acetic acid/H_2O (12:3:5, v/v).

in the corresponding soluble fraction. In leaf blades, the insoluble fraction contained a lower portion of the total ^3H, from 1/20 to 1/2 of the levels in the soluble fraction. Because such large amounts of the ^3H appeared in the insoluble material from all of the tissues analyzed, it seemed essential to analyze this further. When hydrolyzed, most of the solubilized ^3H from leaf blades co-chromatographed with A (Fig. 4); however, a minor (less than 5% of the total DPM) yet distinct peak occurred in zone 7, which corresponds to guanine (G). Less than 3% was in the Z zone and that was probably a small spillover from the large A peak. No ^3H could be detected in the Z zone from the hydrolyzate of insoluble material from embryos, but some activity corresponded to adenine and guanine. In general, the two procedures used gave similar results; ^3H chromatographed like A and G was also found in both the RNA and DNA extracts. The KOH treatment (Schmidt-Thannhauser procedure) does not seem to have caused any major loss of ^3H from the purine rings.

Distribution of ^3H from ^3H-Adenosine

When ^3H-adenosine was supplied via the xylem in early-podfill explants (just as ^3H-cytokinin was applied), the distribution of the soluble ^3H in different parts was similar to that observed with ^3H-cytokinin (Table 6). With ^3H-aden-

Table 5. Analysis of the composition of extracts from soybean explants (cv. Bragg) supplied ^{3}H-zeatin riboside via the cut base.[a] Percentages of ^{3}H that co-chromatographed with zeatin riboside and its principal known metabolites.[b]

Tissue and time of sampling	% of extractable ^{3}H corresponding to known metabolites								DPM/explant	DPM/explant as a % of total ^{3}H applied
	(D)ZR	(D)Z	(D)ZMP	O-glucosides + 7GZ	9GZ	(D)LA	A	AR		
Embryos										
prepodfill (24 h)	0.5	0.03	2.2[d]	4.3	2.5	1.0	0.6	3.5	1,060	0.019
early podfill (24 h)	1.9	0.7	0.0[c]	1.2	2.4	1.4	1.0	1.5	1,950	0.034
Seed coats										
prepodfill (0 h)	4.0[e]	1.3	11.7[c,e]	4.4[e]	0.0	1.7	1.7[e]	2.2[e]	7,420	0.13
(24 h)	0.4	0.0	1.6[c]	2.9[e]	0.7	1.8	1.6[e]	2.9[e]	8,400	0.15
early podfill (0 h)	12.8[e]	5.0[e]	15.7[c,e]	3.1	0.0	1.1	2.5	2.3[e]	1,750	0.031
(24 h)	0.3	1.1	7.9[c,e]	3.8[e]	0.3	0.9	1.0	3.7[e]	8,100	0.14
Leaf blades										
early podfill (0 h)	22.4[e]	8.6[e]	17.9[d,e]	1.9	0.1	1.1	6.6[e]	6.0[e]	1,218,500	21
Carpels										
early podfill (0 h)	6.6[e]	15.6[e]	4.8[d]	1.5	0.3	1.1	8.5[e]	5.2[e]	45,800	0.80

[a] From experiments shown in Tables 1 and 3.

[b] From two-dimensional TLC in solvent A, then solvent B.

[c] The second dimension was developed with solvent C following B in order to separate (D)ZMP and AMP completely.

[d] Some ^{3}H-AMP would probably be included in this value.

[e] A clear peak of ^{3}H corresponded to these compounds. These discrete peaks of ^{3}H seem more significant.

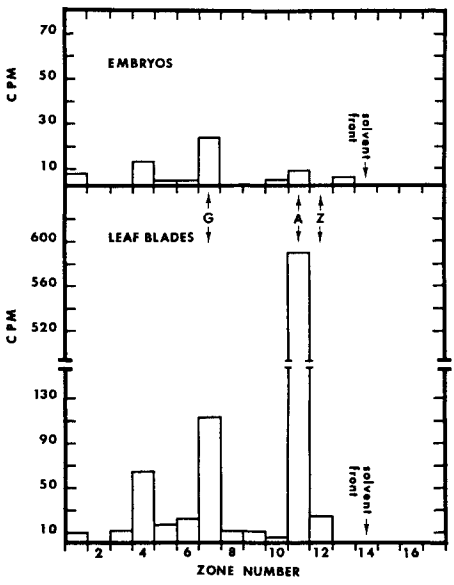


Fig. 4. Thin-layer chromatogram profiles of the ^3H in the hot $HClO_4$ extract (purine bases freed from RNA and DNA) from the insoluble material from the embryos and leaf blades of early podfill soybean explants (cv. Anoka) supplied with ^3H-zeatin riboside for 1 h and then incubated for 24 h. Silica gel plates sprayed with sodium borate and developed with *n*-butanol/conc. NH_4OH/H_2O. The locations of guanine, adenine, and zeatin are indicated.

Table 6. Distribution of ^3H from ^3H (ring-labeled)-adenosine given as a 1-hour pulse via the base of soybean explants (cv. Anoka) cut at early podfill.

	Soluble ^3H			
	At end of pulse		After 24 h	
Explant part	DPM	% of soluble DPM	DPM	% of soluble DPM
Blades	8,119,900	45.6	3,410,700	47.3
Petiole	2,120,400	11.9	535,000	7.4
Stem	6,872,400	38.6	1,555,100	21.6
Carpels	556,200	3.1	959,500	13.3
Seed coats	95,300	0.5	337,400	4.7
Embryos	38,700	0.2	411,000	5.7

osine, however, much higher levels of soluble ^3H were present in the seed coats and embryos.

Discussion

The soybean explant system used in the present study of cytokinin translocation exhibits seed-induced leaf senescence that closely resembles that found in intact soybean plants except that it is accelerated (Neumann et al. 1983). Cytokinins supplied through the xylem (via the cut base) appear to mimic those supplied by the roots; they delay (normalize) leaf senescence and pod devel-

opment (Neumann et al. 1983, Garrison et al. 1984). Because a defined solution including labeled cytokinins can easily be substituted for the roots, the explant system seems well suited to determine: (a) whether or not the developing seeds can divert the supply of cytokinins coming up from the roots away from the leaves and/or remobilize leaf cytokinins, and (b) whether or not the high levels of cytokinins in young seeds (Letham 1978, Lindoo and Noodén 1978, Davey and van Staden 1979) come from the roots. The explants were excised at stages when the foliar cytokinin titer was declining (Lindoo and Noodén 1978), and cytokinin diversion, if any, should have been taking place. Since there is evidence that significant quantities of Z and ZR occur in the xylem (Heindl et al. 1982) and Z or ZR supplied to soybean explants via the xylem is able to delay senescence as the roots would (Garrison et al. 1984), root-produced cytokinins, in particular Z and ZR, must play a role in maintaining the foliage of fruiting soybeans. We did not include minerals in the solution supplied to the explants here; however, these cytokinins behave similarly with or without the minerals (Garrison et al. 1984). Moreover, our experiments were conducted over the first 25 h after excision, during which time the effects of excision from the whole plant were minimal (Neumann et al. 1983).

Most of the soluble ^{3}H from the ^{3}H(ring-labeled) ZR supplied to an early podfill explant is in the leaf blades and petiole at the end of the 1-hour pulse and a large amount is also in the stem, while very little is in the seeds, especially in embryos. The levels of soluble ^{3}H in the leaves, stem, and petiole decrease during the subsequent 24-h incubation without a corresponding increase in the embryos. Generally, the seed coats contain a larger amount of soluble ^{3}H than do the embryos, especially expressed on a per g fresh weight basis, but it is still quite small (0.034–0.037% of the DPM applied). Although the carpels do not contain a high level of soluble ^{3}H, the level is significant (0.4–0.8% of the DPM applied). To test the possibility that only the very young pods are effective accumulators of ^{3}H-cytokinin, the ^{3}H-ZR pulse was also given to explants taken during pod extension (prepodfill), but the distribution is similar. ^{3}H-Z, another form of cytokinin in the xylem of soybean, shows the same distribution pattern. Thus, the ZR and Z in the xylem do not travel directly to the seeds in appreciable quantities, and the seeds do not compete with the leaves for these cytokinins as they come up from the roots.

The observed net decline of soluble ^{3}H over the 24-h incubation suggests metabolism of the ^{3}H-cytokinin and its incorporation into the insoluble fraction. Binding of the ^{3}H into the insoluble materials occurs not only in the leaf blades but also in the petiole and stem, and even in the embryos. Simple chemical methods for extracting RNA and DNA and/or release of the free purine bases indicate that the bound ^{3}H is mainly adenine and sometimes material that co-chromatographs with guanine, but not Z. Thus, the ^{3}H from ZR or Z is not simply bound as cytokinin but is first converted to A or AR (and possibly guanine or guanosine) and then incorporated into both RNA and DNA. A similar metabolism and incorporation into nucleic acids has been observed for ^{14}C (ring-labeled)-kinetin supplied to the roots of pea seedlings (Morris 1981 a,b).

TLC of extracts from all parts of the explants show that even at the end of the pulse relatively little (less than 22%) of the soluble ^{3}H is still in the original

cytokinin or dihydro-derivatives, and this declines to negligible proportions by the end of the 24-h incubation. In the leaf blades, ZR is rapidly metabolized to Z, DZ, DZR, adenine, and adenosine. In both the carpels and seed coats, there does appear to be some of the parent ^{3}H-ZR (or -DZR) at the end of the pulse but probably not after 24 h. ^{3}H-cytokinin nucleotide does seem to exist in the seed coat at the end of the pulse, but it also is metabolized. Since the carpels are connected directly to the xylem of the main vascular system (Thorne 1980, 1981), the appearance of ^{3}H-cytokinin in these structures is not surprising. The tiny amount of ^{3}H-ZR that occurs in the seed coat probably enters through the two small vascular strands that are connected with the main vascular system (Thorne 1980). Although there was some soluble ^{3}H in the embryos, no clear peaks were observed in two-dimensional TLC, and there was not enough in any one fraction to identify it with any of the known cytokinin metabolites. As indicated under *Results,* the data of Table 5 are useful in setting upper limits for the translocation of ^{3}H-cytokinin into the seeds, and this seems vanishingly small. The ^{3}H-ZR in the embryos would be less than 0.0006% of the ^{3}H-ZR applied. Since DZ, DZR, ZMP, and O-glucosides are formed in explants, the restriction of movement to the embryo is not confined to Z and ZR, hence it is likely that DZ and DZR, which occur in the transpiration stream, would be distributed much as Z and ZR.

Since low levels of ^{3}H-labeled materials that could be A and AR occur in extracts from embryos, and the embryos do contain considerable ^{3}H bound in the purine bases of RNA and DNA, one wonders if AR is translocated differently from ZR and Z. Indeed, that is the case; ^{3}H (ring-labelled)-AR is readily translocated into the seeds. Even at the end of the pulse with ^{3}H-AR, there is substantial ^{3}H in the soluble extract from embryos, and this increases over a 24-h period. Thus, the presence of the side chain on the 6 position of the adenine ring not only greatly influences its physiological activity but also its translocation pattern.

After these experiments with ^{3}H-cytokinin translocation had been done, a similar type of experiment was reported for fruiting white lupin plants by Davey and van Staden (1981) using ^{14}C(ring-labelled)-Z injected into the stem. The lupin pods were not subdivided into carpels, seed coats, and embryos; however, if we pool the counts observed for the parts of soybean pods, our distribution data look similar to theirs. Their data on extracts from the whole fruit indicate that none of the radioactivity in the whole pod can be identified with the parent compound; we do find some in the carpels and relatively small amounts in the seed coats, but none in the embryos.

In conclusion, the pods do not compete with the leaves for the supply of cytokinin coming up from the xylem in soybean explants and very little (or more likely none) of this cytokinin enters the embryo, though a small amount does enter the carpels and a very small proportion enters even the seed coats. Likewise, the cytokinin translocated into the leaves is not redistributed to the seeds. Since embryos excised during their early growth (before cell division has been completed) do not seem to require exogenous cytokinin in culture (Thompson et al. 1977, Egli and Wardlaw 1980, Hsu and Obendorf 1982), it can be questioned whether the developing seeds get any of their cytokinin from the roots. Inasmuch as cytokinin supplied via the xylem seems to serve

in lieu of the roots, we believe these observations with excised parts apply to the intact soybean plant.

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