Plant Physiol. (1971) 48, 146–149

Auxin Transport within Intact Dormant and Active White Ash Shoots[1]

Received for publication August 26, 1970

CHARLES A. HOLLIS[2] AND HERBERT B. TEPPER
Department of Forest Botany and Pathology and Plant Physiology-Biochemistry Group, State University College of Forestry, Syracuse, New York 13210

ABSTRACT

Transport of indoleacetic acid-1-^{14}C following application to the buds of intact white ash (*Fraxinus americana* L.) shoots proceeds at a velocity of about 1.3 centimeters per hour in actively growing seedlings, but only 0.3 centimeter per hour in dormant seedlings. The rapid movement is metabolically controlled, and at 1 C or in a nitrogen environment it is reduced to 0.2 centimeter per hour, suggesting that the slower movement is due to diffusion. The transport profile for growing shoots shows a logarithmic decrease in activity in stems treated for 3 hours. However, over longer treatment intervals, especially after 12 hours, a steady state of recoverable activity occurs in the more basal stem segments. Cold-treated shoots acquire the capacity for rapid transport 7 days after they are placed into favorable growing conditions, at which time dormancy callose disappears from the phloem, respiratory activity of the stem tissue increases, and mitotic reactivation occurs in the bud. Following shoot reactivation, the velocity and amount of exogenously supplied indoleacetic acid transported remained relatively uniform until the onset of the succeeding dormant period. Five per cent, or less, of the applied tracer moves into the shoot, with substantial portions remaining as indoleacetic acid.

The classic donor-receiver block method of studying auxin transport through excised plant parts is beset with the problems associated with the use of unnatural experimental conditions. Although information obtained from these studies is useful, extrapolation of the results to explain auxin transport mechanisms in intact plants is difficult. McCready (13) noted that a knowledge of the transport of plant hormones within *intact* shoots is essential to understanding the ways in which they serve to integrate the whole organism.

Evidence indicates that cambial activity and subsequent wood formation are related in part to the production and basipetal transport of auxin from the bud (9, 24, 25). Diffusible auxin has been found in actively growing shoots but is undetectable in the dormant system (9, 15). Presumably, the resumption of cambial activity in woody shoots following dormancy can be correlated with the onset of production and transport of auxin. Thus, it was of interest to study the movement of auxin as a function of the physiological state of the *intact* white ash shoot (*Fraxinus americana* L.). This phase of the study concentrated upon the quantity and velocity of IAA transported through intact shoots, and the influence of certain environmental factors on auxin movement in this system.

MATERIALS AND METHODS

Pot-grown white ash seedlings were utilized. At the end of their 2nd year of growth, one-half of the seedlings were cold-treated at −2 C for 3 months. The remainder were maintained continuously at about 21 C in a glasshouse. The former group eventually provided actively growing seedlings, while the latter remained dormant (no sign of bud break) during the experimental period.

Labeled IAA (1-^{14}C) at a specific radioactivity of 30 to 50 mc/mmole was used (Amersham/Searle Corp.) after being checked chromatographically for purity. The tracer was dissolved in a mixture of 20% ethanol-10% Carbowax 1500-70% water to which unlabeled IAA was added so that the concentration of the hormone was 10^{-5} M. A treatment solution containing 0.5 μc of IAA was applied in a 10-μl droplet to the undamaged bud of each shoot, which had been previously ringed with lanolin to prevent spillage.

Intact shoots of dormant and nondormant seedlings were treated in either of two artificially lighted environments: 25 C, relative humidity 92%; or 1 C, ambient humidity. Following application of the tracer, seedlings were harvested at predetermined intervals and subdivided into 1-cm segments. Each segment was placed into a vial and extracted with 3 ml of methanol for 24 hr. In most instances, stem sections from three plants receiving similar treatment were pooled for extraction. Subsequently, the stem segments were removed, and 10 ml of a standard toluene-based counting fluid were added to each extract. The radioactivity in each was counted in a Packard Tri-Carb liquid scintillation spectrometer, and data were corrected for background and quenching. To assess the distance the tracer moved, a segment of stem tissue was considered radioactive if the counts detected were 2 standard deviations above background.

Procedures for determining the percentage of label transported as IAA were adapted from the techniques of Perley and Stowe (17), Stowe *et al.* (20), and Thomas (23). A group of 18 actively growing seedlings each treated with 0.5 μc of IAA-1-^{14}C were harvested after 6 hr. In addition, aliquots of the tracer were added to frozen stem tissue plus extracting fluid and run in parallel with the treated tissues as a control.

At harvest, appropriate stem regions were cut directly into 100 ml of 70% methanol, acidified to pH 2.5 with H_3PO_4, at −70 C and homogenized. The tissues were re-extracted at

[1] This work was supported in part by funds from the McIntire-Stennis Program.

[2] Present address: Department of Soils, G169 McCarty Hall, University of Florida, Gainesville, Fla. 32601.

−20 C two additional times with 50 ml of acidified methanol during a 24-hr extraction period to yield a final extract of 200 ml. The extracts were filtered and concentrated under vacuum at 40 C to 30 ml of an acidic aqueous solution. Lipids were removed from the extracts by partitioning with *n*-hexane.

Solvent partitioning, essentially as described by Perley and Stowe (17), was used to extract the acidic fraction. Ethyl acetate was substituted for methylene chloride as the organic solvent. To minimize degradation of the indolic compounds, separation was conducted in the dark at 4 C, using redistilled solvents, under nitrogen.

The acidic fraction of each extract, known to contain the free IAA, was concentrated under vacuum at 40 C and further separated into its respective labeled components by thin layer chromatography. A 10-μl aliquot of each acidic extract was spotted, under nitrogen, onto chromatographic plates covered with 0.25 mm Silica Gel G. The solvent systems used to develop the chromatograms were: (*a*) chloroform-ethyl acetate-formic acid (35:55:10, v/v); (*b*) 1-butanol-acetic acid-water (40:10:50, v/v); (*c*) 2-butanone-*n*-hexane (1:3, v/v); and (*d*) 1-butanol-chloroform (3:2, v/v) saturated with 0.5 N formic acid. The position of the labeled IAA on the chromatograms was identified by cochromatography with authentic IAA. The labeled thin layer chromatography spot corresponding to free IAA was scraped into a vial containing 1 ml of methanol to which 10 ml of a toluene-based counting fluid was added, and the quantity of radioactivity was determined by liquid scintillation counting. Where concentrations permitted, the presence of IAA was verified by elution of the appropriate spot and observation of the ultraviolet absorbance peaks.

RESULTS

The velocity of IAA transport following apical application to intact white ash shoots in active or dormant phases of their growth cycle can be calculated from the data presented in Figures 1 and 2, respectively. Considering the maximal transport distance for 3-, 6-, and 12-hr treatments, an average velocity of about 1.3 cm/hr can be calculated for IAA movement in the active and 0.3 cm/hr for the dormant system.

To assess the importance of metabolism for transport in intact plants, two groups of six actively growing seedlings were treated with labeled IAA for 6 hr under low temperature

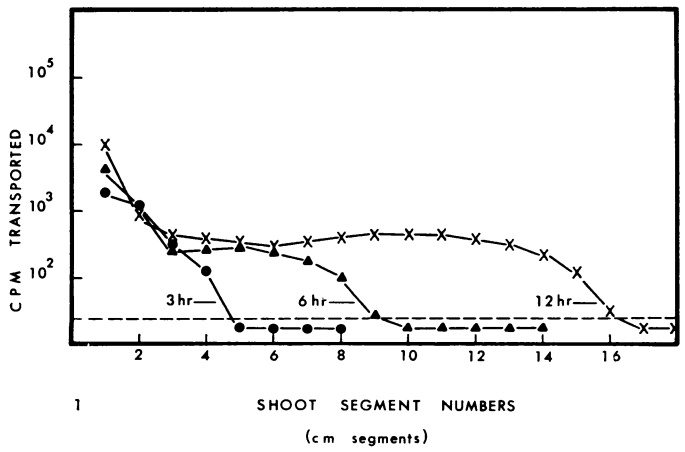

FIG. 1. Activity recovered from 1-cm segments cut from intact, actively growing seedlings treated for 3, 6, or 12 hr. Each point represents the average of three treated stems. ----: Level of background plus 2 SD. IAA-1-^{14}C (0.5 μc) was applied to each apex.

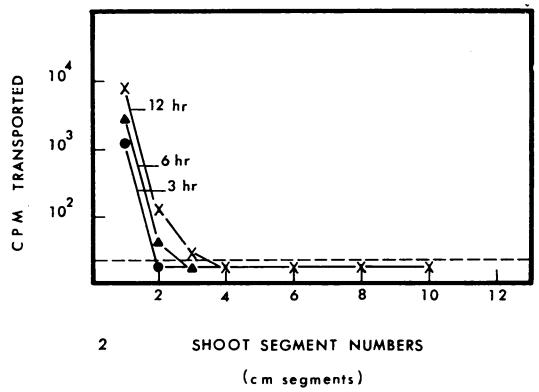

FIG. 2. Activity recovered from 1-cm segments cut from intact, dormant seedlings treated for 3, 6, or 12 hr. Each point represents the average of three treated stems. ----: Background plus 2 SD. IAA-1-^{14}C (0.5 μc) was applied to each apex.

Table I. *Metabolic Requirements for IAA Transport in Intact Ash Shoots*

Plants were conditioned for 12 hr prior to treatment with 0.5 μc of IAA-1-^{14}C.

Experimental Conditions	Average Transport Velocity
	cm/hr
Actively growing shoots at 25 C	1.3 ± 0.1
Actively growing shoots at 1 C	0.2 ± 0.1
Actively growing shoots in N_2 at 25 C	0.2 ± 0.1
Dormant shoots at 25 C	0.3 ± 0.1
Dormant shoots at 1 C	0.2 ± 0.1[1]

[1] Based on one treatment with 0.5 μc of IAA-2-^{14}C.

Table II. *Changes in Transport Velocity during Shoot Reactivation*

Days at 25 C after Cold Storage[1]	Average Transport Velocity[2]
	cm/hr
0	0.3 ± 0.1
2	0.3 ± 0.0
4	0.3 ± 0.0
6	0.3 ± 0.0
7	1.2 ± 0.1
8	1.2 ± 0.1
10 (bud break)	1.2 ± 0.1

[1] Plants were stored in a cold room (−2 C) for 3 months.

[2] Calculations were based on treatment of six plants for 6 hr. Experiment was replicated twice with similar results.

(1 C) or a nitrogen environment. The results (Table I) show that inhibition of metabolism in the active system reduces the rate of IAA transport to a level commensurate with that in the dormant system. Metabolic inhibition had little effect, however, on the rate of IAA movement in dormant shoots.

In order to determine when transport rate increases in relation to reactivation, seedlings were removed from a cold room after 3 months of cold storage and placed into a growth chamber (25 C). Subsequently, six seedlings were treated with labeled IAA on sequential days until the morphological signs of shoot reactivation (bud break) occurred. As Table II shows, a period of 7 days is required prior to the commencement of rapid transport.

In addition to increased rate of auxin transport, other changes occurred during shoot reactivation. Manometric determination of CO_2 evolution and O_2 consumption in the upper 12 cm of stem tissue and buds indicated a marked increase in respiratory activity 7 days after the seedlings were removed from cold storage. Also, histological examination of the reactivating stem tissue showed that dormancy callose disappeared from the phloem and mitotic activity commenced in the bud during that 24-hr period characterized by the increased rate of IAA transport.

The velocity of IAA movement out of the shoot tip was observed at selected intervals during the growing season (Table III). Transport velocity remains relatively constant during active shoot growth. However, when leaves begin to senesce and overwintering buds appear fully developed, the velocity quickly decreases to a rate similar to the dormant system.

The average amount of tracer transported through actively growing stems can be seen in the profile of activity recovered from the individual stem segments (Fig. 1). This profile shows a logarithmic decrease in activity between the point of application and the moving front in stems treated for 3 hr. However, in the longer treatment intervals, especially after 12 hr, logarithmic decreases in activity ensue only near the site of application and at the moving front, whereas relatively uniform amounts of tracer can be recovered from the segments in between.

The amount of label transported into the stem is relatively minimal at all stages of the growth cycle (Table III). Also, the percentage of applied label that is transported remains relatively constant during the growing season but decreases as the shoots enter the dormant phase and is very low in the dormant shoot.

The percentage of activity moving as IAA was determined from chromatograms of the acidic extracts of actively growing shoots treated for 6 hr (Table IV). The results show that about 5% of the applied label was transported out of the bud. The activity recovered in each of the acid fractions remained a relatively constant percentage of the total radioactivity recovered per shoot region. However, the percentage of activity recovered as free IAA from the acid fractions increased toward the basal regions, where most of the label was associated with IAA.

DISCUSSION

The velocity of IAA transport (about 1.3 cm/hr) through actively growing white ash shoots compares favorably with values reported for metabolically dependent polar transport through excised shoot and root segments (6). Inhibition of respiratory activity by low temperature or a nitrogen atmosphere resulted in reduction of the IAA transport velocity in intact shoots. This indicates that transport of exogenously supplied IAA through intact systems requires the expenditure of metabolic energy in a manner similar to polar transport through excised segments (2, 5, 7). Also, the transport velocity is independent of transport distance, which agrees with the findings of McCready (12) and Pilet (18) for polar transport through excised segments. The relatively uniform amount of label recovered in the more basal stem segments, especially the 12-hr treatment, indicates that the transport system is saturated at a relatively low concentration of the hormone. These characteristics of the transport process in white ash also have been demonstrated by workers studying IAA movement in excised systems (4). Our results, when compared with the demonstration of polar movement in other species (6, 13), suggests that metabolically dependent polar transport probably is operative in the intact white ash stem.

The observed velocity of IAA transport following apical application to intact white ash shoots is considerably slower than the 15 to 25 cm/hr reported for IAA transport in other intact systems (1, 3, 10). However, these rapid rates of movement occurred when IAA was applied to one or more leaves of an intact plant and presumably was due to transport along with assimilates in the phloem. In contrast, Morris *et al.* (16), using apical applications of IAA to intact pea plants, have also observed slow transport rates and an apparent lack of movement in the phloem.

If IAA is capable of being rapidly transported in the phloem (1, 3), then it is paradoxical that we did not observe faster transport velocities. Possibly, loading of the sieve tubes is a selective process (14). Therefore, a lack of movement in the sieve tubes might arise from a system controlling entry into these elements. Also, it is conceivable that some IAA did enter

Table III. *Translocation Rates at Selected Stages of One Annual Growth Cycle*

Stage of Shoot Development	Average Transport Velocity[1]	Percentage of Total Applied IAA Transported[2]
	cm/hr	
Dormant	0.3 ± 0.1	0.16 ± 0.02
Actively growing, first internode visible (2 days after bud break)	1.3 ± 0.1	2.33 ± 0.40
Elongation growth one-half complete (15 days after bud break)	1.3 ± 0.1	1.89 ± 0.34
Elongation growth complete (30 days after bud break)	1.2 ± 0.1	2.13 ± 0.62
Leaves senescing, overwintering buds developed (4 months after bud break)	0.5 ± 0.3	1.34 ± 0.42

[1] Calculations were based on treatment of six plants for 6 hr; replicated twice with similar results.

[2] IAA-1-^{14}C, 0.5 μc, was applied to each shoot.

Table IV. *Extraction of IAA-^{14}C from Actively Growing Shoots*

Results are based on extraction of 18 plants treated for 6 hr. Results are expressed in terms of average activity per single plant region.

Extracted Shoot Region	Radioactivity		
	Recovered per shoot region	Recovered in acidic fraction	IAA-^{14}C
		cpm	
Terminal bud	224,500	212,500 (95)[1]	146,200 (65)
1–3 cm	25,800	24,500 (95)	20,500 (79)
4–6 cm	2,200	2,100 (95)	1,950 (88)
7–10 cm	325	300 (92)	295 (91)
Frozen tissue (control)	308,000	295,600 (96)	277,000 (90)
cpm applied	540,000		

[1] Figures in parentheses represent percentage of recovered regional activity.

the sieve tubes but was too small to be detected by our methods.

As has been suggested previously (19), two possible IAA transport mechanisms exist in intact plants. One of these mechanisms, as indicated by our results and those of Morris *et al.* (16), appears to be similar to polar transport between adjacent parenchymatous cells, whereas the second is a relatively rapid, long distance transport possibly in conjunction with assimilate transfer in the phloem (1, 3).

The quantity of IAA transported following an apical application of the tracer is less than 5% of the amount applied (Table IV). This value agrees favorably with the findings of Winter and Thimann (26) for pea segments, and Morris *et al.* (16) for intact pea plants. The average amount of activity recovered from the more basal regions of active shoots treated for 12 hr was 5×10^2 cpm/cm, which represents approximately 5×10^{-3} μmole of IAA per cm of shoot segment. However, more rigorous extraction procedures, as used to obtain data in Table IV, suggest that the actual amount of IAA transported in the basal regions may be five times greater. This value falls within the range of concentrations capable of stimulating growth processes in stems (22).

The observation that 90% of the extracted label represents IAA in the segment containing the moving front suggests that IAA is primarily the substance transported.

The observed movement of IAA in the intact dormant shoot (0.3 cm/hr) was not appreciably affected by low temperature treatment and indicated that such movement was not metabolically dependent. Furthermore, Mirov (15) and Larson (9) reported that endogenous auxin cannot be detected in dormant shoots. This suggests that the movement of the tracer is the result of an artificial situation representing the diffusion of exogenously applied IAA and does not represent a normal physiological occurrence at this stage of the growth cycle.

The transition to a rapid IAA transport velocity occurs following a 7-day period in growing conditions and coincides with increased respiratory activity of stem tissue and the initiation of mitotic activity in the bud. However, the rate of cambial reactivation (appearance of mitoses) noted in white ash (21) is about five times slower than the rate at which IAA is transported. Thus, the observed transport velocity of IAA in the reactivating shoot may be indicative only of a metabolically dependent transport process similar to "active basipetal polar transport," whereas the amount, duration, and maintenance of a polar transport pathway may represent the important regulatory factors in auxin-mediated growth processes.

The observation that dormancy callose disappears from the phloem during shoot reactivation is consistent with the suggestion by Hill (8) that some hormonal factor produced in reactivating buds is responsible for reactivation of the sieve tubes.

LITERATURE CITED

1. Brossard, D. and H. Tepper. 1969. Vitesse de transport de l'auxine exogéne dan les racines du *Zea mays*. C. R. Acad. Sci. Paris (D) 269: 1301–1303.
2. Brown, C. L. and R. H. Wetmore. 1959. Auxin transport in the long shoots of pine. Amer. J. Bot. 46: 586–590.
3. Eschrich, W. 1968. Translokation radioaktiv markierter Indolyl-3-essigsäure in Siebröhren von *Vicia faba*. Planta 78: 144–157.
4. de la Fuente, R. K. and A. C. Leopold. 1970. The transportable auxin pool. Plant Physiol. 45: 19–24.
5. Goldsmith, M. H. M. 1966. Maintenance of polarity of auxin movement by basipetal transport. Plant Physiol. 41: 749–754.
6. Goldsmith, M. H. M. 1969. Transport of plant growth regulators. *In:* M. Wilkins, ed., The Physiology of Plant Growth and Development. McGraw-Hill, New York. pp. 127–162.
7. Gregory, F. G. and C. R. Hancock. 1955. The rate of transport of natural auxin in woody shoots. Ann. Bot. N. S. 76: 451–465.
8. Hill, G. R. 1962. Exudation from aphid stylets during the period from dormancy to bud break in *Tilia americana*. J. Exp. Bot. 13: 144–151.
9. Larson, P. R. 1962. Auxin gradients and the regulation of cambial activity. *In:* T. T. Kozlowski, ed., Tree Growth. Ronald Press, New York. pp. 97–117.
10. Little, E. C. S. and G. E. Blackman. 1963. The movement of growth regulators in plants. III. Comparative studies of transport in *Phaseolus vulgaris*. New Phytol. 62: 173–197.
11. Lodewick, J. E. 1928. Seasonal activity of the cambium in some northeastern trees. N. Y. Coll. Forest. Technol. Publ. 23: 1–87.
12. McCready, C. C. 1963. Movement of growth regulators in plants. I. Polar transport of 2,4-dichlorophenoxyacetic acid in segments from the petioles of *Phaseolus vulgaris*. New Phytol. 62: 3–18.
13. McCready, C. C. 1966. Translocation of growth regulators. Annu. Rev. Plant Physiol. 17: 283–294.
14. Milthorpe, F. L. and J. Moorby. 1969. Vascular transport and its significance in plant growth. Annu. Rev. Plant Physiol. 20: 117–138.
15. Mirov, N. T. 1941. Distribution of growth hormone in shoots of two species of pine. J. Forest. 39: 457–464.
16. Morris, D. A., R. E. Briant, and P. G. Thomson. 1969. The transport and metabolism of ^{14}C-labelled indoleacetic acid in intact pea seedlings. Planta 89: 178–197.
17. Perley, J. E. and B. B. Stowe. 1966. The production of tryptamine from tryptophan by *Bacillus cereus* (KVT). Biochem. J. 100: 169–174.
18. Pilet, P. E. 1965. Polar transport of radioactivity from ^{14}C-labelled β-indolylacetic acid in stems of *Lens culinaris*. Physiol. Plant. 18: 687–702.
19. Smith, C. W. and W. P. Jacobs. 1969. The movement of IAA-^{14}C in the hypocotyl of *Phaseolus vulgaris*. Amer. J. Bot. 56: 492–497.
20. Stowe, B. B., M. Vendrell, and E. Epstein. 1968. Separation and identification of indoles of maize and woad. *In:* F. Wightman and G. Setterfield, eds., Biochemistry and Physiology of Plant Growth Substances. Runge Press, Ottawa. pp. 173–182.
21. Tepper, H. B. and C. A. Hollis. 1967. Mitotic reactivation of the terminal bud and cambium of white ash. Science 156: 1635–1636.
22. Thimann, K. V. 1969. The auxins. *In:* M. Wilkins, ed., The Physiology of Plant Growth and Development. McGraw-Hill, New York. pp. 3–45.
23. Thomas, T. H. 1969. The role of growth substances in the regulation of onion bulb dormancy. J. Exp. Bot. 20: 124–137.
24. Wareing, P. F., C. E. A. Haney, and J. Digby. 1964. The role of endogenous hormones in cambial activity and xylem differentiation. *In:* M. H. Zimmerman, ed., The Formation of Wood in Forest Trees. Academic Press, New York. pp. 323–344.
25. Wilcox, H. 1962. Cambial growth characteristics. *In:* T. T. Kozlowski, ed., Tree Growth. Ronald Press, New York. pp. 57–88.
26. Winter, A. and K. V. Thimann. 1966. Bound indoleacetic acid in *Avena* coleoptiles. Plant Physiol. 41:335–342.