# AUXIN IN *ZEA MAYS* L. COLEOPTILE SEGMENTS: SATURATION OF ABSORPTION AND TRANSPORT AT VARIOUS CONCENTRATIONS

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**Abstract**

This paper re-examines the effects of various concentrations of indoleacetic acid-2-^{14}C on its absorption and translocation efficiencies. Using *Zea mays* L. coleoptile segments and by considering absorption and translocation as discrete phenomena it has been found that the maximum efficiency of the tissue is achieved between 0.2—0.4 mg/1.

## Introduction

The effect of increasing concentrations of the applied auxin on saturation of transport has been the subject of a number of investigations (Goldsmith & Thimann, 1962; Gillespie & Thimann, 1963; Hertel & Leopold, 1963; Scott & Jacobs, 1963). On the basis of the absolute biological activity or radioactivity of the receivers the saturation of transport has invariably been shown to occur on or about $10^{-5}$M (1.75 mg/1) IAA. It is, however, interesting to note that the above investigations take no cognizance of auxin absorption and auxin content of the tissues as factors influencing transport characteristics. The importance and significance of these factors have been well emphasized in auxin transport studies (Naqvi, Dedolph & Gordon, 1965; Kaldewey, 1968). It was, therefore, considered worthwhile to re-investigate the problem on the basis of the above mentioned approach.

## Materials and Methods

*Zea mays* L. (ORLA—266) seeds were soaked overnight in $10^{3}$-M $CaCl_2$ and planted embryo up on cellulose papers (Cellucotton), saturated with the same solution, in plastic boxes. The boxes were covered and placed in the dark room maintained at $25\pm1°C$. To inhibit mesocotyl growth the planted seeds were exposed to red-light (Philips TL 20 W/15, red-fluorescent tube) between 48 and 54 hr and then kept in complete darkness till they were used for transport determination (94 hr. after planting).

Indoleacetic acid—2—[14]C (sp. act. 48.5 mCi/mM; Amersham, England) was used in appropriate concentrations and incorporated in 1.5% agar cylinders (ca. 21 μ'). The purity of the stock solution of ^{14}C-IAA in acetonitrile was periodically checked and no sign of impurity was observed.

Ten mm sections were excised from coleoptiles (30-35mm) commencing 1-2mm below the tip and the leaf was pushed out. The sections were then placed on receivers (plain 1.5% agar; ca. 22 μl) in normal vertical orientation and the physiological apex was covered with donor cylinder of appropriate auxin concentrations. At the end

of each time period two segments were pooled and the radioactivity in donors, 8.0mm tissues and the remaining 2mm adjacent tissues together with the receiver blocks was placed in three separate polyethylene vials containing 10 ml of Naqvi's scintillant (toluene: liquifluor: ethanol in a ratio of 71: 4: 25, v/v/v) and the radioactivity was analysed by liquid scintillation counting (Naqvi, 1966, 1971). Following corrections for the background net activities and the data derived from them were analysed by the analysis of variance. Unless otherwise stated all the experiments were replicated three times and the manipulations and transport determinations were carried out at $25 \pm 1$ °C under safe green-light (Naqvi, 1972).

## Results

A ninety minute transport study was carried out to determine the effects of various concentrations of $^{14}C$—IAA on transport through the coleoptile segments. On the basis of absolute radioactivity the counts lost from the donor are a linear function of the applied auxin concentration up to about 0.8 mg/l (Fig. 1). However, out of the applied $^{14}C$—IAA 18 to 26 % is absorbed, and between 25 and 37% of that absorbed is translocated at all concentrations tested (Table 1): unabsorbed radioactivity remained in the donor blocks. The percentage values for absorption at 0.2 and 0.4 mg/l is significantly greater than those at both lower and higher concentrations but not amongst themselves. Similarly the percentage values for the portion of the absorbed auxin which is translocated at these two concentrations are not materially different but are significantly more than those at both lower and higher concentrations.

In a separate set of experiments the effect     .2 mg/l and 0.4 mg/l of the auxin on the kinetics of transport was determined (Fig. 2). The calculated X-axis
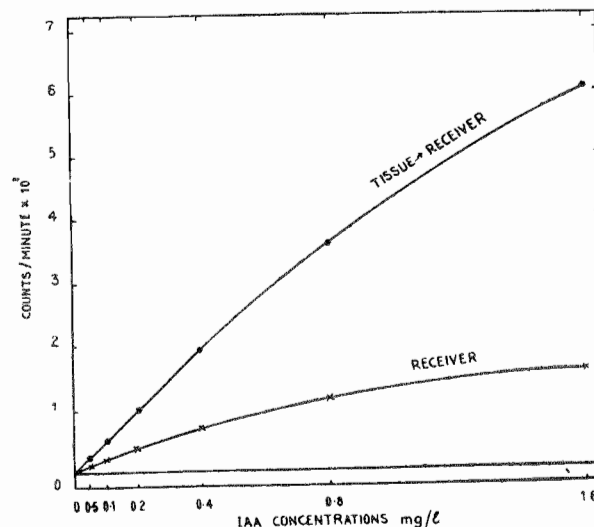


Fig. 1.  Effect of $^{14}C$-IAA concentrations on absorption and translocation through *Zea mays* L. coleoptile segments.

**TABLE 1.  Effect of Increasing Concentrations of  Auxin on Absorption and Transport Efficiency of** *Zea mays* **Coleoptile Segments**

| IAA mg/l | | 0.05 | 0.1 | 0 2 | 0 4 | 0.8 | 1.6 |
|---|---|---|---|---|---|---|---|
| % of donor | in tissue | 14.93 | 16.21 | 16.44 | 16.39 | 13.92 | 13.98 |
| | in receiver | 7.11 | 8.23 | 9.76 | 9.34 | 7.06 | 4.79 |
| % Absorbed | | 22.04 | 24.44 | 26.20 | 25.73 | 22.98 | 18.77 |
| % Transported | | 32.34 | 33.61 | 37.19 | 36.31 | 30.72 | 25.50 |

intercept for the linear radioactivity in the receiver versus transport period was taken as the index of the time required for the radioactivity to move through the 8.0mm length of the tissue.   Since the loss of radioactivity from the donor is also a linear function, for the transport period used, we can calculate the time period when the first  radioactivity left the donor and appeared in the tissue.   Thus the calculated intercept for
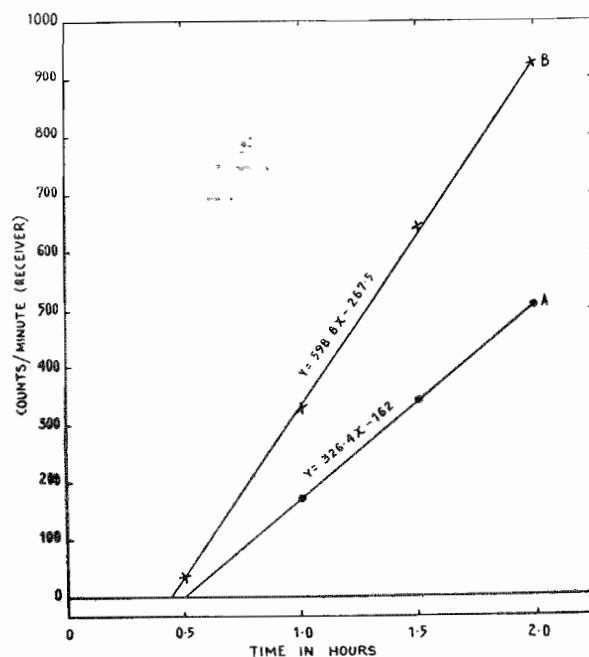


Fig.   2.   Effect of $^{14}$C-IAA concentrations on transport velocity and intensity through *Zea mays* L. coleoptile segments.

uptake was determined to be 0.05 hr. (3 minutes) at both these concentrations. By substracting this time period from the X-axis intercept for transport velocity a corrected value of 0.45 and 0.39 hr. representing a velocity of 18.2 (0.2 mg/l) and 20.0mm hr-[1] (0.4 mg/l) is obtained. The slope of the line *i.e.* the transport intensity shows 326 CPM hr-[1] and 599 CPM hr-[1] respectively.

### Discussion

Calculating the percentage of the applied IAA that appeared in receivers, a number of workers have shown that while the absolute amount of auxin in the receiver increases with the concentrations applied the percentage appearing in the receivers actually decreases (Goldsmith & Thimann, 1962; Hertel & Leopold, 1963, Gillespie & Thimann, 1963; McCready & Jacobs, 1963; Naqvi, 1963(. In the present studies the data were analysed on the basis of, (1) absolute radioactivity (2) the actual percentage absorbed by the tissue and (3) the portion of that which is translocated.

As shown in Figure 1 there is a constant increase, initially linear, in the radioactivity lost from the donors upto the highest concentrations tested. The radioactivity which appear in the receivers behaves similarly. These observations corroborate those of Gillespie & Thimann (1963) who also used *Zea mays* L.However, on the basis of the percentage of the applied auxin that is absorbed, and the portion of that absorbed which is translocated (Table I), it is concluded that the maximum efficiency of absorption and of translocation was achieved when the donor concentration reached 0.2 mg/l. The efficiency remained unchanged upto 0.4 mg/l and then started decreasing when the concentration was further increased. McCready & Jacobs (1963) and Pilet (1965) have also reported similar observations although they used concentrations of 5-50 µM (0.875-8.750 mg/l). From the present observations it is suggested that in the bove two studies the saturation of absorption and translocation efficiency may also have occurred in similar concentration ranges as observed in the present investigations for *Zea mays* L. Jacobs (1961) suggestion that "Saturation capacity could act as a controlling valve which prevents excess amounts of auxin from being transported around the plant and thereby disturbing the balanced coordination of normal development" is further supported from the present studies.

Van der Weij (1934) concluded that the velocity of auxin transport was independent of the concentration of exogenously supplied auxin, though his data shows a wide range & variance. McCready & Jacobs (1963), using bean petiole segments, concluded that the velocity of 4.86 mm hr—[1] (5 µM) was not significantly different than 6.80 mm hr—[1] (50 µM). Pilet (1965) also concluded that the velocity of IAA movement through *Lens* shoot did not change when the concentration of donor was increased from 5 µM (5.34mm hr—[1]) to 50 µM (6.42 mm hr—[1]). However, it is interesting to note that velocity of IAA movement through bean petiole as well as *Lens* shoot segment was always higher with increase in donor concentrations even though they were considered to be non-significant. With the more precise measurements enabled by the use of optimal donor concentrations, correction for the initial delay in uptake, and consideration of tissue length at the end of transport period it is concluded that the velocity is significantly faster when the donor concentration is increased from 0.2 mg/l (Fig. 2A; 18.19±0.13mm hr—[1]) to 0.4 mg/l (Fig. 2B; 20.01±0.09 mm hr—[1]). An analogous effect of concentration on transport velocity had been observed with *Coleus* segment (Naqvi, 1963). Also the initial delay of 0.05 hr. in uptake is identical with that observed by Newman (1959) for the

movement of a peak of electrical potential after the application of IAA donor blocks to decapitated *Avena* coleoptile segments. Similarly Smith & Jacobs (1968) observed a delay of 30-45 minutes before the donor radioactivity appeared in bean hypocotyl segments. Thus all the velocity measurements assuming that the absorption starts immediately are underestimates of the true value.

From the considerations made above it may be stated that the difference in the slopes of the transport curves shown by 0.2 mg/l and 0.4 mg/l (Fig. 2A and B) may not represent an increase of transport intensity, but only reflects a difference in the *amounts* of auxin leaving the donor blocks. From Table I, at both 0.2 mg/l and 0.4 mg/l approximately 26% of the applied auxin was absorbed, and 37% of that was translocated. Thus it may be inferred that doubling the concentration of auxin had no effect on increasing the transport efficiency of the tissues. Doubling the auxin concentration only increased the amount of auxin absorbed and translocated.

Therefore, the above discussed considerations suggest the desirability of recognizing absorption as a discrete phenomenon in auxin transport studies. Erroneous conclusions can be drawn from auxin transport studies if one considers only absolute auxin titers, or the percent of the applied auxin which appears in tissues and receivers, and overlooks the amounts of auxin absorbed from the donor and the portion of that actually translocated.

## Acknowledgements

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