DIFFERENCES IN APPLIED AUXIN TRANSPORT AND METABOLISM RELATED TO ROOTING OF BLACK WALNUT CUTTINGS 1/

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Abstract.--Indolebutyric acid labeled with tritium (IBA ^{3}H) was transported from the base of cuttings from visible bud shoots more rapidly than from cuttings from adventitious shoots, and chromatograms showed that quantities and, probably, the kind of metabolites differed from those in adventitious cuttings. After cuttings were treated 60 hours with IBA ^{3}H, 53.3 percent of the radioactivity in adventitious cuttings was in their indoleacetic acid (IAA) zones and 46.5 percent in their indolebutyric acid (IBA) zones, while respective percentages from visible bud cuttings were 46 and 53.8. That finding suggests that the plant, before using IBA may change it to IAA.

This study was conducted to gain insight into the physiology of root initiation in black walnut cuttings from shoots of visible and adventitious origin.

A method for growing genetic duplicates of black walnut, *Juglans nigra*, timber and nut types by rooting softwood cuttings from adventitious shoots has been demonstrated (Shreve and Miles 1972). They found that cuttings from terminal shoots of current-year seedlings and adventitious shoots, regardless of ortet age, readily formed roots when the base was quick-dipped into the synthetic auxin, indolebutyric acid (IBA) at 5,000 or 8,000 ppm in 95 percent ethanol and placed under mist. Ramets transplanted to the field grew satisfactorily. Cuttings made from shoots originating from visible buds did not form roots. The natural auxin, indoleacetic acid (IAA), caused no significant root formation. Shreve (1972) reported similar results with Chinese chestnut, *Castanea mollissima*, and probably pecan, *Carya illinoensis*.

Hess (1965) identified four rooting co-factors in easy-to-root juvenile forms of *Hedera helix*, and postulated that the absence of any co-factor, nutrients, or IAA would retard root formation in a cutting.

Hackett (1969) found that adult cuttings of *Hedera helix* would form roots as readily in the absence of light as juvenile cuttings of the same species would form roots in light. He also found that methanol extract of adult and juvenile stems caused similar rooting in juvenile cuttings of the same species but not in adult cuttings, which indicated that neither extract limited root initiation in the adult form.

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When Knoop (1904) administered phenyl derivatives of fatty acids containing from one to five carbon atoms to dogs, phenyl derivatives of even-numbered acids always led to the excretion of phenyl acetic acid, but phenyl derivatives of odd-numbered fatty acids were degraded to benzoic acid. He postulated that successive removal of two carbon units could explain the results, and the phenomenon was termed Beta-oxidation.

Synerholm and Zimmerman (1947) and Wain (1953) showed that the auxin activity of indole-3-butyric acid was due to oxidation at the position Beta to the carboxyl which reduced the chain length to that of acetic acid.

Moser et al. (1964) labeled carbohydrates by bombardment with energetic tritium (^3H) atoms. Useful amounts of nonspecifically-labeled glucose, sucrose, and glucose-1-phosphate were obtained.

MATERIALS AND METHODS

Our studies on observed differences in response of cuttings originating from different tissues in the tree were from July 1971 through March 1972.

One-year-old black walnut seedlings that had remained in cold storage during the summer of 1971 were planted in pots in the greenhouse early in December 1971. The tops were removed on half of the seedlings to one-half inch above the root collar--to force adventitious shoots in an area of no visible buds. The remaining half were pruned to ten inches above the root collar. The two groups were the source of adventitious and visible bud shoots.

In January 1972, IBA was nonspecifically labeled with tritium, ^3H, using the exposure-to-energetic-tritium-atoms technique developed by Moser et al. (1964). The labeled IBA was prepared at a concentration of 5,000 ppm so that a 25-microliter sample's radioactivity was IBA ^3H = 105,000 cpm when counted in a liquid scintillator.

Cuttings were two to eight inches tall; those of visible bud origin were usually shorter than those of adventitious origin. However, cuttings of both types were the same in height (8 inches) for the 60-hour treatment.

Treatment times are given in Table 1. Labeled IBA in a solution of 95 percent ethanol was applied to the base of each cutting with a disposable micropipette. The cuttings were then placed in a flask containing 0.5 cm of distilled water (1 cutting per flask) and kept in a closed hood the desired time. Water was added as needed.

Nine adventitious cuttings and 8 visible bud cuttings were treated with labeled IBA to compare the recovered radioactivity. After treatment, cuttings were divided into five sections: (a) the first centimeter at the base of the cutting; (b) the second and third centimeter above the base; (c) the remaining stem; (d) the leaves; and (e) the shoot terminal. Each section was ground with a mortar and pestle, soaked in 95 percent ethanol at least two hours, and filtered. The filtrate was reduced in volume (by heating) and adjusted to 1 ml per sample except the d-sections (leaves) of the

visible bud cuttings were treated for 48 hours and adjusted to a standard 2 ml per sample. Ninety microliters of each sample were streaked on thin-layered (silica gel) sheets, along with reference spots of IBA. When cuttings were treated 48 or 60 hours, reference spots of IAA were added.

Table 1.--Percentage of total labeled auxin (IBA ^{3}H) count recovered from each section (a, basal centimeter; b, second and third centimeters of stem; c, remainder of stem; d, the leaves; and e, apical bud) of black walnut cuttings 24, 48, and 60 hours after applying 5 microliters of IBA ^{3}H

Section of cutting	Shoot origin	Percentage of total IBA ^{3}H activity		
		24 hours	48 hours	60 hours
a	adventitious	44.9 (3)[a]	33.8 (4)	37.4 (2)
	visible bud	23.8 (3)	27.7 (3)	19.3 (2)
b	adventitious	19.0	22.0	15.7
	visible bud	27.8	14.2	21.2
c	adventitious	12.0	17.4	21.1
	visible bud	--	4.7	11.5
d	adventitious	13.0	22.0	16.6
	visible bud	34.8	50.4	44.6
e	adventitious	10.4	4.6	9.2
	visible bud	13.9	3.0	3.3

[a]Figures in parentheses equal number of samples.

Chromatograms were developed in a solvent of isopropanol, ammonia, and water (8:1:1 v/v) (Nitsch 1956). Sheets were left in the chamber until the solvent advanced beyond 15 cm of the 20 cm sheet (5 to 7 hours). After drying, chromatograms were examined under short wave, ultraviolet light (286 nm) and reference spots of IAA and IBA outlined. Then, recovery zones of each auxin (radioactive) were drawn on the sheet by extending the upper and lower reference lines for each auxin across the sheet. Chromatogrammed plant extract in the desired recovery zone was scraped into a test tube containing 2 ml of ethanol. After a minimum of one hour, one ml of the alcohol extract was removed and placed in scintillation fluid (3gPP0: .3g POPOP: 1,000 ml toluene) for cutting. In one case, more than 1 ml of the ethanol extract was used. When we examined the radioactive content of the entire shoot treated for 48 hours with IBA ^{3}H, we added 4 ml of extract to the silica gel scraped from the chromatogram and counted 3 ml for each sample zone.

Additionally, the radioactive contents of entire plants was determined for an adventitious shoot treated for 48 hours with IBA ^{3}H and for two adventitious cuttings and two visible bud cuttings treated for 60 hours. Chromatograms of cuttings treated 48 hours were divided to 1- to 3-cm and the radioactivity contents of sections of cuttings treated with IBA3 for 60 hours were determined by taking a 2-ml aliquot of the filtered plant extract from every sample of each section and placing it in bottles of scintillation fluid for counting. Each sample examined for radioactivity was counted twice in a scintillator for one minute. The average of the two counts was used for comparisons.

All samples were compared by percent of the total count per minute in each section of plants (cpm of section/cpm of cutting).

RESULTS

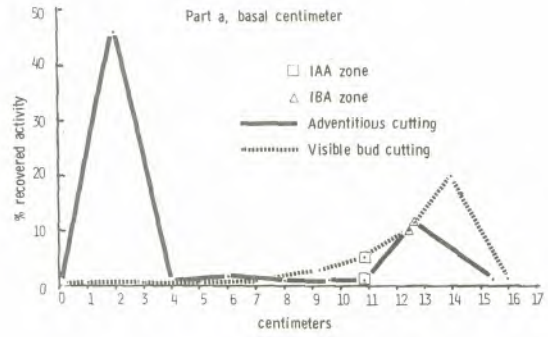
Amounts of IBA in each section of the cuttings are shown in Table 1. IBA in the basal centimeter (section a) of adventitious shoots remained constant and higher than in the same section of visible bud cuttings. The same was true for section b (second and third centimeters of stem) and section e (remainder of stem) for the 48-hour period, but not for the 24 and 60 hour periods. Then more IBA was in section b of visible bud shoots than in adventitious shoots. No comparison was possible for the section c, 24-hour period because the stems of visible bud cuttings did not exceed three centimeters. IBA was much higher in section d (the leaves) of visible bud shoots than adventitious shoots for all three time periods. In section e (apical bud), visible bud shoots contained more IBA than adventitious shoots did for the 24-hour trials, but adventitious shoots contained more than visible bud shoots for the 48- and 60-hour periods.

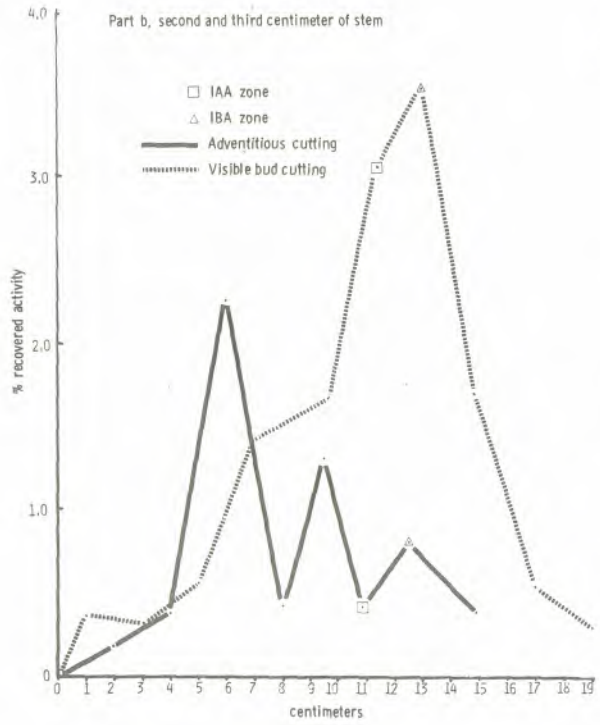
Data in Table 1 show that IBA ^{3}H is transported upward faster from bases of visible bud cuttings than from bases of adventitious cuttings.

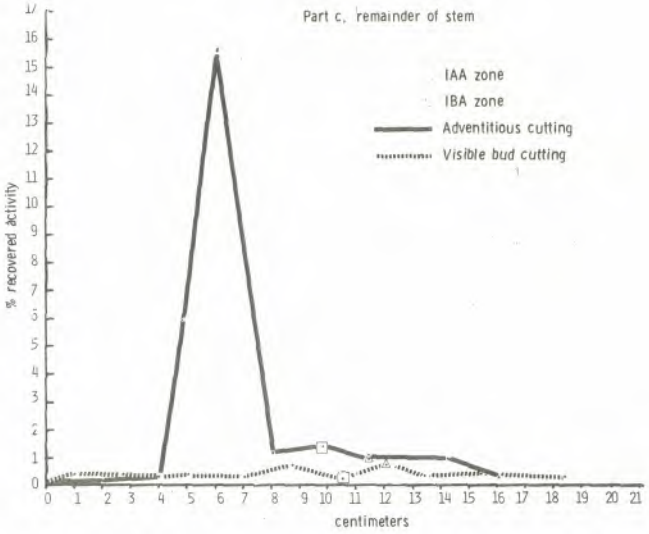
Parts a, b, c, d, and e of figure 1 show the percentage of total radioactivity recovered from specific areas of the chromatograms for each section of the cuttings 48 hours after adventitious and visible bud cuttings were treated with IBA ^{3}H. The data show that IBA ^{3}H was transported more rapidly in visible bud cuttings and indicate that applied IBA may result in different quantities and entirely different substances in the two types of cuttings.

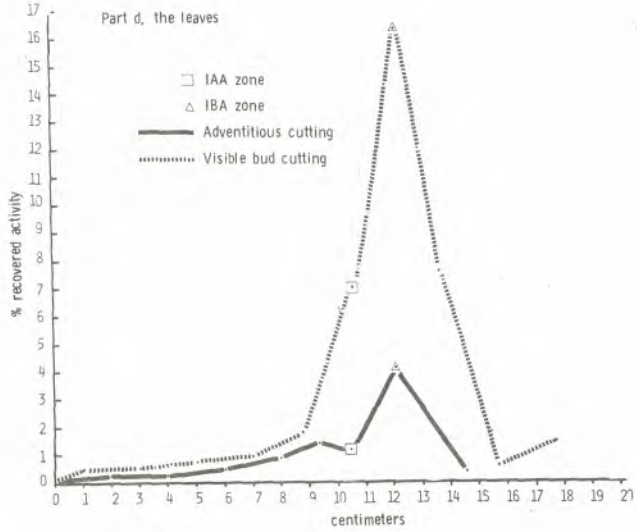
Comparing figure 2 with data in Table 1 supports the idea of different quantities and substances in the two types of cuttings. Percentages of IBA ^{3}H recovered over 60 hours differ strikingly from percentages of total radioactivity recovered from each section. For example, total radioactivity in section a of adventitious shoots was 63 percent of the total but only 37 percent of the total IBA was recovered from section a. Comparable percentages for section b of visible buds were 37 and 21. In section d of visible bud cuttings the percentages reversed: 25 for radioactivity; 44.6 for IBA.

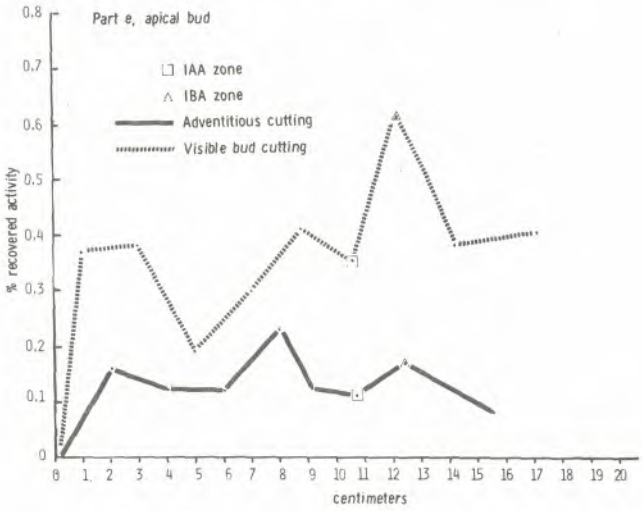
Figure 1. Percentage of radioactivity recovered from black walnut cuttings of adventitious origin and visible bud origin after treatment with IBA-^{3}H 48 hours. Each point on a graph expresses the percentage of the total recovered radioactivity from the cutting in that segment of the chromatogram. Part a, basal centimeter of cutting; part b, second and third centimeter of the stem; part c, remainder of stem; part d, the leaves; and part e, apical bud.











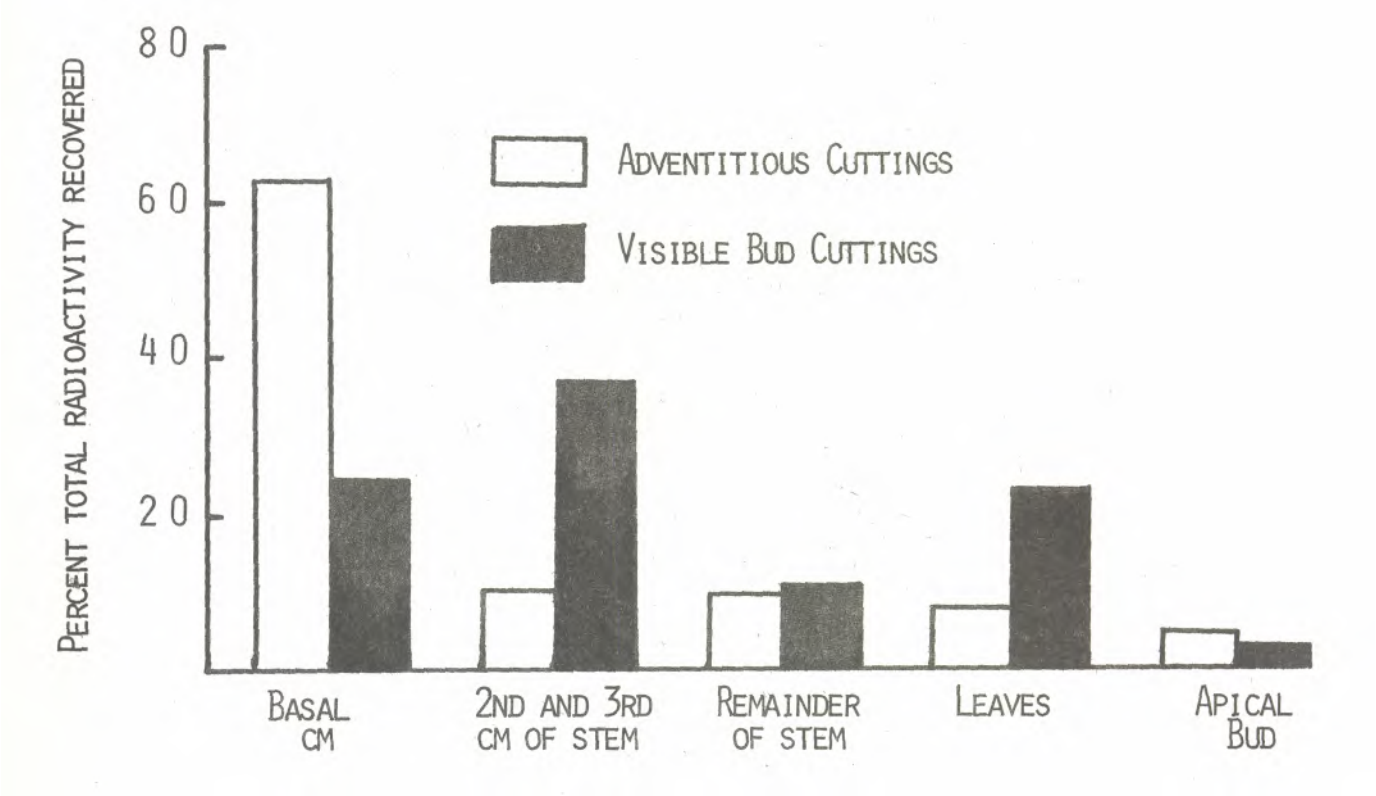


Figure 2.--Percent of total activity recovered from each section of black walnut cuttings of adventitious and visible bud origin 60 hours after treatment with IBA3H.

Table 2 shows that applied IBA^{3}H was recovered as IAA^{3}H. Percentages of IAA^{3}H recovered increased from 41.4 and 43.3 for adventitious and visible bud cuttings, respectively, 48 hours after treatment to 53.3 and 46.0 60 hours after treatment.

Table 2.--Percentages of total auxin recovered at rf for IBA and rf for IAA after treatment with 5 microliters IBA^{3}H after 48 and 60 hours

Hours after treatment	Shoot origin	rf recovery zones	Percentage of total activity in each section					
			a	b	c	d	e	Total
48	adventitious	IBA	14.2[a]	11.8	10.6	17.7	3.9	58.2
		IAA	8.1	8.0	6.0	14.3	5.0	41.4
	visible bud	IBA	11.3	5.3	1.9	36.8	0.8	56.1
		IAA	7.0	3.9	1.6	30.1	0.7	43.3
60	adventitious	IBA	17.4[b]	7.3	9.8	7.7	4.3	46.5
		IAA	14.2	10.1	15.6	9.8	3.6	53.3
	visible bud	IBA	10.4	11.4	6.2	24.0	1.8	53.8
		IAA	9.6	6.6	5.8	21.6	2.4	46.0

[a]Each figure represents the average of three samples.
[b]Each figure represents the average of two samples.

DISCUSSION

Our laboratory data help explain why adventitious cuttings form roots when treated with IBA while visible bud cuttings do not. IBA is transported much more rapidly from bases of visible bud cuttings (Table 1) than from bases of adventitious cuttings. Chromatograms reveal that adventitious cuttings and visible bud cuttings either form different metabolites from applied IBA, or different quantities (Fig. 1). Greater quantities of IBA metabolites remain in bases of adventitious cuttings than in bases of visible bud cuttings after 60 hours treatment (Fig. 2).

There are two possible explanations of the data in Table 2: (1) metabolites of IBA may have rf similar to IAA, and (2) IBA could be metabolically changed into IAA by the cutting. Knoop (1904) postulated that successive removal of two carbon units could explain why phenyl derivatives of even numbered fatty acids always led to excretion of phenylacetic acid in canine urine, and phenyl derivatives of odd numbered fatty acids were oxidized to benzoic acid. Subsequent experiments have shown that both plants and animals possess that ability with the mitochondria being the site of fatty acid oxidation (Conn and Stumpf 1967), so IBA could be oxidized by the walnut cutting to form IAA.

Cuttings of adventitious and visible bud origin from the same clone and labeling IBA with ^{14}C at a known location on the molecule would add precision to future similar research, as would facilities to duplicate the greenhouse situation to use with radioactive auxins. In future studies, total cutting should be examined for radioactivity and all products containing radioactive isotopes identified.

LITERATURE CITED

Conn, Eric E. and P. K. Stumpf. 1967. Lipid metabolism, p. 274-294. In Outlines of biochemistry. 2nd Edition. John Wiley & Sons, Inc., New York.

Hackett, Wesley P. 1969. The influence of auxin, catechol, and methanolic tissue extracts on root initiation in aseptically cultured shoot aspices of the juvenile and adult forms of Hedera helix. Int. Plant Propagators Soc. Proc. 19:57-68.

Hess, Charles E. 1965. Rooting co-factors--identification and functions. Int. Plant Propagators Soc. 15:181-186.

Knoop, F. 1904. Beta-oxidation theory, p. 275-276. In Outlines of biochemistry. 2nd Edition. John Wiley & Sons, Inc., New York.

Moser, H. C., P. Nordin and J. K. Senne. 1964. Labeling carbohydrates by exposure to energetic tritium atoms. Int. J. Applied Radiation and Isotopes 15(1):557-559.

Nitsch, J. P. 1956. Methods for the investigation of natural auxins and growth inhibitors, p. 3-31. In The chemistry and mode of action of plant growth substances, R. L. Wain and F. Wightman, Editors. Butterworth's Scientific Publications, London.

Shreve, Loy W. 1972. Propagation of walnut, chestnut, and pecan by rooted cuttings. Proc. Eighth Cent. States For. Tree Improv. Conf. (In press)

Shreve, Loy W. and Neil W. Miles. 1972. Propagating black walnut clones from rooted cuttings. The Plant Propagator 18(3):4-7.

Synerholm, M. and P. W. Zimmerman. 1947. The auxins, p. 5-43. In The physiology of plant growth and development. McGraw Hill, New York.

Wain, R. L. 1953. The auxins, p. 5-43. In The physiology of plant growth and development. McGraw Hill, New York.