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THE ROLE OF PLANT GROWTH REGULATORS IN THE TRANSLOCATION OF SUCROSE IN *PHASEOLUS VULGARIS* L.

by

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ABSTRACT

The effects of exogenous applications of hormones on translocation of radioactive sugars were studied in intact plants of *Phaseolus vulgaris* L. Indoleacetic acid (IAA) facilitated the uptake of labelled sugars in the fed leaf and promoted basipetal transport of the label in the petiole and stem. Acropetal transport of the label, in contrast, was unaffected. The site of hormone application had a marked effect on the uptake and subsequent basipetal transport of the label. Higher concentrations of the hormone (1000 ppm rather than 100 ppm) and longer durations of treatment (96 h vs. 72 h) had a further promotive effect. The addition of gibberellic acid (GA_3) did not have any enhancement effect on basipetal transport but facilitated the uptake of the label, presumably by creation of a "sink". The synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D) had no promotive effect on basipetal transport. Application of 2, 3, 5 - triiodobenzoic acid (TIBA) decreased the basipetal transport of sugars and this effect was partly overcome by application of IAA following TIBA application. TIBA, however, did not toally inhibit the basipetal transport of ^{14}C - IAA. Data on translocation to sink areas and mature tissues are also presented. In all these experiments only a small amount of the fed label was actually translocated, the highest values for translocation being obtained when IAA was applied for 48 or more h at the site of subsequent feeding of labelled sugar. For highly colored leaf samples, a change from the normal ethanol extraction to NCS solubilization of ethanol extracts revealed that a good deal of label was not available for counting.

This thesis is dedicated to

my parents

and

loving husband

who

provided the spark of

encouragement

and

enlightenment

throughout

the course of its

fulfillment

Labor omnia vincit

Labor conquers everything.

Virgil, Georgics, I.

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

Introduction

Translocation of photoassimilates is a complex phenomenon involving at least three sites. In a green leaf photosynthesis is carried out in the chloroplast-containing mesophyll cells. Phloem strands end more or less blindly among the mesophyll cells and, consequently, among the first processes is the loading of photoassimilate from the mesophyll and intervening parenchyma cells into the sieve elements. The photoassimilate then moves to the other plant tissues including roots and growing meristems. This movement, the long distance transport, is believed to occur in the longitudinal files of sieve elements. Periodically, from the longitudinally moving stream, the photoassimilate is unloaded via parenchyma cells and moved to metabolic sinks where it is utilized. It is generally agreed that the intercellular transfer of assimilate between parenchyma cells, and the phenomena of loading and unloading are energy-requiring processes. The mechanism of long-distance transport in the sieve elements, in contrast, is a highly controversial topic. Despite intensive research on the structure of sieve elements, constitution of the sieve tube sap, and measurement of rates and velocities of transport under different conditions there is no concensus at the present time on the exact mechanism of long distance transport (see reviews by Canny, 1960, 1971; Crafts and Crisp, 1971; Esau, 1969; Esau, Currier and Cheadle, 1957; MacRobbie, 1971; Weatherley and Johnson, 1968 and Zimmermann, 1960).

Plant hormones such as auxins, gibberellins and cytokinins are known to affect various processes of growth and development (Cleland, 1961; Goldsmith, 1969; Kende, 1971 and Thimann, 1972). They are also known to affect the translocation of photoassimilates though this effect is much less investigated. Much of the work on translocation of hormones and the effect of hormones on the translocation of photoassimilates has been done on isolated segments of stems or petioles but these studies, while yielding valuable data on velocities and polarity of transport, do not provide information pertinent to intact plants. Accordingly, in this thesis, an attempt is made to study the effect of selected hormones on translocation of radioactive sugars in intact bean plants. The data presented in this thesis indicate that an auxin, indoleacetic acid (IAA) enhances the basipetal transport of labelled sugars and also affects the profile of distribution of the label in various parts of the plant. The data also indicate that among the hormones tested this effect is specific for IAA and that there is a close parallelism between the movement of IAA itself and the translocation of labelled sugars.

Literature review

In the following review some of the important information on the constitution of the sieve-tube sap and kinetics of transport are summarized for background information. The translocation of hormones and the effects of hormones on translocation of assimilates are reviewed in detail.

Composition of the sieve tube sap

The analysis of sieve-tube sap in higher plants, obtained by tapping the bark near the base of the stem (Zimmermann, 1964) or by aphid stylet method (Mittler, 1953), has consistently shown that the principal sugar translocated in the majority of higher plants is sucrose which may occur in concentrations as high as 20% w/v. Zimmermann (1960) also noted small amounts of raffinose and related sugars in the sieve-tube sap of various trees, but reported that hexoses and sugar phosphates were absent. Amino acids (10-100 mM) as well as various cations, 20-85 mM K^+, 2.3-23 mM Mg^{++}, 0.06-0.3 mM Na^+ and 0.25-

0.5 mM Ca^{++}, are present (MacRobbie, 1971). More recently, several phosphorylated products including ATP have been shown to be formed by incubation of the sieve-tube exudate with ^{32}P (Becker et al, 1971; Ho and Peel, 1969; Peel et al, 1969, and Ziegler, 1956; see also Schmitz and Srivastava, 1974). The formation of these products indicates the presence of several enzymes in the sieve-tube sap. Gilder and Cronshaw (1973) have shown cytochemically the presence of ATPase on various membranes in the sieve-tubes of Cucurbita. These and various other studies indicate that whereas sucrose is the principal sugar translocated in the higher plants, various nitrogenous compounds including enzymes may be translocated and still other enzymes may occur bound to membranes in the sieve tubes.

Rates and Velocities of Translocation

Table I represents data compiled by Canny (1960) for the specific mass transfer and transport velocities of labelled compounds in various plants. It is clear that substantial amounts of material are transported at fairly high velocities. Various factors including environmental factors influence the rates and velocities of translocation (see review by Zimmermann, 1969). In addition, it has been calculated that if the dose of radioactivity is doubled there is a 30% increase in distance of the 'front' of activity down the stem and if the dose is squared in value the distance of the front is doubled (Canny, 1960). Velocity measurements therefore, should be viewed in conjunction with isotope information.

Theories of Translocation

Data from velocity and specific mass transfer experiments preclude protoplasmic streaming as the mechanism for long distance transport for, under

TABLE I - RATES AND VELOCITIES OF TRANSLOCATION IN PHLOEM

1. Rate of translocation as measured by mass transfer of dry weight

Plant System	Specific mass transfer (g dry w/cm^2 phloem/h)
Stems	
Solanum tuber	4.5
Dioscorea tuber	4.4
Solanum	2.1
Kegelia fruit peduncle	2.6
Cucurbita fruit peduncle	3.3
Cucurbita fruit peduncle	4.8
Petioles	
Phaseolus	.56
Phaseolus	.70
Tropaeolum	.70

2. Velocities of translocation as determined by the use of radioactive tracers

Plant System	Velocity (cm/h)
Phaseolus vulgaris	107
Beta vulgaris	85-100
Vitus labrusca (Concord)	60
Salix sp.	100
Saccharum officinarum	270
Saccharum officinarum	84
Cucurbita melopepo	290
Glycine max	86
Cucurbita pepo	40-60

Note:

Rate = weight transfer per unit time

Velocity = distance travelled per unit time

a. The data for specific mass transfer were obtained by calculating the change in fresh weight over time divided by cross sectional area of phloem.

b. In most of these experiments $^{14}CO_2$ was supplied to the leaflet, activity measured in plant parts distal to fed leaf.

Adapted from Canny, M.J. 1960 The rate of translocation, Biol. Rev. 35: 507-535.

the best of conditions, velocities of more than 5 cm/h have not been recorded for protoplasmic streaming (see MacRobbie, 1971; and Weatherely and Johnson, 1968). Furthermore, reports on protoplasmic streaming in 'mature' sieve elements are contradictory. Currier, Esau and Cheadle (1955) investigated a number of plants at various times of the year and observed no streaming, but Fensom (1972) reported streaming within individual sieve elements of Heracleum mantegazzianum at velocities of 1.5–2.5 cm/h, occasionally up to 5.0 cm/h. Alternative mechanisms for long distance transport include the pressure or mass flow hypothesis of Munch (1930). According to this hypothesis, accumulation of photoassimilates in the 'source' region creates a gradient in turgor pressure relative to 'sink' areas, where assimilates are utilized, with a resultant mass flow of solution through the phloem system. This flow requires the presence of open pores in the sieve plates of the sieve-tubes. In many studies, pores have been reported to be filled by fibrillar material; also the parenchyma cells associated with sieve elements have been reported to be rich in mitochondria. These studies and others which sho the presence of enzymes in sieve-tubes have led many authors to question the pressure flow hypothesis. An activated diffusion process especially across the sieve plates has been postulated by Fensom (1972) and Spanner (1958). Hejnowicz (1970) has visualized the generation of electrical waves on microfibrils longitudinally oriented in sieve elements, and recently a theory, the reciprocating flow hypothesis, involving protoplasmic streaming and osmotically driven mass flow was put forward by Miller, 1973).

Translocation of Hormones

A. Indoleacetic acid (IAA)

It is well known that IAA is transported in a polar fashion toward the base of the plant (Goldsmith, 1969). Most work on IAA transport, however, has been done with isolated segments of petioles or stems which are 'capped' by agar blocks containing labelled auxin (McCready, 1968; Goldsmith, 1968). These studies, while confirming the polar transport of IAA toward the morphological base of the segment, further reveal that the segment length and auxin concentration affect the degree of polar movement. It appears that high concentrations of IAA, that is above the physiological range of 3.5-8.8 mgIAA/l lanolin, decrease whereas longer segments increase the degree of polar movement in bean stem segments (McCready, 1968; McCready and Jacobs, 1963). Similar results are reported for *Avena* stem segments by Goldsmith (1968).

The rates and velocities of auxin transport have been investigated by various authors. Thimann (1972) reported a specific mass transfer of labelled IAA, applied at a concentration of 3.2 mg/l, of 0.21 $g/h/cm^2$ in *Avena* stem segments. The velocity of IAA transport as measured by different authors in different systems varies widely from 5.7 to 14 mm/h (see review by Goldsmith, 1969). Some of the more recent papers have given similar velocities. For example, Smith and Jacobs (1969) and Jacobs (1970) reported velocities of 5.7 mm/h and 5.3-5.8 mm/h in petiole segments of *Phaseolus vulgaris* and of *Coleus* stem segments, respectively. In contrast to these reports, Newman (1970) found a much higher velocity of 9.5 cm/h for ^{14}C-IAA in *Avena* stem segments.

Velocities of IAA transport may be different for intact plants versus isolated segments. For instance, Little and Blackman (1963) reported a velocity of 20-24 cm/h for intact hypocotyls of Phaseolus vulgaris but only 6 mm/h for excised petioles. The movement of IAA is affected by various other factors including age of the tissue, light and darkness, and length of time the plant segment has been excised. For example, the basipetal component of IAA transport is reported to decline in bean stems as the plant ages (McCready and Jacobs, 1963; Smith and Jacobs, 1969). By appropriate darkening of plant parts the usual basipetal transport of photoassimilate can be reversed to an acropetal transport (see Hartt, 1965). Under the same experimental conditions a reversal from basipetal to acropetal transport of ^{14}C-IAA has also been reported (see Wardlaw, 1968). In Coleus, the vegetative shoots had a basipetal/acropetal transport ratio of 3 whereas floral shoots had values of 1.3 (Naqvi and Gordon, 1965). Osborne and Mullins (1969) reported that, 5 h after excision, the petiole segments of Phaseolus vulgaris showed a 50% decrease in the basipetal transport of IAA; after a 10 h period, the basipetal IAA transport was reduced to 10% of its original value.

One of the important aspects of the polar transport of IAA in isolated segments is that this transport is independent of the concentration gradient. For instance, IAA continues to accumulate at the morphological base of the segment irrespective of the fact that the concentration of IAA at the base is much higher than at the apex (Leopold, 1967). This rules out a simple passive diffusion and indicates a cellular control over the movement of IAA. It is not known whether the movement of IAA in intact plants is independent of the concentration gradient.

Most people think that IAA moves in living parenchyma cells of the vascular tissues though movement in sieve elements and tracheary cells is not ruled out (dela Fuenta and Leopold, 1966; Goldsmith, 1969; Leopold, 1967 and Salisbury and Ross, 1969, pg. 454).

B. 2,4-dichlorophenoxyacetic acid (2,4-D)

In comparison with IAA, the synthetic auxin 2,4-D is a relatively stable molecule. Furthermore it has been shown to be absorbed continuously in a linear fashion over the concentration range of 0.1-200 mg/l lanolin over a period of 24 h (Audus, 1964; Pallas and Williams, 1962; and Yamaguchi, 1965). For these reasons, 2,4-D is the synthetic auxin that is most often used for investigations.

The velocity of 2,4-D transport varies but is generally lower than that for IAA. McCready (1963) and McCready and Jacobs (1963) used petiolar segments of Phaseolus vulgaris and reported velocities of 0.6-1.0 mm/h for 2,4-D and 6 mm/h for IAA in comparable experiments. Application of ^{14}C-2,4-D to the cotyledonary node of Phaseolus vulgaris gave a velocity of 10-12 cm/h; and for ^{14}C-IAA a velocity of 20-24 cm/h (Little and Blackman, 1963). Jacobs, McCready and Osborne (1966) noted that different plant parts may show differing velocities of transport for 2,4-D. For instance, in petiole segments of bean velocities of 1.5 mm/h and in pulvinar segments of 0.8 mm/h were recorded. Leonard et al (1968) obtained similar results for bean leaves and reported that a decline in basipetal 2,4-D transport occurred with increasing age of the tissue. Due to the relatively slow velocity of 2,4-D higher concentrations and/or longer treatment times are often necessary for adequate amounts to be translocated. For instance, in Salix viminalis radioactivity from ^{14}C-2, 4-D fed to the morphological apex of bark strips was not noted after 24 h but readings at 36 h revealed activity (Field and Peel, 1972). In similar experiments with Populus tremula twigs Eliasson and Hallman (1973) noted relatively long times for adequate transport of 2,4-D. These authors noted an upward transport of ^{14}C-2,4-D into the growing shoot apex, however. Similar acropetal transport for ^{14}C-2,4-D in stem and petiole segments of Phaseolus vulgaris was reported by Jacobs (1967). According to Jacobs (1967) transport of 2,4-D was greater in vascular strands than parenchyma cells of bean and Coleus segments, and changes in flux (weight of compound moved per unit time) and concentration rather than velocity or cellular transport site may be more important in polarity investigations. For example, high 2,4-D concentrations were required for labelling of roots in soybean (Crafts, 1967). Label moved from the primary leaf to other trifoliate leaflets, the shoot apex, and the roots, but the primary leaf opposite to the treated leaf was almost always bypassed.

C. Gibberellic acid (GA)

In contrast to 2,4-D and IAA, the movement of ^{14}C-GA is reported to be nonpolar (see Crafts and Crisp, 1971; Galston and Warburg, 1959; and Lazer et al, 1961). However, Jacobs and Kaldewey (1970) recorded a 10:1 ratio for basipetal: acropetal ^{14}C-GA transport in petiole segments of Coleus. Zweig, Yamaguchi, and Mason (1961) treated the primary leaf blade of Phaseolus vulgaris with GA_3 under high humidity conditions for periods ranging from I h to 9 days. ^{14}C-activity accumulated in both shoot and root apices after 8 h but no label appeared in the opposite primary leaf. Musgrave et al (1969) determined the distribution of ^{3}H-GA_1, ^{3}H-GA_5 and other GAs of low biological activity in dwarf pea plants. More activity was located in the apical than the basal parts of the plants and higher levels of radioactivity were recovered in both regions from the GA_5 than the GA_1 treatment. Derivatives of GA with low biological activity were not preferentially taken up by the apex.

Several authors have reported velocities of GA movement in the range of 10-50 mm/h (Evans and Wardlaw, 1966 for Lolium temulentum; Galston and Warburg, 1959 for pea stems; McComb, 1964 for elongating pea stems; Neely and Phinney, 1957 for dwarf-1 maize).

As for IAA and 2,4-D, GA is also believed to move in parenchyma cells but movement through the xylem or phloem conducting cells is not excluded. (Lang, 1970).

D. Triiodobenzoic acid (2,3,5-TIBA, TIBA)

TIBA is known to inhibit various responses induced by IAA but whether it acts as a competitive inhibitor or as a general inhibitor of IAA-induced reactions is not known (Goldsmith, 1968, 1969). Goldsmith (1969) found that TIBA inhibited both basipetal and acropetal transport of IAA in isolated pea stem segments. In contrast, McCready (1963) noted that TIBA promoted acropetal but inhibited basipetal transport of IAA in petioles of beans. Penny et al (1972) reported that TIBA applied around bean stems as a ring at concentrations of 10^{-3}-10^{-5}M, induced an accumulation of radioactivity above the girdle when ^{14}C-IAA was applied to the decapitated apex. Winter (1968) suggested that TIBA promoted the immobilization of IAA in *Avena*, but increased the decarboxylation of IAA in *Pisum*. Hertel and Leopold (1963) reported that a 10^{-6}M TIBA pretreatment of *Zea mays* caused a promotion of ^{14}C-IAA uptake and a subsequent decrease in the transport of label. Mullins (1970) also noted that TIBA did not inhibit the uptake of ^{14}C-IAA by petiole segments of bean but did inhibit the subsequent transport of the label.

Effects of Hormones on the transport of photoassimilates

The information on transport of hormones has obvious bearing on the effect of hormones on the translocation of photoassimilates. As mentioned earlier, however, there are very few studies on this subject.

Seth and Wareing (1964, 1967) applied hormone pastes to decapitated shoots or de-fruited peduncles of Phaseolus vulgaris plants prior to feeding ^{32}P to the node of the primary leaves. They found that GA (unspecified as to type) and kinetin at 1000 ppm each did not affect the acropetal transport of ^{32}P when applied singly. IAA (1000 ppm) alone augmented the transport but IAA applied together with GA and kinetin further augmented the transport of ^{32}P. The combination GA and IAA treatment did not elicit as much ^{32}P transport as IAA alone. The authors further noted that the movement of ^{14}C-labelled products was similarly stimulated by IAA and the synergistic action of the combined hormones. In Meteor pea also, Davies and Wareing (1965) noted that GA or kinetin, at a concentration of 0.1% in lanolin, were ineffective in stimulating acropetal transport of ^{32}P applied to nodes 10 cm below the cut stump. IAA at 0.1% concentration stimulated ^{32}P transport, and steam-girdling experiments verified the IAA effect to be phloem mediated. Use of 1% TIBA negated the IAA effect of ^{32}P transport in these experiments. Mullins (1970), using a similar experimental design for hormone and ^{14}C-sucrose feeding to bean plants, reported an increase of ^{14}C transport with combination IAA (1000 ppm) + GA (200 ppm) + benzyl-adenine (BA, 200 ppm) over IAA alone or control treatments. Morris and Thomas (1968) noted a similar enhancement of ^{14}C movement in peas after IAA treatment. Hew (1965) and Hew et al (1967) pretreated decapitated stumps of soybean with 5 ppm IAA and combination 5ppm IAA + 50 ppm GA for 30 m and subsequently fed $^{14}CO_2$ for 30 m to one primary leaf. They reported a 6% increase in ^{14}C transport from the leaves with the combination IAA + GA treatment relative to controls and the IAA treatment.

In some other experiments, GA alone has been reported to increase the transport of assimilates presumably by the well known action of GA in creating and/or enhancing metabolic sinks. Jeffcoat and Harris (1972) found that GA_3 alone promoted the translocation of $^{14}CO_2$ products in decapitated *Dianthus* flowering shoots. Halevy et al (1964) sprayed *Phaseolus vulgaris* plants at the 19 and 25 day stage of development with 3×10^{-4}M GA. Subsequently on the 28th day the primary leaves were fed ^{14}C-sucrose for 4 and 20 h and the percent of ^{14}C transported was calculated. They found that in GA treated plants after a 4 h incubation only 0.9% of the ^{14}C was translocated, as opposed to 3.4% in controls, but after a 20 h incubation the GA treated plants transported 8.6% of the fed label versus 4.5% in controls. Sampling sites included the treated and untreated primary leaves, the stem above and below the primary leaves, the second trifoliate and the roots. GA hastened translocation of ^{14}C compounds to upper stems and leaves and reduced transport to lower stems and roots. That 100 ppm GA created metabolic sinks for assimilate movement in *Vitis vinifera* was noted by Shindy and Weaver (1967). Quinlan and Weaver (1970) applied GA to one of a pair of shoots of *Vitis* for 48 h before administration of $^{14}CO_2$ to the opposite shoot of the pair. Incubation time with $^{14}CO_2$ was 24 h, and plants at the pre-bloom (i.e. two weeks before blossoming) stage of development were used. ^{14}C was translocated to the shoot sprayed with GA whereas in control no such movement was recorded. Further it was shown that the GA-induced and control profiles for distribution of ^{14}C products were markedly affected by darkening or defoliation of adjacent shoot parts.

It will be recalled that, in bean plants, labelled 2, 4-D supplied to a primary leaf moved up and down the stem including roots but not to the opposite primary leaf (Crafts, 1967), and that $^{14}C\text{-}GA_3$ applied to the primary leaf resulted in an accumulation of the label in the root and shoot apices but not in the opposite primary leaf (Zweig et al, 1961). Leonard et al (1968) induced the movement of ^{14}C-assimilate from a primary leaf of bean to the opposite leaf by pretreating the latter with 2,4-D. Extremely high concentrations of 2,4-D (10,000 ppm 2,4-D for 3 days in the dark) were required, however, and the radioautographs revealed ^{14}C activity in the 2,4-D treated leaf 2 days after the application of $^{14}CO_2$ to the other primary leaf.

The papers reviewed above indicate that hormones, particularly IAA and GA, affect translocation of photoassimilate but in nearly all these studies the hormones have been used in such a way that metabolic sinks have been created or augmented. The movement of ^{14}C or ^{32}P toward these sinks is not typical of assimilate movement in intact plants. Accordingly, in this thesis, it was considered necessary ~~to design~~ experiments which tested the effects of hormones on assimilate movement which more truly reflected the situation in intact plants. Since this approach was new and there was little pertinent information in the literature, a considerable length of time was spent in devising criteria for uniformity of plant growth and in determining the optimal environmental conditions as well as appropriate times and methods for feeding hormones and labelled sugars. Labelled sucrose rather than $^{14}CO_2$ was used. Preliminary experiments tested the effects of concentration and duration of hormone treatments as well as the site of hormone application on subsequent sucrose movement. Later experiments determined the effects of hormones on distribution of label in different parts of the plant and experiments with TIBA tested the specificity of IAA action in translocation phenomena. It was hoped that these experiments with hormones would shed some further light on the mechanism of long distance transport of photoassimilates.

Materials and Methods

Growth conditions

Seeds of *Phaseolus vulgaris* L. var Topcrop Stringless were germinated on moist filter paper in petri plates. After 3 to 4 days seedlings were selected for uniform growth and planted in vermiculite pots held on a defined photoperiod in a growth chamber under a balanced regime of fluorescent cool white and tungsten filament lamps. They were watered every 3 days with half-strength Hoagland-Arnon solution (Hoagland and Arnon, 1950) in which ferric nitrate substituted for iron tartrate. Photoperiod regimes for each experiment are indicated at the appropriate places but day/night temperatures of $23^{o}/20^{o}C$ were maintained until treatment time at which the second or third trifoliate leaflet was half-expanded. The time to reach this stage varied with daylength and duration of the maximal light intensity (see below) but generally took from 12 to 18 days.

Preliminary experiments indicated the optimal photoperiod and light intensity conditions for plant growth. For instance, a simulated natural daylength with lower intensities of 800 ft-c pre- and post-dating the midday period at 1200 ft-c was found to be superior to a 12-12 or 18-6 h photoperiod at a uniform 1200 ft-c. A lengthening of the midday period at 1200 ft-c from 6 to 11 h favoured more uniform plant growth.

Hormone treatment

Before each experiment, plants were selected for uniformity on the basis of trifoliate and/or primary leaf area criterion. Lanolin pastes containing growth regulators were applied to stem, petiole, or laminar sites for varying incubation times from 24 to 96 h under the photoperiod regime relevant to the experiment. Control plants received pastes of pure lanolin or no application of lanolin pastes prior to administration of isotopically labelled compounds.

Administration of radioactive sucrose

Radioactive sucrose was administered at the midpoint of the light cycle when, under natural conditions, translocation of photoassimilate is beginning to increase in the diurnal cycle. Preliminary tests indicated that high concentrations of isotopically-labelled substances, high light intensity during the feeding period, and longer feeding periods facilitated the uptake of label and its subsequent partitioning into labelled compounds in various metabolic pools. Accordingly, the hormone-treated and control plants were transferred to the laboratory and acclimatized to a light intensity of 3000 ft.c. provided by Miller-Sylvania photoflood (color temperature 3400 K) or Dicrolite (Quartz-Iodide, 3300 K) lamps having standardized spectral emissions. During this stabilization period and subsequent isotope administration near infra-red radiation intensities were reduced by interposing 10 cm water filters, allowing maintenance of 21 ± 2 C air temperatures for the plants.

Two methods were employed to administer labelled sucrose: flap feeding and scrape feeding. In the flap feeding method (Nelson and Gorham, 1957) the midrib vascular bundle of the middle leaflet of a trifoliate was severed and the cut stump immersed into the radioactive solution for the appropriate feeding time. In the scrape method the upper epidermis one cm from the midvein-petiole junction of the middle leaflet was removed with a scalpel. Direct application of compounds to the unwounded vascular bundle could then be done. A solution of boron (5ppm) and 0.08% Tween 20 (sodium lauryl sulfate) was used to augment entry of isotope following the methodology of

Nelson and Gorham (1957) and Yamaguchi (1961).

Sampling and determination of isotopic content

Tissue samples were extracted in hot 80% (v/v) ethanol 3 times and the volume was reduced by evaporation to a 2 ml aqueous solution. Aliquots of 200 µl were taken for triplicate 5 min counts in a modified Bray's Cocktail solution on a calibrated Packard Tri-Carb Scintillation Counter. This method proved adequate for non-color quenched samples from petioles and stems. For highly color-quenched laminar samples from the fed leaflets counting efficiency was low. In the last experiment addition of NCS solubilizer (New England Nuclear) was used to improve counting efficiency in these leaf samples.

Data analysis

For the first three experiments data are presented with deviations from the mean. For the last two experiments, which involved an increased use of sampling sites and a larger number of replicates, a more detailed statistical analysis was carried out. Two way analysis of variance and a Bartlett's test for homogeneity of the variances as well as the New Duncan's Multiple Range test were employed. The Bartlett's Test was chosen over the similar Neumann-Keuls Test since less stringent limits were established at higher degrees of freedom thus allowing a more open approach to comparison of treatments at the 1% level of significance (Alder and Roessler, 1968 and Li, 1966). Alternatively, sample count data expressed as percent of administered isotope levels and percent of total ethanol soluble fraction recovered as well as the arc sine transformation of these percentages were used for data analysis. The raw dpm and arc sine analyses provided only restricted information and did not permit comparison between treatments. For this reason $\log_e$ transformation of dpm was utilized in the grouped-site analysis. Profile distribution studies conducted by numerous workers have indicated that a logarithmic decrease in recoverable activity occurrs as distance from the site of isotope administration increases (Vernon and Aronoff, 1952; Biddulph and Cory, 1957; Whittle, 1971).

Experiments and Results

I. Influence of exogeneously applied hormones on net photosynthesis rates (P_n)

It was considered desirable to test whether hormone application to decapitated stem apex affected the photosynthetic rates (P_n) of adjacent leaflets. Accordingly, the following hormones were applied to the cut stem apex: 100 ppm IAA with 20 ppm GA and plain lanolin controls for 72 h, and 1000 ppm IAA for 96 h. For plants receiving hormones, a plain lanolin paste replaced the hormone paste during P_n determinations.

Following the methods of Lister, Krotkov and Nelson (1961), a 250 ml closed circuit system, at normal 300 ppm CO_2 concentrations, was used with a Beckman Model 315 Infra-Red Gas Analyzer (Figure 1) calibrated against standard gas mixtures.

The net photosynthetic rates in Table II are the means of six consecutive determinations in the rate of change of CO_2 concentrations between 350 and 250 ppm for each of the three replicate plants in each treatment. It should be noted that no appreciable differences appeared between the treated and control plants at this level of sensitivity within this analysis system.

FIGURE 1 - APPARATUS FOR NET PHOTOSYNTHETIC MEASUREMENTS

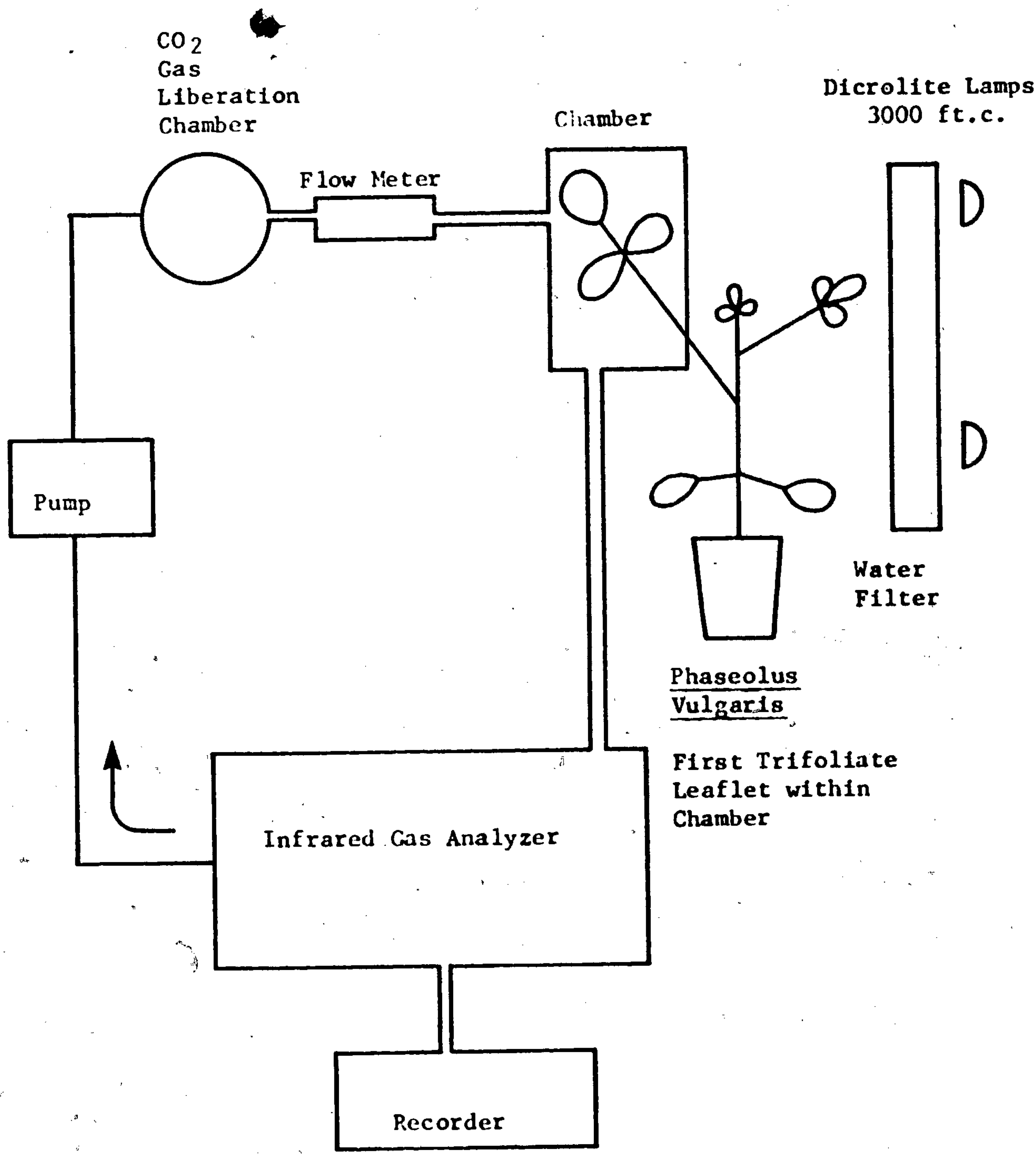
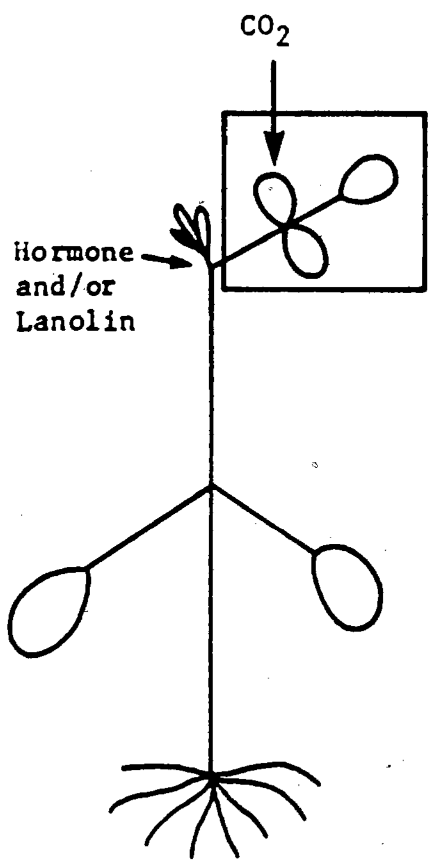


TABLE II - NET PHOTOSYNTHETIC RATE OF FIRST TRIFOLIATE LEAFLET AFTER HORMONE PRETREATMENT



	72 H PRETREATMENT		96 H PRETREATMENT WITH
	PLAIN LANOLIN CONTROL	100 ppm IAA + 20 ppm GA	1000 ppm IAA
AVERAGE CO_2 RATE (ppm/MIN.)	125	128	100
LEAF AREA (TOTAL GRID SQUARES)	160	147	137
AVERAGE CO_2/UNIT LEAF AREA $\frac{\text{(ppm/MIN.)}}{\text{(GRID UNIT)}}$	0.76	0.72	0.76
PERCENT STANDARD DEVIATION FROM MEAN	40.5%	50.0%	43.4%

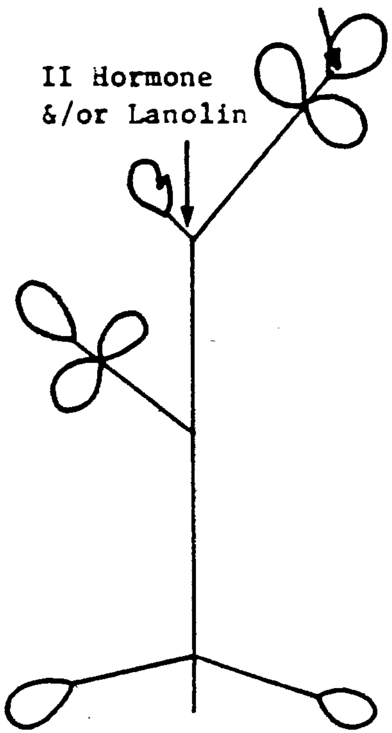
REPLICATE OF THREE PLANTS - = 6 TRIALS PER PLANT

2. Effect of site of hormone pretreatment on the distribution of flap-fed sucrose-^3H

Hormones were applied to decapitated stems above the third internode or to the petiolar stump of the second trifoliate middle leaflet. Gibberellic acid treatment of petiolar stumps resulted in some callus growth which was removed prior to sucrose feeding. In the case of stem applied hormones, sucrose administration was preceded by application of fresh pastes of plain lanolin to the decapitated stems. Sucrose-6,6'-^3H was administered to the second trifoliate middle leaflet for 1 h following the hormone pretreatment. Other experimental details are presented with the results in Table III.

Two trends are evident: 1. Pretreatment with IAA markedly increased the uptake of sucrose in the fed lamina and adjacent petiole and augmented the concentration of label in the second internode. This was evident from comparisons of dpm in the lanolin control and the 72 h IAA treatment at the petiole site (columns 4 and 5) and from an increase in the length of pretreatment with IAA from 24 to 72 h at the stem site (columns 1 and 3).
2. The site of hormone application had a marked effect on uptake and transport of label. If IAA was applied at the site of subsequent sucrose administration, more than ten times the dpm levels were obtained in extracts from the petiole and upper stem segments than in comparable samples from stem pretreatments (compare columns 3 and 5). The effect of GA in association with IAA was not very clear. It seemed to facilitate entry of label into the system and perhaps depressed the translocation of label into stem sites farther from the site of isotope feeding (compare dpm for rows 1 and 3 in columns 1 and 2 and columns 5 and 6 Table III).

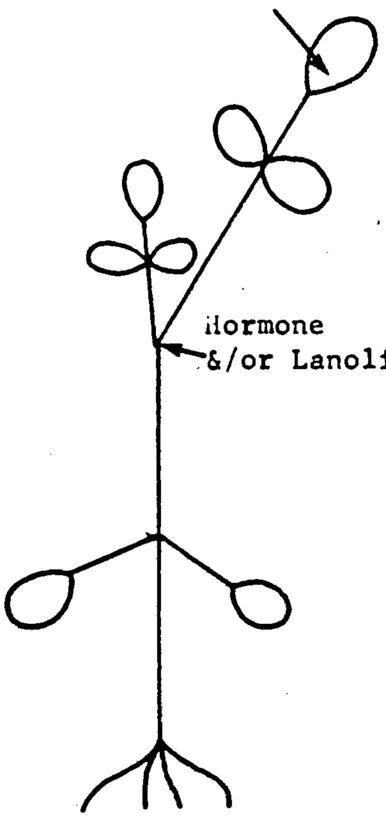
TABLE III - EFFECT OF SITE OF HORMONE PRE-TREATMENT (*dpm RECOVERED IN ETHANOL EXTRACTION) ON THE DISTRIBUTION OF FLAP-FED SUCROSE - ^{3}H



SAMPLING SITE	STEM: 24 H PRETREATMENT 100 ppm 1AA (1)	STEM: 24 H PRETREATMENT 100 ppm 1AA+ 20 ppm GA (2)	STEM: 72 H PRETREATMENT OF 100 ppm 1AA (3)	PETIOLE: 72 H PRETREATMENT PLAIN LANOLIN CONTROL (4)	PETIOLE: 72 H PRETREATMENT 100 ppm 1AA (5)	PETIOLE: 72 H PRETREATMENT 100 ppm 1AA+ 20 ppm GA (6)
2ND TRIFOLIATE PETIOLE	142	262	230	100	3127	3285
INTERNODE - 2ND TRIFOLIATE TO 1ST TRIFOLIATE	107	180	180	51	4263	2467
INTERNODE - 1ST TRIFOLIATE TO PRIMARY LEAVES	59	49	49	28	N/A	17

AVERAGE PERCENT VARIATION FROM THE MEAN dpm VALUE FOR THREE REPLICATES 43.0%

TABLE IV - EFFECT OF HORMONE CONCENTRATION AND DURATION OF HORMONE PRETREATMENT ON THE DISTRIBUTION OF FLAP-FED SUCROSE - ^{3}H (dpm FROM ETHANOL EXTRACTION)



SAMPLING SITE	72 H PRETREATMENT				96H PRETREATMENT OF
	PLAIN LANOLIN CONTROL (1)	100 ppm IAA (2)	100 ppm IAA+ 20 ppm GA (3)	1000ppm IAA+ 200 ppm GA (4)	1000ppm IAA (5)
1ST TRIFOLIATE PETIOLETTE (PI)	1954	1685	945	2059	1969
1ST TRIFOLIATE PETIOLE(PII)	2572	2034	1058	2282	1595
INTER-NODE 1ST TRIFOLIATE TO PRIMARY LEAVES	470	962	127	1096	2385
HYPOCOTYL PRIMARY LEAVES TO GROUND	22	113	105	750	549

Photoperiod 14H Light
1200 FT-C
10H Dark
Dicrolite Lamps - ^{3}H Admin.

AVERAGE PERCENT VARIATION FROM THE MEAN dpm VALUE FOR THREE REPLICATES 31.3% SEPARATE PI, PII, 4.9% SUMMED PI, PII SAMPLES

3. Effect of concentration and duration of hormone pretreatment on distribution of flap-fed sucrose-^{3}H

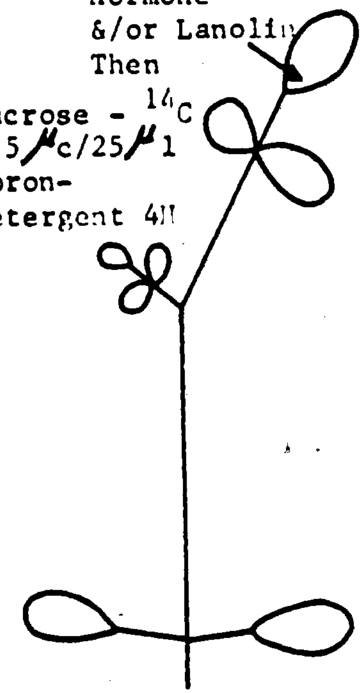
The experimental details and results are presented in Table IV. Treatment with IAA markedly increased the amount of recovered label in stem parts below the site of hormone application, and a longer treatment with IAA for 96 h rather than 72 h, further increased the amount of the label at these sites (compare internode and hypocotyl in columns 1, 2 and 5). The addition of GA at a concentration of 20 to 200 ppm to IAA did not further affect the distribution of label below the site of hormone application (compare column 2 with columns 3 and 4) except for one reading (column 4, row 4).

The amount of label in parts above the site of hormone application showed no clear trend either with respect to IAA concentration,duration of treatment, or addition of GA (compare columns 1-5, rows 1 and 2). These treatments are comparable with respect to site of hormone application and sucrose feeding with those in Table III (row 1, columns 1, 2, and 3) and seem to indicate that IAA or IAA + GA application does not affect the uptake and transport of sucrose above the site of hormone application in any significant way.

4. The influence of growth regulators on distribution profiles of scrape-fed sucrose- ^{14}C

Previous experiments using cut petiolar stumps had shown that a wound-repair response occurred as callus tissue was observed after pretreatments with hormones especially those involving gibberellic acid. They had also indicated the need for an increase in the number of sampling sites and the number of plants within each treatment. For these reasons, the scrape method (see Materials and Methods) for application of hormones and sucrose was adop-

TABLE V - DISTRIBUTION OF SCRAPE-FED SUCROSE - ^{14}C AFTER 48H PRETREATMENT WITH HORMONES * dpm



SAMPLING SITE	48 H PRETREATMENT				72H PRETREATMENT
	NO LANOLIN CONTROL (1)	PLAIN LANOLIN CONTROL (2)	1000 ppm IAA (3)	1000 ppm IAA+ 200 ppm GA (4)	OF 5ppm 2, 4-D (5)
1ST TRIFOLIATE: FED LEAFLET	13.30	9.48	12.15	13.90	11.60
LATERAL LEAFLETS	1.76	0.32	2.15	0.12	0.18
PETIOLETTE) PETIOLE)	1.08	1.36	0.77	0.67	1.01
HYPOCOTYL 1ST TRIFOLIATE TO PRIMARY LEAVES	0.25	0.20	0.37	0.21	0.25
% RECOVERY ISOTOPE FED IN ETOH ASSAY	16.89	11.31	15.44	14.90	13.04
% TRANS-PORTED ISOTOPE	3.09	1.89	3.29	1.00	1.45
% TRANS-PORTED/% RECOVERED	18.3	16.7	21.3	6.7	11.1%

MEAN dpm VALUE FOR EIGHT REPLICATES EXPRESSED AS PERCENTAGE OF ADMINISTERED ISOTOPE LEVELS ted, the number of replicates and sampling sites were increased, and in addition to IAA and GA, the synthetic auxin 2,4-D was used. Control plants received plain lanolin or no paste application prior to sucrose-^{14}C administration. After hormone pretreatment the pastes were removed and a boron-detergent solution of sucrose-^{14}C was administered to the scraped surface for 4 h. Eight plants were used in each treatment.

The mean dpm values for the eight plants in each treatment are presented as percentages of administered isotope levels in Table V. The control treatments of sucrose-^{14}C (column 1) and the plain lanolin with sucrose-^{14}C (column 2) illustrate the range of total recoverable label from 16.89% to 11.31%, respectively. There was a similarity between 1000 ppm IAA (column 3) and the sucrose control (column 1) in the percent of label translocated (3.29% and 3.09%, respectively) and in the movement of label to lateral leaflets of the treated leaf (1.76% for controls and 2.15% for IAA treatment. As expected, IAA (column 3) enhanced the basipetal transport of label over the lanolin control (column 2) but surprisingly, the synthetic auxin 2,4-D did not induce a similar increase: in fact, if anything, it depressed the total amount transported.

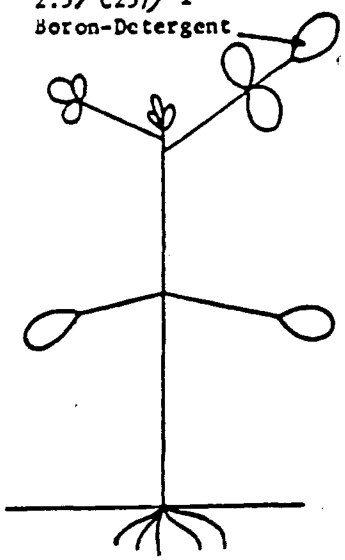
IAA and GA combination treatment (column 4) resulted in maximum retention of label in the fed leaflet and the least amount transported. This may be related to the callusing effect of GA and the creation of a metabolic sink in the fed leaflet.

5. Mediation of IAA and TIBA in isotope distribution from the sites of scrape-fed sucrose-^{14}C

The effect of IAA on the distribution of sucrose-^{14}C was further investigated with and without the use of 2,3,5 triiodobenzoic acid (TIBA) which acts

TABLE VI - MEDIATION OF IAA AND TIBA PRETREATMENTS IN ISOTOPE DISTRIBUTION FROM 'SCRAPE' ADMINISTERED - ^{14}C SUCROSE * - PERCENT OF ADMINISTERED ISOTOPE ACTIVITY

I Inhibitor or Lanolin
Hormone or Lanolin
Then II Sucrose - ^{14}C
2.5 μC 25 μl
Boron-Detergent



Photoperiod
3.5/11/3.5H
(800/1200/800 FT-C)+
6H Dark
Dicrolite Lamp-^{14}C
Admin.

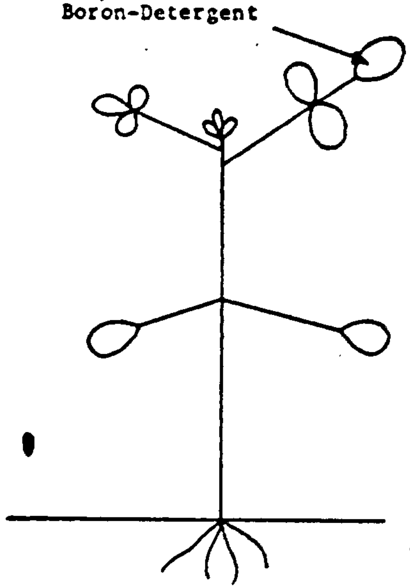
SAMPLING SITE	SUCROSE ^{14}C ADMINISTRATION					IAA ^{14}C ADMINISTRATION 4H PRETREATMENT HORMONE +/OR LANOLIN	
	NO PRETREATMENT	48H PRETREATMENT	52H PRETREATMENT 4H INHIBITOR 48H HORMONE OR LANOLIN				
		1000 ppm IAA	LANOLIN + LANOLIN	500 ppm TIBA + LANOLIN	500 ppm TIBA + 100 ppm IAA	LANOLIN	500 ppm TIBA
COLUMN	(1)	(2)	(3)	(4)	(5)	(6)	(7)
1ST TRIFOLIATE FED LEAF	64.50	30.05	33.57	17.80	19.60	32.72	44.35
1ST TRIFOLIATE LATERAL LEAFLETS	.02	.05	.02	.24	.02	.47	.10
PETIOLE	.46	1.84	.23	.29	.46	.83	.27
2ND TRIFOLIATE	.33	.45	.07	0.00	.09	.11	.19
APEX	.34	.46	.80	.10	.32	.11	.17
INTERNODE 1	.31	.96	.14	.14	.26	.59	.16
PRIMARY LEAVES	.02	.22	.03	.01	.02	.28	.11
INTERNODE 2	.47	.72	.32	.12	.43	1.57	.12
% TRANSPORTED (ETOH SOLUBLE)	1.95	4.19	1.60	.90	1.60	2.54	1.12
AMOUNT RECOVERED / AMOUNT ADMINISTERED	66.45	34.24	35.17	18.70	21.20	35.26	45.47

* MEAN VALUE FOR EIGHT REPLICATE PLANTS (i.e. SUCROSE - ^{14}C 4.8×10^6 dpm; IAA - ^{14}C 4.8×10^5 dpm)

TABLE VII - MEDIATION OF 1AA AND TIBA PRETREATMENTS IN ISOTOPE DISTRIBUTION FROM 'SCRAPE' ADMINISTERED - ^{14}C SUCROSE * - PERCENT OF ~~ADMINISTERED~~ RECOVERED ISOTOPE ACTIVITY

SUCROSE ^{14}C ADMINISTRATION

I Inhibitor or Lanolin
Hormone or Lanolin Then
II Sucrose - ^{14}C
2.5 μC/25 μl
Boron-Detergent



Photoperiod
3.5/11/3.5H
(800/1200/800 FT-C)+
Dicrolite Lamp - ^{14}C Admin.

SAMPLING SITE	NO PRETREATMENT	48H PRETREATMENT 1000 ppm 1AA	52H PRETREATMENT 4H INHIBITOR 48H HORMONE OR LANOLIN: LANOLIN + LANOLIN	52H PRETREATMENT: 500 ppm TIBA + LANOLIN	52H PRETREATMENT: 500 ppm TIBA + 100 ppm 1AA	1AA ^{14}C ADMINISTRATION 4H PRETREATMENT HORMONE +/OR LANOLIN: LANOLIN	1AA ^{14}C ADMINISTRATION: 500 ppm TIBA
COLUMN	(1)	(2)	(3)	(4)	(5)	(6)	(7)
1ST TRIFOLIATE FED LEAF	97.00	88.00	95.45	95.20	92.45	92.63	97.58
1ST TRIFOLIATE LATERAL LEAFLETS	.03	.15	.06	1.28	.09	1.33	.22
PETIOLE	.70	5.38	.65	1.55	2.17	2.35	.59
2ND TRIFOLIATE	.50	1.32	.20	.0	.42	.31	.42
APEX	.51	1.35	2.27	.53	1.51	.31	.37
INTERNODE 1	.47	2.80	.40	.75	1.23	1.67	.35
PRIMARY LEAVES	.03	.64	.09	.05	.09	.79	.124
INTERNODE 2	.71	2.10	.91	.64	2.03	.44	.26
% TRANSPORTED (ETOH SOLUBLE)	2.95	13.74	4.58	4.80	7.54	7.20	2.45

* MEAN VALUE FOR EIGHT REPLICATE PLANTS

as an inhibitor of the basipetal transport of IAA (see Literature review). Additional experiments determined the movement of sucrose after combination TIBA/IAA treatments. The dependence of sucrose transport on IAA transport per se and the mode of IAA action on the transport of assimilates could thus be investigated.

The schedule of treatments was as follows:

a. a 4/48/4 h application of

500 ppm TIBA + 1000 ppm IAA + sucrose-^{14}C

500 ppm TIBA + lanolin + sucrose-^{14}C

Lanolin + lanolin + sucrose-^{14}C

b. a 4/48 h application of

500 ppm TIBA + IAA-^{14}C

lanolin + IAA-^{14}C

c. a 48/4 application of

1000 ppm IAA + sucrose-^{14}C

d. a 4 h application of sucrose-^{14}C without any hormone pretreatment.

The sucrose solution was applied as a boron-detergent mixture to the leaf surface after removal of the lanolin paste.

The results are presented as percentages of administered (Table VI) and recovered (Table VII) isotope in the 80% aqueous ethanol-soluble fraction. Either of the two percentages is adequate for a comparison of isotope levels at different sites within a treatment, but for comparison of isotope levels at the same site between treatments it is better to use the percentage of recovered activity. This is necessary because the total activity recovered, after sucrose-^{14}C feeding and aqueous ethanol extraction varied from as low as 18.70% to as high as 66.45% (Table VI). To a large extent this variation in recovery seems to be due to the extraction procedure for highly colored leaf samples. Further discussion of the NCS solubilization step follows later.

Both on the basis of percent administered (Table VI) and percent recovered ethanol soluble label (Table VII), it seems clear that only small quantities of label were transported in the intact plant.

As shown in Table VII, IAA pretreatment at 1000 ppm led to an increased transport of label out of the fed leaflet to all the sampling sites (cf. different sites in column 2 with those in column 1). This movement was not only noticeable for the petiole and stem sites (internodes 1 and 2) but also for the shoot apex and the second trifoliate as well as the mature primary leaves. Although ^{14}C-IAA moved into the mature leaves and lateral leaflets, no similar distribution into the apex and second trifoliate segments was observed. This distribution could be explained by a passive diffusion between ^{14}C-IAA and the endogeneous IAA wherein the radioactivity would be mixed and transported basipetally at the primary leaf node. The subsequent distribution of ^{14}C-sucrose after IAA treatment (column 2) into the apex and second trifoliate, as well as the mature leaves and lateral leaflets could then be explained. TIBA treatments at 500 ppm had higher values of ^{14}C-sucrose label in petiole and the lateral leaflets the first trifoliate, but farther down the stem in the internodes and the primary leaves no marked difference in the amounts of label with the treatment and the comparable lanolin control (compare columns 3 and 4) was noticeable. However, TIBA seemed to depress the translocation of the ^{14}C-sucrose to the shoot apex and the second trifoliate (rows 4 and 5, columns 3 and 4). IAA treatment following the TIBA treatment improved the label count in the petiole and internodes 1 and 2 in comparison to both the TIBA treatment and lanolin control, and for the laterals of the first trifoliate, shoot apex and secondary trifoliate and primary leaves gave more or less the same profile as that for the lanolin control (compare column 5 with columns 3 and 4).

The effect of 500 ppm TIBA on the translocation of ^{14}C-IAA was very marked. TIBA not only inhibited the total amount of label translocated but particularly inhibited the ^{14}C-IAA movement into the petiole and stem segments but also into the laterals of the first trifoliate and the mature primary leaves. Only in respect to the shoot apex and the second trifoliate was there no marked differences between the TIBA treated and the lanolin control (compare columns 6 and 7, Table VII).

Further grouping of sampling sites into mainline (petiole, internodes 1 and 2), sink (second trifoliate, apex and lateral leaflets) and mature tissues (the primary leaves) led to further information on the physiological activity in the sampling sites the effects upon the distribution of isotopically-labelled compounds within the intact plant.

Statistical analysis of percent administered and percent recovered data for all sites did not reveal significant differences between treatments (columns 1 and 2, Table VIII), except that for percent recovered data (column 2) IAA stimulated sucrose-^{14}C transport to all sites. Since significant differences were not revealed by these two analyses, $\log_e$ transformation of the raw dpm data were utilized. Also, sites were grouped according to mainline, sink and mature tissues. This analysis gave significant differences at the 1% level between some sites and treatments (columns 3-5). For sink areas there was no distinction between control and IAA treatment patterns of sucrose ^{14}C distribution implying the predominance of a basipetal rather than apical influence of IAA (column 4). A change in the ^{14}C sucrose profile was

TABLE VIII - STATISTICAL ANALYSIS OF RECOVERY DATA

1. BARLETT'S TEST FOR HOMOGENEITY OF VARIANCES
2. NEW DUNCAN'S MULTIPLE RANGE TEST
* ONE WAY ANALYSIS OF VARIANCE

TREATMENTS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER

TREATMENTS	% ADMINISTERED (ALL SITES) (1)	% RECOVERED (ALL SITES) (2)	$\log_e$ RAW DPM MAIN LINE (3)	SINK (4)	MATURE * TISSUES (5)
ONLY SUCROSE - ^{14}C	bd	b	b	bc	b
IAA + SUCROSE - ^{14}C	bc	a	a	b	a
LANOLIN + LANOLIN + SUCROSE - ^{14}C	bd	b	b	bcd	a
TIBA + LANOLIN + SUCROSE - ^{14}C	ad	c	c	ef	b
TIBA + IAA + SUCROSE - ^{14}C	bd	b	b	cde	b
LANOLIN + IAA - ^{14}C	ad	c	c	de	a
TIBA + IAA - ^{14}C	ad	c	c	e	a

N.B. - ALL SITES - EXCLUSIVE OF FED LEAFLET; 'MAINLINE' = PETIOLE, STEM 1 + 2
'SINK' = SECOND TRIFOLIATE, APEX LATERAL LEAFLETS OF FIRST TRIFOLIATE
'MATURE TISSUES' = PRIMARY LEAVES

evident after TIBA + lanolin treatment relative to lanolin control, and furthermore IAA reversed TIBA action for the mainline and sink areas but not for the primary leaves (rows 5-7, columns 3-5). Despite the evident enhancement of basipetal sucrose-^{14}C movement by IAA, there was no distinction between the TIBA treatment and lanolin control with respect to the movement of IAA-^{14}C for any of the sampling sites (rows 6 and 7).

As previously mentioned, the amount of label recovered expressed as a percent of the label administered, following ethanol extraction, varied from 18.70-66.45% (Table VI). Percent recovery of label was improved markedly by NCS solubilization of the fed lamina samples as the ethanol extract-aqueous aliquot (Table IX). In addition, the range of variation between treatments was reduced to a low of 62.60% (column 2) and a high of 75.00% (column 1). These results suggest that a good deal of label activity was 'bound' and not available for counting. For sucrose-^{14}C studies this binding was particularly evident in the TIBA + lanolin and the TIBA + IAA treatments (columns 4 and 5). Treatments involving lanolin only (column 3) or IAA (column 2) also indicated substantial amounts of bound activity in relation to the 'no pretreatment' control (column 1). For IAA-^{14}C studies also there was substantial activity bound in the lanolin control (column 6). Lanolin application by itself (column 3) or with hormones (columns 2, 4, 5 and 6) may have something to do with the binding of the label, but this assumption was negated by the figures in column 7 where lanolin was used both for IAA and the subsequent IAA-^{14}C application and yet there was only marginal improvement in dpm following NCS solubilization procedures. It may be that in this particular case the 'unrecovered' activity was present in non-base soluble or ethanol insoluble complexes.

TABLE IX - ISOTOPE RECOVERY DATA WITH ETHANOL AND ETHANOL - NCS SOLUBILIZATION PROCEDURES (EXPRESSED AS PERCENT OF ADMINISTERED ISOTOPE LEVELS)*

	SUCROSE ^{14}C ADMINISTRATION					IAA ^{14}C ADMINISTRATION	
	NO PRETREATMENT	48H PRETREATMENT	52H PRETREATMENT 4H INHIBITOR 48H HORMONE OR LANOLIN			4H PRETREATMENT HORMONE +/OR LANOLIN	
		1000 ppm IAA	LANOLIN + LANOLIN	500 ppm TIBA + LANOLIN	500 ppm TIBA + 100 ppm IAA	LANOLIN	500 ppm TIBA
COLUMN	(1)	(2)	(3)	(4)	(5)	(6)	(7)
RECOVERY OF ACTIVITY IN ETHANOL FRACTION	66.45	34.24	35.17	18.70	21.20	35.26	45.47
RECOVERY AFTER ETHANOL AND NCS TREATMENT	75.00	62.60	74.60	67.50	72.50	43.64	46.32
QUANTITY OF LABEL FROM NCS ONLY	11.40	45.30	52.86	72.29	70.76	19.20	1.84

* MEAN OF EIGHT REPLICATE PLANTS

The relative distribution of label between the petiole and stem internodes 1 and 2 is presented in the form of histograms of dpm/g fresh wt (Figures 2-4). The bars for the petiole and internodes for each treatment are shown adjacent to each other and separate from similar bars for other treatments. In this presentation, a comparison between different treatments can be misleading without the numerical data of dpm and fresh weight (see Appendix 5 & 6). Hormones of the auxin-gibberellin type, as well as TIBA, are known to have strong secondary effects on growth and some of these effects are evident in data of fresh weight (see Appendix 5 & 6).

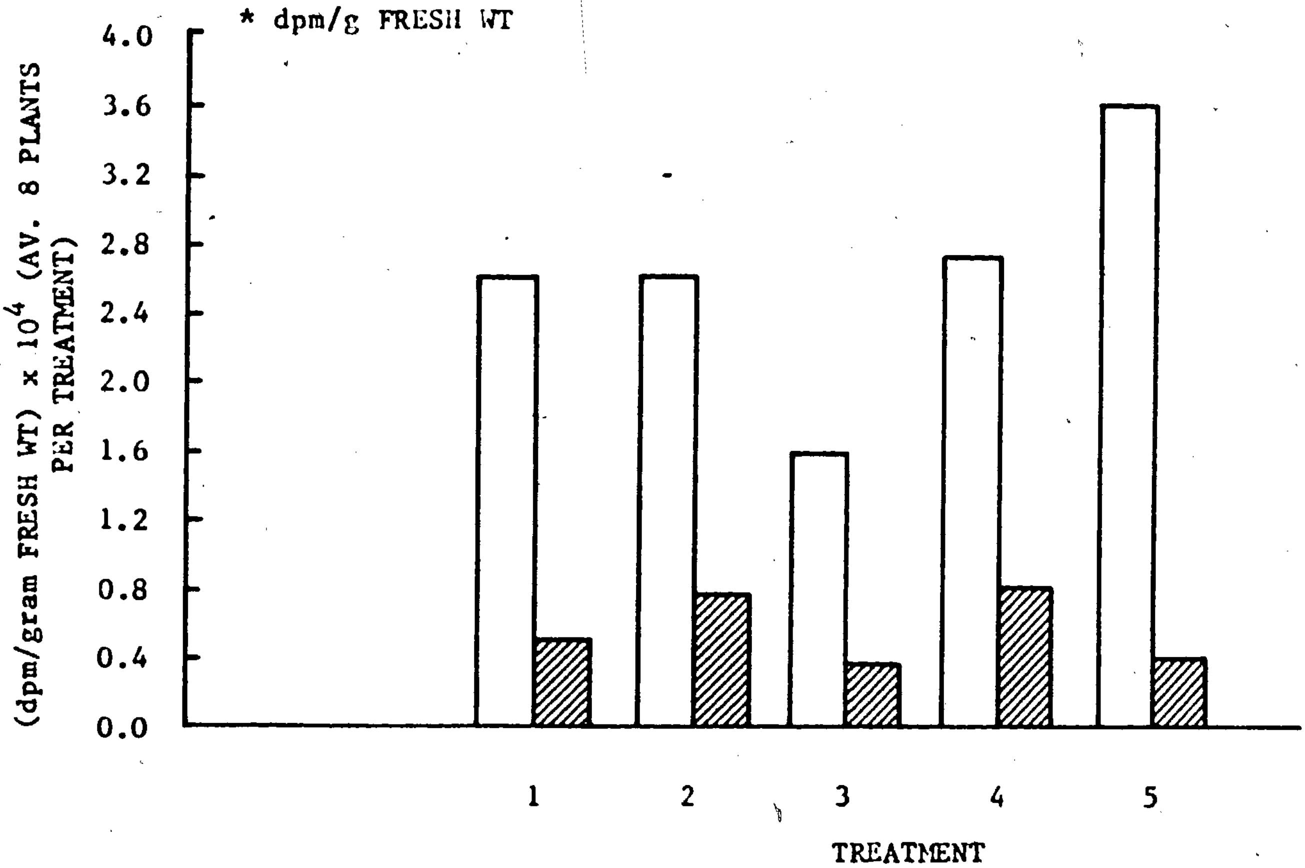
Strict comparisons of petiole/internode 1 ratios in different experiments are not possible. Generally the ratios varied from 2.05 to 5.71 for hormone and inhibitor treatments. A high petiole/internode 1 ratio indicates a low transport through the petiole into the more distant stem segments. The large variability extant in the control treatments, for instance, for lanolin-ratios of 8.62 (Figure 2) and 1.51 (Figure 3) and for the sucrose-^{14}C only ratios of 3.61 (Figure 2) and 0.85 (Figure 3) is not explained by a 'lanolin effect' and no immediate explanation is evident. Standardization of growth and experimental treatment regimes were maintained in both experiments. Generally, low transport ratios were shown by all treatments but plain lanolin + sucrose (Figure 2) and IAA + GA (Figure 2). TIBA treatments permitted less transport of labelled IAA-^{14}C into stem segments than controls receiving only lanolin and IAA-^{14}C. The marked stimulation of sucrose-^{14}C distribution into stem segments was demonstrated in IAA treatments (Figure 2).

A general reversal of ratio occurs for IAA-^{14}C profiles after lanolin or TIBA treatment when internode 1/internode 2 rather than the previous petiole/internode 1 ratios are examined (Figures 4). A similar reversal occurs for IAA-stimulated sucrose-^{14}C transport (bars 6 and 7, Figure 3). These examples

FIGURE 2 - HISTOGRAM DATA FOR ^{14}C TREATMENTS WITH SUCROSE

RATIO* PETIOLE / INTERNODE	5.28	2.31	5.71	3.61	8.62
TREATMENT NO.	1	2	3	4	5

* dpm/g FRESH WT



TREATMENT

1 2,4-D + SUCROSE - ^{14}C

2 IAA + SUCROSE - ^{14}C

3 IAA + GA + SUCROSE - ^{14}C

4 ONLY SUCROSE - ^{14}C

5 PLAIN LANOLIN + SUCROSE - ^{14}C

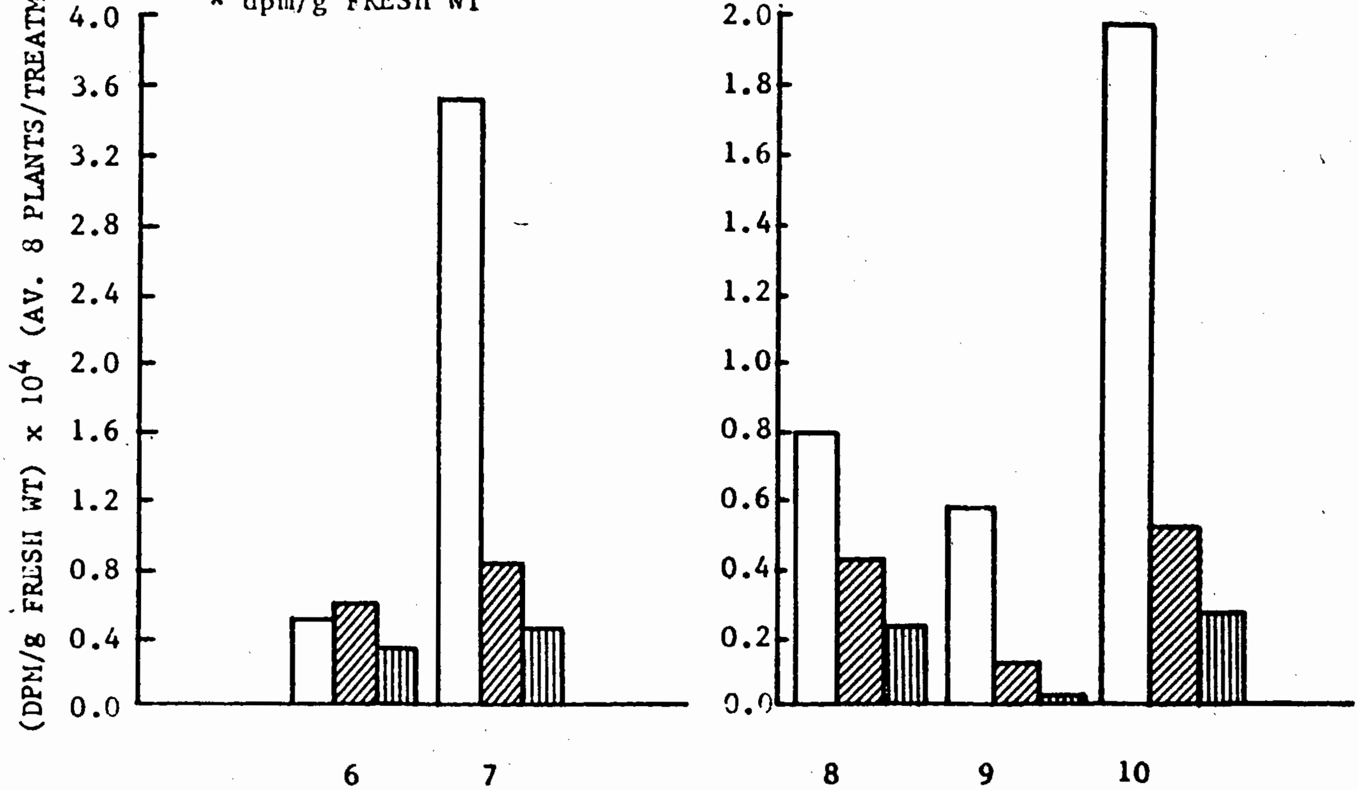
☐ PETIOLE

▨ INTERNODE I - 1ST TRIFOLIATE LEAF NODE TO PRIMARY LEAVES

FIGURE 3 - MEDIATION OF TIBA/IAA IN DISTRIBUTION OF ^{14}C SUCROSE - HISTOGRAM PRESENTATION SITE DISTINCTION IN dpm/g FRESH WT FOR 8 REPLICATE PLANTS WITHIN A TREATMENT

RATIO*					
PETIOLE / INTERNODE 1	0.85	4.00	1.51	4.42	3.98
INTERNODE 1 / INTERNODE 2	2.00	1.81	1.79	4.00	1.66
TREATMENT NO.	6	7	8	9	10

* dpm/g FRESH WT



TREATMENT NUMBER

TREATMENT

6 ONLY SUCROSE - ^{14}C
7 IAA + SUCROSE - ^{14}C
8 PLAIN LANOLIN + PLAIN LANOLIN + SUCROSE - ^{14}C
9 TIBA + PLAIN LANOLIN + SUCROSE - ^{14}C
10 TIBA + IAA + SUCROSE - ^{14}C

PETIOLE

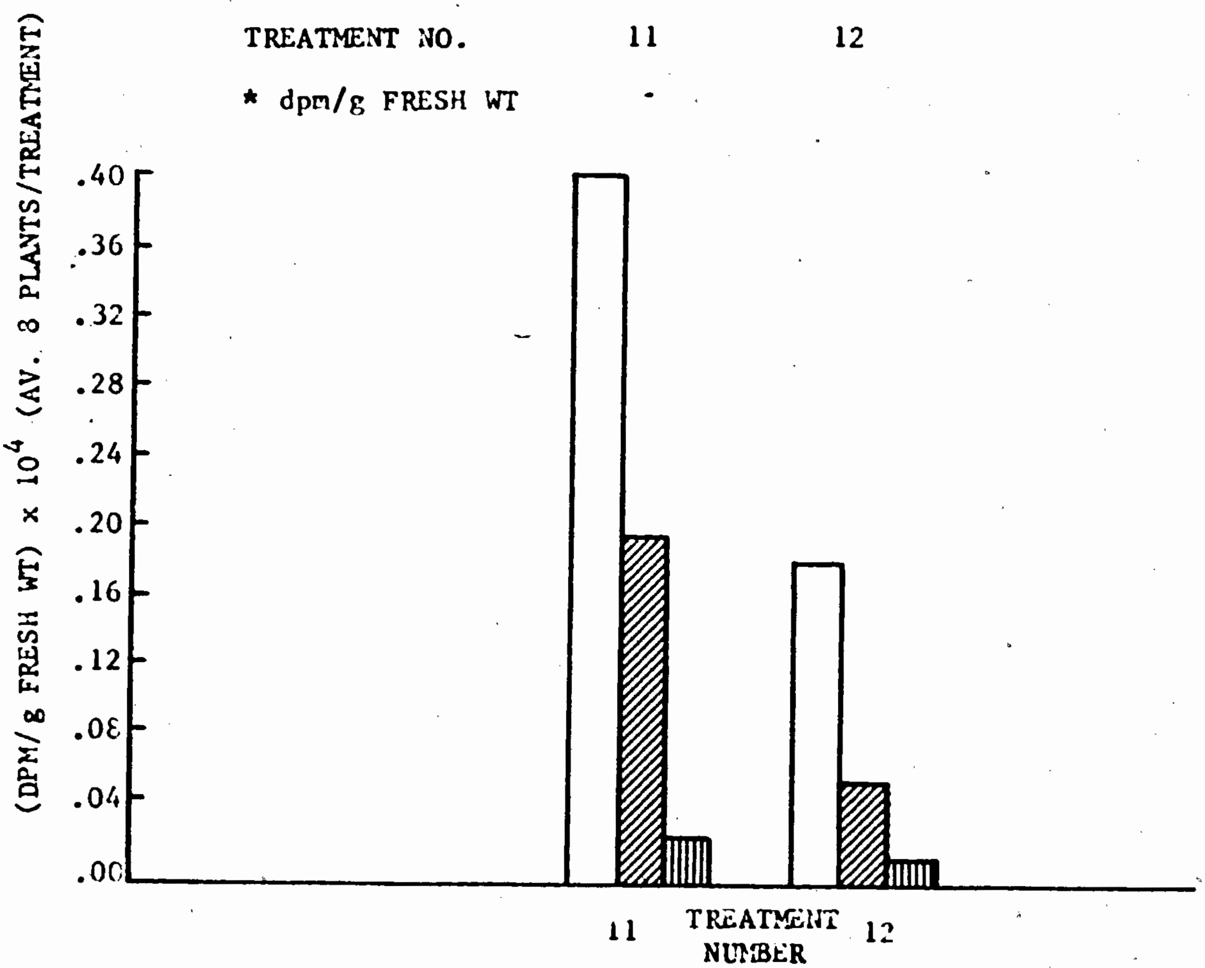
INTERNODE 1 - FIRST TRIFOLIATE LEAF NODE TO PRIMARY LEAVES

INTERNODE 2 - PRIMARY LEAVES TO GROUND LEVEL

FIGURE 4 - HISTOGRAM DATA FOR IAA - ^{14}C TREATMENTS SITE DISTINCTION IN dpm/g FRESH WT - FOR 8 REPLICATE PLANTS WITHIN A TREATMENT

RATIO*			
	PETIOLE / INTERNODE 1	2.05	3.40
	INTERNODE 1 / INTERNODE 2	9.5	5.00
	TREATMENT NO.	11	12

* dpm/g FRESH WT



TREATMENT

11 PLAIN LANOLIN + IAA - ^{14}C

12 TIBA + IAA - ^{14}C

PETIOLE

INTERNODE 1 - 1ST TRIFOLIATE LEAF NODE TO PRIMARY LEAVES

INTERNODE 2 - PRIMARY LEAVES TO GROUND LEVEL demonstrate a low transport initially followed by high transport in the lower stem areas. This effect would be expected for TIBA-inhibited IAA-^{14}C movement as TIBA influence seemed to markedly decline with increasing distance from the site of administration. Isotope levels in the internode 2 segment would then make the ratio larger. The low initial transport of sucrose-^{14}C with IAA is indicative of the large increase in label in stem regions of the internode 2 area.

Discussion

An attempt was made in this thesis to study the effects of selected hormones on translocation of labelled sucrose and translocation of ^{14}C-IAA in intact bean plants. In contrast to the work with isolated stem or petiolar segments and donor-receiver agar blocks, which has the advantage of quick analysis under rigorously controlled experimental conditions, the use of intact plants, despite careful attention to the uniformity of seed source and growth conditions, introduces a large inherent variability factor. In my experiments there were often large variations between individual plants of a particular treatment which precluded statistical analysis and strict comparisons between different experiments. Consequently, the results should be taken more as indicative of trends rather than as absolute values. Different methods were used for data expression— dpm values, percent of radioactivity administered, percent of the ethanol soluble activity recovered, and histograms of dpm/gm fresh wt—which graphically illustrate the trends. These trends are discussed in the following pages, supplemented whereever possible by statistical analyses.

Among the hormones tested, the natural auxin IAA definitely promoted the basipetal transport of labelled sucrose (Tables II - VIII). The combination of IAA + with GA did not enhance the transport of the label more than IAA or control treatments, and in fact, resulted in a greater retention of the label in the fed leaf (Table V). These results are in contrast to those reported by Wareing and his associates (Davies and Wareing, 1965; Seth and Wareing, 1964, 1967) and Mullins (1970) where IAA and GA in combination with kinetin or benzyl-adenine were reported to enhance the translocation of ^{14}C or ^{32}P over IAA or control treatments. They are also in contrast to those reported by Hew (1965) and Hew et al (1967) where continued IAA+ GA treatments resulted in greater translocation of ^{14}C than by IAA alone. The explanation seems to be that all the above-named authors applied the hormones to the decapitated stem apex and the label to the basal primary leaf. A condition similar to the metabolic sink was thus created at the stem apex and the label moved acropetally to the sink. Both GA and cytokinins, such as kinetin and benzyl-adenine, are known to induce cell divisions and growth (see Cleland and Burstrom, 1961; Kende, 1971), and Shindy and Weaver (1967) and Quinlan and Weaver (1970) have shown that GA alone can cause ^{14}C assimilate movement in grape vine presumably by creating metabolic sinks. In my experiments also the higher retention of labelled sucrose in the fed leaf following IAA + GA treatment in comparison to in IAA or control treatments is probably due to this GA effect. What is striking is that in the experiments of Wareing and associates and Mullins referred to above GA or cytokinins applied singly did not, whereas IAA applied alone did promote the acropetal transport of label. From these and my experiments, therefore, it seems that IAA promotes both basipetal and acropetal transport of label but that the acropetal transport is further enhanced by a synergistic action of GA and/or cytokinins.

My results further indicate that the site of application of IAA as well as the concentration of hormone and duration of treatment have an effect on the translocation of labelled sucrose. As shown in experiment 2, if IAA is applied at the same site as the subsequent application of sucrose, in this case the petiolar stump of the second trifoliate, far more label is translocated basipetally than if IAA is applied to the decapitated stem apex and the sucrose is applied to the second trifoliate. Furthermore, both experiments 2 and 3 show that the movement of the label in sites topographically above the site of the hormone application is unaffected by the hormone. Although strict controls are lacking, these two experiments further suggest that an increase in IAA concentration from 100 to 1000 ppm and duration of treatment from 24 to 72 or 72 to 96 h have a further promotive effect on transport of label below the site of hormone application (compare columns 1 and 3 in Table III and 2 and 5 in Table IV).

In the literature it has been reported that a one order of magnitude increase in concentration of 2,4-D induced greater growth of plant tissue than a four-fold increase in IAA when concentrations ranged between 0.01-0.10 mg/l and 0.001-10.00 mg/l lanolin, respectively (Crafts, 1961). Eames (1950) also reported a proliferation of phloem parenchyma with high doses of 2,4-D; and Leonard et al (1968) were able to induce movement of ^{14}C assimilate from a primary leaf of bean to the opposite primary leaf by pretreating the latter with 10,000 ppm 2,4-D. In my experiments 2,4-D had little or no effect on transport of labelled sucrose; if anything, it depressed the total amount transported (Table V). Davies and Wareing (1968) also noted the lack of 2,4-D effect on acropetal transport of ^{14}C in pea plants. Because of the known low velocity of 2,4-D movement (see Literature review) and the possible herbicidal damage caused by large concentrations, only 5 ppm 2,4-D was used in my experiments but the incubation time was increased to 72 h as opposed to 48 h for IAA. It is possible that this concentration was too low to have any effect on basipetal transport of the label. It is also possible that some of the applied 2,4-D was metabolized for Fang et al (1951) reported a loss of 17.5% activity from ^{131}I-labelled 2,4-D after a 3 day period.

As analysed in detail earlier (Table VI and VII), IAA at 1000 ppm significantly enhanced the total amount of ^{14}C-sucrose translocated from the fed leaf with it being evident most in mainline segments of petiole and stem. In contrast the translocation of label to the sink areas, particularly shoot apex, was not statistically different from the two controls. For translocation of label to the mature primary leaves the IAA treatment differed significantly from one control but not the other. It is well known that shoot apices and young leaves are rich sources of endogeneous auxins and that the transport of IAA in intact plants as well as isolated segments is strongly polar and basipetal (see Literature review). It appears, therefore, that IAA stimulates some process or processes in long distance transport of the photoassimilate. That the translocation to sink areas remained unaffected under IAA treatment is probably due to the fact that these areas are themselves rich in endogeneous auxin.

To further ascertain that IAA is involved in transport of photoassimilate, the transport of ^{14}C-sucrose under the influence of IAA with and without the use of TIBA was studied. A 4 h application of 500 ppm TIBA, 48 h before the application of ^{14}C- sucrose, significantly altered the distribution of the label to all 3 sites-- the mainline, sink and mature regions-- in respect to lanolin control (Table VIII; see Results for details). A 48 h application of 1000 ppm IAA following the TIBA application, however, restored the distribution seen in the lanolin control, except for the mature tissues (Table VII). This similarity between the lanolin control and the TIBA + IAA treatment in the distribution of ^{14}C activity implies a 'neutralization' role for the exogeneous IAA on the inhibitory action of the TIBA. Table VI further revealed a higher recovery value for ^{14}C activity in the mainline sites for TIBA + IAA treatment than for TIBA or lanolin control and furthermore that the recovery was proportionally higher in the more distant mainline sites, namely internodes 1 and 2, than in the petiole (see also bars 8-10, Figure 3). This may be due to a higher mobility of IAA than for TIBA such that in the lower mainline sites not the inhibitory action of TIBA but only the stimulatory effect of IAA was observed. Interestingly, lateral leaflets of the first trifoliate received more activity after TIBA treatment than in the lanolin controls. If auxin stimulates the distribution of sucrose-^{14}C and TIBA inhibits basipetal auxin distribution how can the lateral leaflets receive higher activity levels than the control? As reviewed by Goldsmith (1969) the weak auxin action of TIBA may explain this apparent anomoly. TIBA apparently abolishes the basipetal and enhances the acropetal transport of auxin.

Transport of ^{14}C-IAA under the influence of TIBA provided further information on the role of auxin in transport processes. TIBA already had a strong inhibitory action on the total mobility as well as the basipetal transport of ^{14}C-IAA (columns 6 and 7, Table VII). For total mobility an inhibition of nearly 65.8% and for mainline transport an inhibition of 70.9% was observed. These values are comparable to though not as high as the 100% inhibition noted my Mullins (1970) for the basipetal translocation of ^{14}C-IAA by 500 ppm TIBA. In contrast to the basipetal movement, the transport of ^{14}C-IAA to the second trifoliate and shoot apex was not inhibited by TIBA. It is possible that as a result of TIBA inhibition of auxin transport, the apical sites are induced to synthesise more auxin. This augmented auxin activity in the apical regions would explain an increased ^{14}C-sucrose transport to the shoot apex under TIBA treatment. It would also explain the presence of ^{14}C-IAA in the shoot apex and second trifoliate as the high levels of exogenous auxins in these regions may be able to exchange with the exogeneously supplied ^{14}C-IAA over the short distances involved. Although the above mentioned conclusions follow from an analysis of Table VII, a statistical analysis of the recovery data revealed no significant differences at the 1% level between the ^{14}C-IAA transport in the lanolin control and TIBA treated material (Table VIII). This lack of statistical confirmation was probably due to the very high variability between individual plants and the small sample size.

How does IAA promote the translocation of photoassimilates? There is no precise answer to this question because, despite extensive investigations, the primary target and mode of action of IAA remain completely unknown. Among the various suggestions in the literature, IAA has been reported to affect gene activation (Moreland, 1967), RNA metabolism (Trewavas, 1968 a, b,), protein and polysaccharide synthesis (Key and Ingle, 1968: Abdul-Baki and Ray, 1970,) and increased respiration rates in the presence of metabolic substrates (Cleland, 1961). Arisz (1969) distinguished between the transport of exogeneous and endogeneous auxins and suggested, after investigations of electrical potential and concentration gradients, that an active membrane was not involved but that energy-integrated relationships with the endoplasmic-reticulum-plasmodesmatal complexes were necessary. Hager et al(1971) suggested that auxin acted co-operatively with GTP or ITP (guanine tri phosphate or inosine tri-phosphate) as an effector of a membrane-bound anisotropic ATPase or a proton pump. Still others have suggested that auxin increased membrane permeability by altering 'pore sizes' (Ursino, Fensom and Nelson, 1964). Suggestions on IAA action on translocation of photoassimilate are rare. Bidwell et al (1968) suggested that IAA stimulated turnover of the Calvin cycle intermediates, and Hew (1965) reported altered photosynthetic rates and increased sucrose movement in soybeans as a result of 100 ppm IAA treatment. Bowen and Wareing (1971) noted that the amounts of IAA and some synthetic auxins travelling down the stem could be related to the quantities of metabolites moved upwards. Finally, Mullins (1970) reported that auxin may affect translocation through a growth senescence effect or 'by direct action on a translocation regulatory centre'.

From these various suggestions, it seems that IAA may stimulate basipetal transport of assimilate by increasing the photosynthetic rates in the fed leaf thereby augmenting the source. This was not evident in my investigations of photosynthetic rates after IAA pretreatment. It may also increase the respiratory rate as well as membrane permeability at the feeding site and/or along the translocation pathway, thus stimulating the loading and unloading phenomena which require energy. If IAA acts in any or all of the above ways its action would be primarily centered in parenchyma cells of the transport system and would not negate the pressure flow hypothesis of long distance transport. Alternatively, IAA may have a more direct effect on the sieve tube protoplast and the enzymes located on sieve tube membranes especially along the sieve plates. If IAA acts at this latter site some form of activated diffusion seems the more likely explanation for phloem transport.

SUMMARY

1. The wound-repair response induced by 'flap-feeding' was circumvented with the 'scrape' administration of radioactive compounds to the first trifoliate leaflets of the intact plant.
2. A variation in net photosynthetic rate (P_n) after hormone pretreatment was not evident.
3. Both the site and duration of hormone pretreatment with IAA affected the distribution of labelled sucrose.
4. IAA, at 100 and 1000 ppm concentrations, augmented the distribution of labelled sucrose but the synthetic auxin, 2,4-D, and combination IAA + GA treatments at lower concentrations did not stimulate transport of label from the fed leaf.
5. The distribution profile for ^{14}C-IAA was established for mainline and sink sites along the transport pathway within the intact plant.
6. Even though the pathway, velocity and mechanism of ^{14}C-IAA transport seems to be different from that of ^{14}C-sucrose, IAA does seem to have a specific effect on the translocation of sucrose.
7. TIBA + lanolin provided maximal inhibition of 65% of auxin basipetal transport relative to the slightly stimulatory action induced by the TIBA + IAA treatments over the contols. In the latter case an apparent 'neutralization' of TIBA inhibition of labelled sucrose movement by the exogeneous IAA supply allowed the endogeneous auxins to stimulate label distribution above levels in the controls.
8. The relationship of 'immobile' to transported activity after ethanol-NCS solubilization procedures varied only slightly between the treatments involved with sucrose- ^{14}C. Values ranged from 62 to 75% for sucrose-

^{14}C recovery while ^{14}C-IAA values remained at 45% of the label translocated. The actual amount of label transported from the fed leaf relative to the quantities of isotope administered varied from 2.93 to 12.25% for sucrose- ^{14}C and from 2.46 to 7.20% for IAA- ^{14}C. In the latter case, formation of IAA-conjugate compounds that may be physiologically inactive could contribute to the 'immobile' phase.

9. Interpretation of data from histogram presentation of dpm/g fresh wt should be conducted with caution. Information on the actual weights and average dpm should be available when petiole/internode 1 and internode 1/internode 2 rations are evaluated for the effects of hormones in transport phenomena.

10. The mediation of growth regulators and inhibitors provides a tool for further investigations of transport phenomena.

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CONTENTS OF THE APPENDIX

1. Specific activity and source of radio-isotopes
2. Raw data as disintegrations per minute from ethanol extractions of tissue samples in experiment 4
3. Raw data as disintegrations per minute from ethanol extractions of tissue samples in experiment 5
4. Fortran programs used for data analysis:
    a. Two way analysis of variance
    b. Bartlett's test for homogeneity of variances
    c. One way analysis of variance
5. Data for histograms as dpm/g fresh wt for experiment 4 data of ^{14}C-sucrose distribution
6. Data for histograms as dpm/g fresh wt for experiment 5 data of ^{14}C-sucrose and ^{14}C-IAA distribution

APPENDIX 1

Specific Activity & Source of Radio-isotopes

	Specific Activity	Source
Sucrose-6,6'T	770 mC/mmol	Amersham-Searle
Sucrose-^{14}C	600 mC/mmol* I	Amersham-Searle
IAA-^{14}C	52 mC/mmol*	Amersham-Searle

NCS tm Amersham Searle - a quaternary ammonium base in toluene

* equivalent to 294 μC/mg as 3-indolyl (acetic acid -2 - ^{14}C)

* equivalent to 1.67 mC/mg or 80% isotopic abundance in all carbon atoms in this uniformly labelled sample

APPENDIX 2

Raw Data (disintegrations per minute) from Ethanol extractions of tissue samples in experiment 4

SAMPLING SITES		TREATMENTS 24D+ SUCROSE ^{14}C	IAA+ SUCROSE ^{14}C	IAA+GA+ SUCROSE ^{14}C	ONLY SUCROSE ^{14}C	LANOLIN SUCROSE ^{14}C
fed leaf	1	56404	48391	74083	65461	24042
(IST)	2	48619	54202	62117	120966	54524
	3	52311	52710	71237	43349	54500
	4	65643	81967	81344	24	53077
	5	56039	55382	43918	70128	52838
	6	66426	62980	68590	67407	42646
	7	56346	69889	84024	56358	35876
	8	45454	47017	49278	95471	43032
lateral	1	414	2093	56	3257	786
leaflets	2	48	70937	38	107	781
(IST)	3	107	502	45	309	512
	4	1074	121	229	1548	106
	5	2344	1126	116	58054	2370
	6	266	476	1838	3812	4191
	7	1038	1119	116	647	2224
	8	1657	1221	2098	563	2334
Petiole	1	2241	6708	4173	9645	3307
	2	2708	3252	2887	2475	2565
	3	1670	3900	1056	1241	3702
	4	3010	5163	920	2872	753
	5	6973	1548	583	8599	5807
	6	6091	2273	4795	8542	9349
	7	7374	1604	2649	3841	8782
	8	8914	4958	8664	4334	17884
Stem 1	1	1041	1858	1143	1623	617
	2	1004	606	863	385	596
	3	608	1540	189	226	409
	4	1404	571	428	1088	281
	5	1155	322	177	1934	1250
	6	902	7571	2162	1697	1364
	7	1262	938	1239	959	1087
	8	2125	524	1889	1554	1796

ADMINISTERED SUCROSE ^{14}C (a) 4.8×10^6 dpm LEVEL

APPENDIX 3 - Raw data (disintegrations per minute) from ethanol extractions of tissue samples in experiment 5.

SAMPLING SITE		TREATMENT TIBA + IAA ^{14}C	PL LAN + IAA ^{14}C	IAA + SUCROSE ^{14}C	ONLY SUCROSE ^{14}C	LAN + LAN + SUCROSE ^{14}C	TIBA + LAN + SUCROSE ^{14}C	TIBA + IAA + SUCROSE ^{14}C
Petiole (IST)	1	124	500	9644	1297	1090	170	5789
	2	230	62	12899	403	696	7437	1136
	3	220	558	6734	92	1072	304	4312
	4	138	700	9579	2241	943	1761	4830
	5	134	1236	7034	1221	1073	11	16924
	6	885	421	11136	878	2606	20	4085
	7	343	174	11500	1728	946	315	372
	8	313	1139	1700	1760	365	1146	3964
Stem 1	1	83	372	1834	316	652	83	1775
	2	133	3	1663	280	1700	1176	577
	3	178	1677	2300	763	273	290	678
	4	33	337	2451	2216	434	675	1373
	5	114	299	2229	1207	1097	5	3109
	6	143	195	4550	1541	3019	15	1081
	7	41	142	1850	2594	141	135	171
	8	160	221	503	2696	492	325	1321
Stem 2	1	42	219	8401	1909	779	128	2744
	2	64	3	1405	410	3210	242	448
	3	76	103	4850	1004	270	53	1762
	4	103	240	2977	3389	1519	1111	1272
	5	56	145	1982	1924	1866	9	6740
	6	74	86	3632	2084	2725	0	1364
	7	22	37	3570	3331	1337	419	333
	8	65	73	546	4041	441	419	1895

APPENDIX 3 - (Continued) (2)

SAMPLING SITE		TREATMENT TIBA + IAA ^{14}C	PL LAN + IAA ^{14}C	IAA + SUCROSE ^{14}C	ONLY SUCROSE ^{14}C	LAN + LAN + SUCROSE ^{14}C	TIBA + LAN + SUCROSE ^{14}C	TIBA + IAA + SUCROSE ^{14}C
Lateral Leaflets (IST)	1	5	107	69	20	42	130	27
	2	43	0	459	131	12	108	9
	3	103	249	42	66	35	530	43
	4	88	992	105	40	59	16	13
	5	265	918	39	34	274	95	129
	6	14	460	67	16	30	0	127
	7	44	16	1116	58	64	8529	261
	8	4	21	607	330	40	9	18
2nd Tri-foliate	1	52	143	3	143	21	93	435
	2	63	4	5154	496	9	28	0
	3	656	59	28	2011	95	32	12
	4	29	188	2319	64	5	0	5
	5	200	73	1441	393	1260	0	38
	6	61	113	6585	1289	190	0	96
	7	18	28	2248	6230	23	0	227
	8	31	7	206	1559	836	0	2670
apex	1	14	64	1639	1656	1190	127	2866
	2	31	5	2183	366	3450	903	157
	3	102	180	401	1031	1480	41	549
	4	61	78	2148	3022	866	1611	2195
	5	66	75	1911	838	4300	13	2267
	6	93	86	6941	1886	16010	61	627
	7	23	77	2183	4100	2101	398	25
	3	631	51	61	126	1343	398	3926

APPENDIX 3 - (Continued) (3)

SAMPLING SITE		TREATMENT TIBA + IAA ^{14}C	PL LAN + IAA ^{14}C	IAA + SUCROSE ^{14}C	ONLY SUCROSE ^{14}C	LAN + LAN + SUCROSE ^{14}C	TIBA + LAN + SUCROSE ^{14}C	TIBA + IAA + SUCROSE ^{14}C
Fed Leaf (IST)	1	34947	56179	136785	34203	153474	32762	292031
	2	31974	180	236964	39597	180904	208909	30842
	3	54181	13407	129937	38099	166762	34495	30140
	4	19483	30949	193317	28387	174000	34664	34450
	5	21792	30705	36508	36734	154630	38636	277079
	6	53444	11627	28310	31163	27729[illegible]	37064	23478
	7	5082	10168	230700	35389	153624	34988	36686
	8	39370	32575	154000	5765	38698	260353	29758
Primary Lvs.	1	45	37	175	242	126	152	27
	2	66	20	6626	37	67	31	3
	3	35	35	181	10	139	140	13
	4	58	493	57	48	123	27	1
	5	136	118	89	0	558	8	62
	6	107	800	229	8	43	21	124
	7	6	74	356	11	46	9	17
	8	141	38	414	142	13	0	353

APPENDIX 4

Fortran programs used for data analysis:

a. Two way analysis of variance

```
 1.Ø1 E
Ø1.Ø5 A ?ROWS  , COLUMNS  , REPS.  ?,!,"EST. GRAND MEAN"EM,!
Ø1.1Ø *;F J=1,RO; T %2,!"ROW"J;D 2:S SR=SR+RS(J)+2
Ø1.12 F K=1,CO;S SC=SC+CS(K)+2
Ø1.15 S GM=GS/(RO*CO*RE);S CI=GS+2/(RO*CO*RE)
Ø1.18 S RC=SR/(CO*RE)+SC/(RO*RE)
Ø1.2Ø T !!,"ANALYSIS OF VARIANCE TABLE:",!!!
Ø1.25 S ST=G2-CT;S SR=SR/(CO*RE)-CT;S SC=SC/(RO*RE)-CT
Ø1.3Ø S SE=G2-(SI/RE);S SI=(SI/RE)+CT-RC
Ø1.35 T "    VARIATION          DF              SS                 MS",!!
Ø1.4Ø T % ,"ROWS:              "RO-1,%,"     "SR,"     "SR/(RO-1),!!
Ø1.5Ø T %4,"COLUMNS:           "CO-1,%,"     "SC,"     "SC/(CO-1),!!
Ø1.6Ø T %4,"INTERACTION:    "(RO-1)*(CO-1),%,"     "SI
Ø1.65 T "    "SI/((RO-1)*(CO-1)),!!
Ø1.7Ø T %4,"ERROR:            "RO*CO*(RE-1),%,'''  "SE
Ø1.75 T "    "SE/(RO*CO*(RE-1)),!!
Ø1.8Ø T %4,"TOTAL:'           "RO*CO*RE-1,%,"     "ST,!!
Ø1.85 T "GRAND MEAN" GM,!!;*;Q

Ø2.Ø5 F K=1,CO;T !," COLUMN"K,!;D 3;S CS(K)=CS(K)+X

Ø3.Ø5 S X=Ø;S X2=Ø
Ø3.1Ø F P=1,RE;A S;S X=X+(S-EM);S X2=X2+(S-EM)+2
Ø3.15 S RS(J)=RS(J)+X;S SI=SI+X+2
03.20 S GS=GS+X;S G2=G2+X2
```

b. Bartlett's test for homogeneity of variances

```
Ø1.Ø1 E
Ø1.Ø5 A ?ROWS ,COLUMNS ,REPS. ?,!,"EST. GRAND MEAN"EM,!
Ø1.1Ø *;F J=1,RO;T %2,!,"ROW"J;D 2
Ø1.12 D 4
Ø1.9Ø *;Q

Ø2.Ø5 F K=1,CO;T !,X2," COLUMN" , ;  DS


Ø3.Ø5 S X=Ø;S X2=Ø
Ø3.1Ø F P=1,RE;A S;S X=X+(S-EM);S X2=X2+(S-EM)+2
Ø3.15 S BA=X2-(X+2/RE);S SP=SP+BA;S S2=S2+(FLOG(BA/(RE-1))*(RE-1))
Ø3.2Ø T !,%,"MEAN"(X+RE*EM)/RE,"    STD.DEV."FSQT(BA/(RE-1)),!
Ø3.25 T "STD.ERROR MEAN"1ØØ*FSQT(BA/(RE-1))/((X+RE*EM)/RE)

Ø4.Ø5 S DV=RO*CO(RE-1)
Ø4.1Ø S KI=DV*FLOG(SP/DV);S KB=K1-S2
Ø4.15 S LI=(CO*RO*(1/(RE-1)))-(1/DV);S L=L1/3*(CO*RO-1)
Ø4.2Ø T '',%,"CHI2"KB/(1+L)
```

APPENDIX 4 (Continued)

c. <u>One way analysis of variance</u>

```
**
****************************/
*G
G:?Ø1.ØØ @ Ø1.Ø2
*W
C-8K FOCAL @1969

Ø1.Ø1 E
Ø1.Ø2 A "G"G;S J=1
Ø1.Ø4 A "N"N(J);S P=1;S S=Ø
Ø1.Ø5 A "S"X(P);S S=S+X(P)
Ø1.Ø7 S P=P+1;I (N(J)-P)1.Ø8,1.Ø5,1.Ø5
Ø1.Ø8 DO 2
Ø1.Ø9 S J=J+1;I (G-J)1.1Ø,1.Ø4,1.Ø4
Ø1.1Ø F J=1,G;S SZ=SZ+(N(J)*M(J))/SN
Ø1.11 T "ANALYSIS OF VARIANCE TABLE:",!!!;G 3.Ø1

Ø2.Ø1 S M (J)=S/N(J);S K=N(J)-1;S SX=Ø;S SN=SN+N(J)
Ø2.Ø2 F P=1,N(J);S SX=SX+(X(P)-M(J))+2
Ø2.Ø3 S V(J)=SX/K;S SD-FSQT(V(J));T !=
Ø2.Ø4 T "GROUP    DF         MEAN              VARIANCE          STAND. DEV.",!
Ø2.Ø6 T %2 J,"  ",K,"  ";T %, M(J),"  ",V(J),"  ",SD,!!!

Ø3.Ø1 F J=1,G;S SB=SB+N(J)*(M(J)-SZ)+2;S SW=SW+(N(J)-1)*V(J)
Ø3.Ø2 S KB=G-1;S MB=SB/KB;S ST=SB+SW
Ø3.Ø3 F J=1,G;S KW=KW+(N(J)-1)
Ø3.Ø4 S KT=KB+KW;S MW=SW/KW
Ø3.Ø5 ! "    VARIATION           DF          SS                    MS",!!
Ø3.Ø6 T %4,"BETWEEN:             "KB;T %,"  "SB,"          "MB,!!
Ø3.Ø7 T %4,"WITHIN:              "KW;T %,"  "SW,"          "MW,!!
Ø3.Ø8 T %4,"TOTAL:               "KT;T %,"  "ST,!!!
Ø3.Ø9 T "RATIO:  BETWEEN/WITHIN",MB/MW,!!;Q
```

APPENDIX 5

Data for Histograms - dpm/gram fresh weight - sucrose ^{14}C distribution patterns. Figure represents average of 8 plants.

TREATMENT	SAMPLING SITE	GRAM FRESH WEIGHT (GM)	AVERAGE dpm	$\frac{dpm}{gram\ fresh\ wt.} \times 10^4$	RATIO
2,4-D + Sucrose ^{14}C	Petiole	.188	4873	2.59	5.28
	Internode 1	.241	1183	.49	
IAA + Sucrose ^{14}C	Petiole	.213	3676	1.73	2.31
	Internode 1	.232	1741	.75	
IAA + GA + Sucrose ^{14}C	Petiole	.202	3216	1.60	5.71
	Internode 1	.363	1011	.28	
Only Sucrose ^{14}C	Petiole	.187	5194	2.78	3.61
	Internode 1	.154	1183	.77	
Lanolin + Sucrose ^{14}C	Petiole	.180	6519	3.62	8.62
	Internode 1	.220	925	.42	

APPENDIX 6

Histogram presentation of TIBA - IAA mediation of sucrose - ^{14}C distribution. Figures represent average of 8 plants.

TREATMENT	SAMPLING SITE	GRAM FRESH WEIGHT (GM)	AVERAGE dpm	$\frac{dpm}{\text{gram fresh wt.}}$ x 10^4	RATIO
Only Sucrose ^{14}C	Petiole	.213	1202	.56	.85
	Internode 1	.230	1514	.66	2.00
	Internode 2	.691	2261	.33	
IAA + Sucrose ^{14}C	Petiole	.251	8778	3.50	4.00
	Internode 1	.249	2173	.87	1.81
	Internode 2	.713	3420	.48	
Lanolin + Lanolin + Sucrose ^{14}C	Petiole	.170	1099	.65	1.51
	Internode 1	.226	976	.43	1.79
	Internode 2	.645	1518	.24	
TIBA + Lanolin + Sucrose ^{14}C	Petiole	.261	1396	.53	4.42
	Internode 1	.290	333	.12	4.00
	Internode 2	.869	298	.03	
TIBA + IAA + Sucrose ^{14}C	Petiole	.271	5183	1.91	3.9[illegible]
	Internode 1	.261	1262	.48	1.66
	Internode 2	.713	2070	.29	

APPENDIX 6 (CONTINUED)

TREATMENT	SAMPLING SITE	GRAM FRESH WEIGHT (GM)	AVERAGE dpm	$\frac{\text{dpm}}{\text{gram fresh wt.}} \times 10^4$	RATIO
Lanolin + IAA ^{14}C	Petiole	.154	599	.39	2.05
	Internode 1	.222	412	.19	9.50
	Internode 2	.631	113	.02	
TIBA + IAA ^{14}C	Petiole	.174	298	.17	3.40
	Internode 1	.258	118	.05	5.00
	Internode 2	.845	68	.01	