

Short Communication

Auxin Transport and Conjugation in Cotton Explants¹Betty J. Robinson, M. Forman², and F. T. Addicott

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Indoleacetic acid (IAA), a naturally-occurring auxin, prevents or retards abscission of plant parts under many conditions (1). When applied to the cotyledonary petiole stumps of explants of cotton (*Gossypium hirsutum* L.) in a commonly used abscission test IAA in moderate physiological concentrations retards abscission markedly (2,6). The disposition within the explant of the auxin applied under these conditions has now been investigated both quantitatively and qualitatively with the use of ¹⁴C-labeled IAA. A variety of related investigations have been reported (see 7,8,11,12), but in this brief paper we will comment only on those of immediate relevance to our results.

In 2 replicate experiments with IAA-1-¹⁴C and a third with IAA-2-¹⁴C (both compounds were obtained from Nuclear Research Chemicals, Orlando, Florida, and had specific activities of 4.0 and 1.0 mc/mole, respectively), explants were treated with 0.5 μg per petiole of the labeled IAA in agar. After selected times (5 min, 30 min, 1 hr, 4 hr, and 24 hr), the explants were divided into hypocotyl portions (10 mm long), stem stumps (3 mm), intervening nodal portions (including axillary buds), and 3 equal portions of each petiole stump (about 1 mm each portion). The anatomy and gross morphology of the explant are shown in Bornman *et al.*, (6) figures 1 to 6. At each time, similar parts were pooled to form 1 sample. The tissue was frozen immediately in absolute methanol and was subsequently extracted. The radioactivity was measured in the methanolic extracts and in the tissue residues by a Beckman Lowbeta II gas flow proportional counter with a counting efficiency of 28%. The amount of radioactivity remaining in the tissue residues was in the order of 1% or less for extractions during the first hour; and in the order of 7 to 8% at 4 and 24 hours. The chemical form of the major part of the radioactivity was investigated by chromatography of the extracts. Thin-layer strip chromatograms were prepared in 2 separate solvent

systems (isopropanol-ammonia-water and butanol-acetic acid-water) and extracts were co-chromatographed with unlabeled IAA and eventually with indoleacetylaspatic acid (IAA_{sp}). Radioactivity was measured along the strips, IAA and IAA_{sp} spots were located by the Prochazka reagent, and autoradiographs were prepared from chromatograms.

To visualize the pattern of isotope transport and determine more precisely the intracellular localization of the label in the abscission zone, micro-autoradiographs were made. Explants were prepared as described above, with the exception that 0.1 μg labeled IAA was added per petiole. After the appropriate incubation times the tissue samples were immediately frozen in a mixture of isopentane (2-methyl-butane) and methylcyclohexane cooled to the temperature of liquid nitrogen (13), sectioned (15-18 μ) in a cryostat, transferred to glass slides and dried. The slides were then dipped in liquid emulsion (Type NTB-2 Eastman Kodak), dried, and exposed in light-tight boxes at 4° for 4 days. Following development, the sections were stained with azure B (13) and viewed with phase contrast optics.

The chromatography indicated that for the first 30 minutes most radioactivity present in any part of the explant was extracted in the form of IAA. By 1 hour after application, some of the IAA had been converted to IAA_{sp}. By 4 hours, almost all of the radioactivity extracted from the base of the petiole and from the axis was in the form of IAA_{sp}. By 24 hours after application, even the portion of the petiole closest to the site of application contained almost no IAA-¹⁴C, but had converted large amounts of it to IAA_{sp}-¹⁴C. The formation of this compound by the conjugation of exogenous IAA with aspartic acid had been observed earlier in peas by Andreae and Good (4). It has been found also in other plants (9) but not previously in cotton. As with the cotton explants, pea and other tissues had required about 2 hours induction period in the presence of exogenous IAA (5,19) or some other active carboxylic acid (15), and maximal conjugative activity was not attained for 4 to 6 hours (5,19).

The absorption and movement of the auxin in our explants is indicated by the results summarized in table I and the micro-autoradiographs in figure 1. Five minutes after application, the IAA-¹⁴C had

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Table I. Total ^{14}C -Activity Extracted From Samples of Cotton Explants Treated with IAA- $1\text{-}^{14}\text{C}$

The agar applied to each petiole contained $0.0114 \mu\text{C}$ of ^{14}C . Evaporated extracts were counted directly. ^{14}C -Activity is expressed as net cpm.

Position sampled	Incubation time before sampling				
	5 min	30 min	1 hr	4 hr	24 hr
Petioles, outer thirds	8190	12,240	12,830	16,505	32,350
Petioles, central thirds	340	1778	2190	10,632	12,590
Petioles, basal thirds	100	330	1208	6435	6370
Nodal regions	72	70	785	2335	1780
Stem stumps	...	3	222	110	55
Hypocotyl segments	56	1068	1171
Total ^{14}C from all tissue extracted (net cpm)	8702	14,421	17,291	37,085	54,316
No. of explants sampled	8	7	7	7	8
Avg total ^{14}C extracted per explant (net cpm)	1088	2060	2470	5298	6790
Total ^{14}C extracted from tissue as percent of ^{14}C applied	10 %	20 %	23 %	50 %	64 %

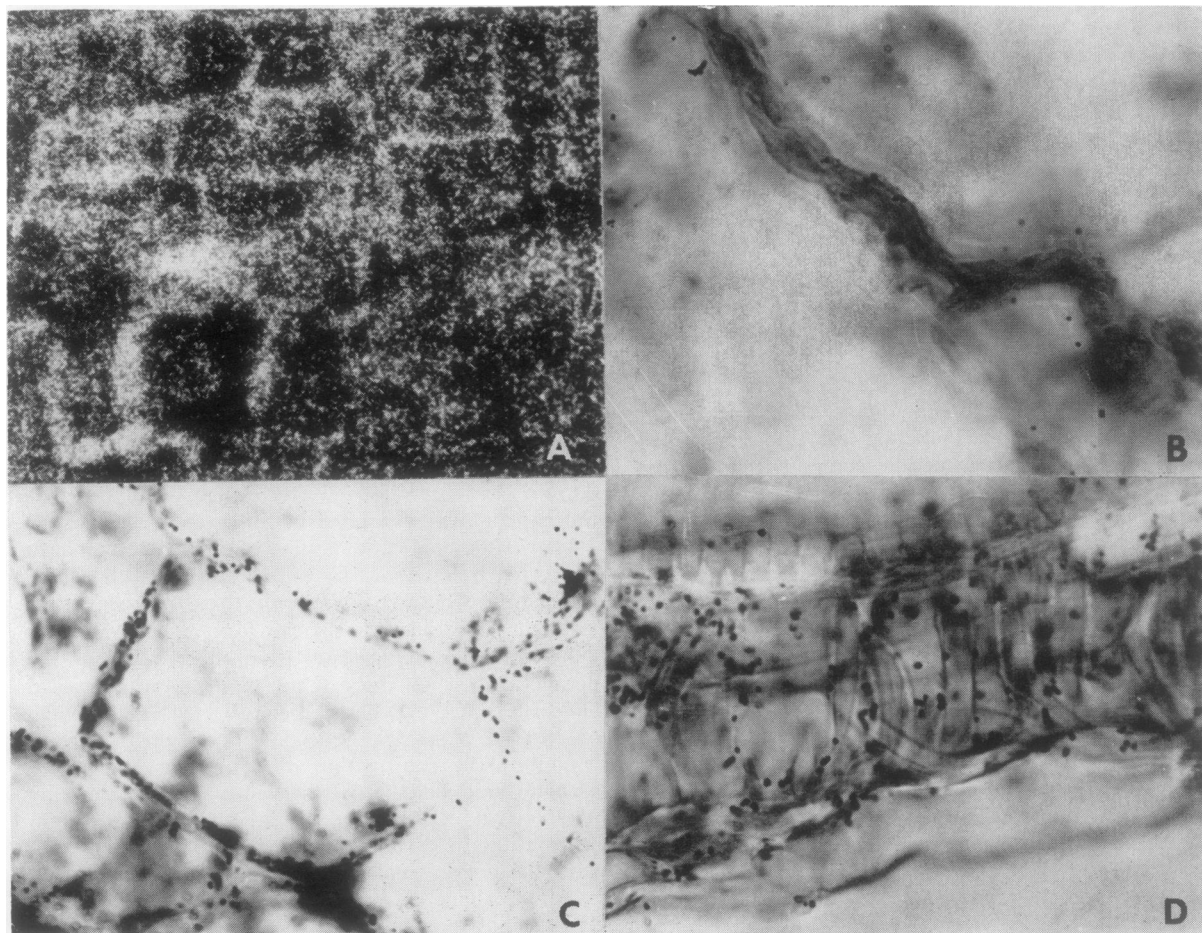


FIG. 1. Micro-autoradiographs of cotton explant petioles showing localization of label after application of IAA- $1\text{-}^{14}\text{C}$. A) Distal end of the petiole, 24 hour incubation. Label generally concentrated at cell peripheries. (Simulated dark field, silver grains appear bright against a dark background). $\times 160$. B) Wall between 2 abscission zone cells, 5 minute incubation. Label is beginning to accumulate at cell peripheries. $\times 1000$ (compare with C). C) Abscission zone, 24 hour incubation. Increased accumulation of label in or near cell walls. $\times 800$. D) Tracheary element in distal portion of petiole, 5 minute incubation. $\times 1000$.

moved in detectable quantity through the 3 mm length of the petiole and into the explant axis. It was distributed so that its concentration was greatest near the site of application and diminished sharply with distance from the site. The labeled IAA moiety continued to enter the petioles and to accumulate farther down the explant. By 4 hours after application appreciable quantities had moved to the hypocotyl, some had moved into the stem, and most was in the form of IAA_{sp} (see above). The microautoradiographs showed the label to be present in all petiolar tissues including conductive tissues (fig 1, A and D). There appeared to be no barrier distal to the abscission layer (fig 1, B and C), in contrast to some (11, 14) but not all (11, 12) other observations. The greatest intensity of label in the petiolar cells was at the periphery of the cells, while localization within the abscission zone proper was in, or near the cell wall, probably associated with the peripheral cytoplasm of the cell. The labeling profile was the same with each of the isomers used.

Other workers (10, 16, 17) have conducted somewhat similar experiments on the disposition of the synthetic auxin, naphthaleneacetic acid (NAA), in *Coleus* nodal explants, after its application to the stem stump. They found also that most of the radioactivity from labeled NAA was fixed close to the site of application, and that the NAA was rapidly conjugated to naphthaleneacetylaspatic acid. The similarities between the disposition of IAA in cotton explants and that of NAA in *Coleus* explants were particularly interesting since physiological responses to IAA and synthetic auxins often differ quantitatively and sometimes qualitatively (1, 11). Other workers have shown that exogenous IAA and NAA are conjugated in various plant tissues by similar or identical mechanisms (3, 15, 18, 19).

IAA_{sp} was tested for its effect on abscission in the cotton explants. It was applied at 1.0, 0.5, 0.25, 0.1, 0.01, and 0.001 μg per petiole, and duplicate tests were performed. While the low concentrations had no significant effect on the rate of abscission, the 3 highest concentrations showed increasing retardation of abscission with increasing concentration. IAA_{sp} at 1.0 μg per petiole retarded abscission to about the same extent as did IAA at 0.1 μg per petiole.

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