TRANSLOCATION OF EXOGENOUS GROWTH-REGULATORS IN THE BEAN SEEDLING

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(WITH THREE FIGURES)

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Introduction

Localized presentation of growth-regulatory chemicals to a particular organ of a plant can give rise to responses in other, more or less distant, parts, a circumstance that may be taken as evidence of the translocation of a stimulating substance which may be termed the "stimulus." In most instances there is no information at hand to permit a decision as to whether the translocated stimulus is the applied chemical itself, a derivative of the applied compound, or some endogenous hormone, the formation or movement of which has been influenced by the treatment. In a few cases, however, there is rather clear-cut evidence that the applied compound is transported as such (**6**, **8**, **17**) and at present there seems to be no cogent argument to gainsay that this may be universally true.

It has been shown (**7**) that the stem curvature response of bean seedlings to leaf application of 2,4-dichlorophenoxyacetic acid requires the exposure of the plants to relatively intense light and carbon dioxide concurrently. Such exposure need not take place while the plant is responding but is effective even though terminated some time before application of the growth-regulator. The response itself may occur in absence of light or of CO_2. No stem curvatures occur in leaf-treated plants which have been maintained in darkness, or in light of low intensity, for several hours preceding treatment, or in plants which have been exposed to bright light in a CO_2-free atmosphere. However, under such environmental conditions, plants do give stem curvature responses to growth-regulators applied directly to the stem, to the cotyledons, or to the roots. Furthermore, under conditions in which leaf presentations do not lead to stem curvatures, there may occur localized torsions of the lamina in the vicinity of the applications.

Conversely, a latent response may be elicited by holding treated carbohydrate-depleted plants in darkness for a considerable period and then exposing them to conditions favorable to photosynthesis as shown by the following experiment. Seven-day-old bean seedlings (*Phaseolus vulgaris* var. Black Valentine) were placed in a dark room for 24 hours after which a 0.01 ml. drop containing 10 micrograms of ammonium 2,4-dichlorophenoxyacetate in 1% aqueous Carbowax 1500 was placed on one primary leaf of each plant. Groups of the treated plants were transferred from the darkroom to the greenhouse at intervals up to 72 hours. No responses, other than localized leaf torsions, were evident while the plants were in darkness but all exhibited characteristic stem curvatures within a few hours after exposure to light. Control plants treated in the same manner, except for having the treated portion of the leaf removed just prior to transfer from dark room to greenhouse, showed no growth-regulator response.

These facts indicate that the favorable influence of light is not directly upon the curvature (growth) response itself or upon the entry of growth-regulator into the plant, but rather upon movement from the leaf blade to and through the stem. The experiments of RICE (**9**, see especially fig. 4) indicate that entry into the leaf is actually greater in darkness than in light even though export from the carbohydrate-depleted leaf does not occur under the former condition. It has been suggested by various investigators that translocation of the growth-regulatory stimulus from the leaf is associated with concurrent transport of organic food materials produced by photosynthesis. Evidence consistent with this view has been presented by the demonstration that the magnitude of response induced by leaf application was correlated with the sugar content of the leaf blade (**7**), and was virtually prevented by drastically decreasing the leaf area available for photosynthesis (**15**). It was shown also that movement of the stimulus from the leaf took place only through living cells.

Role of nutrients in growth-regulator transport

The following experiments were undertaken to ascertain whether translocation of applied growth-regulators might be influenced by specific food materials artificially supplied to the leaf. That sugar can enter an intact leaf from solutions in which it is immersed and be utilized for shoot development in darkness has been shown for various species (for literature see **16**).

In general, the technique employed was as follows. Bean seedlings were cultured in soil in the greenhouse until the second internode had begun to elongate (usually eight days after planting). The cotyledons were removed by gentle pressure with the fingers and the plants were placed in darkness for 24 or more hours in order to deplete the leaves of reserve carbohydrates. Subsequent manipulations were conducted in light of low intensity (5 foot candles or less), the duration of illumination being kept as short as possible. All darkroom experiments were conducted at approximately 26° C. Under these conditions there was very little stem elongation in unfed plants. One or both of the primary leaves were gently rolled and inserted into vials of 30 to 60 ml. capacity into which the desired solutions (unsterilized) were then introduced. All liquids supplied to the leaves contained 0.025% sulfanilamide, as used by WENT and CARTER (**16**), for the purpose of minimizing microbial development. The sulfanilamide appeared to be without influence upon the beans, as shown by: (1) comparison of sucrose solutions containing 0.025 and 0.05% sulfanilamide, (2) an experiment in the greenhouse in which equal shoot growth was made during a 5-day period by untreated plants and by those with one primary leaf immersed in aqueous sulfanilamide solution, and (3) comparison of sugar solutions with and without sulfanilamide in shorter tests (two to three days) during which microbial contamination was not apparent.

Figure 1 presents results of an experiment in which stem elongation varied linearly with concentration of supplied sucrose up to 0.75 M. In this concentration range the 2-day increment was very nearly two fifths that in five days, indicating that the growth rate was substantially constant. Proportionality between growth and sugar concentration has been observed repeatedly, although the absolute amount of growth obtained with a particular concentration may vary considerably from experiment to experiment unless the plants are very carefully selected for uniformity.

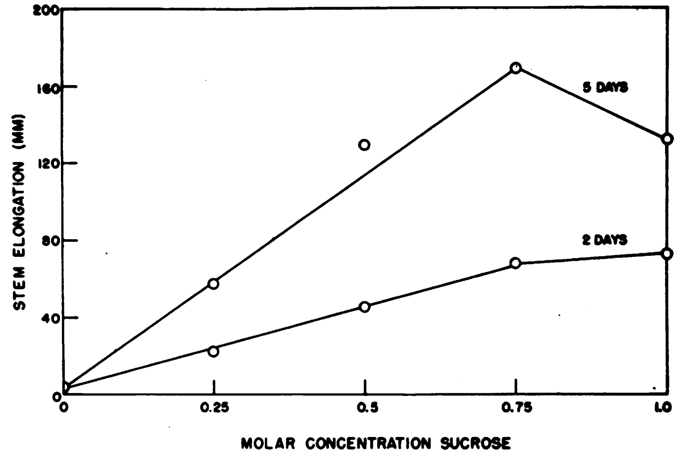

FIG. 1. Shoot elongation in darkness as influenced by concentration of sucrose supplied to leaf. Each point represents the mean of seven plants.

Glucose and maltose are approximately equal to sucrose in supporting growth, whereas fructose is somewhat inferior; lactose and galactose are much less satisfactory, possibly owing, in part at least, to the leaf injury which they induce. Whether extracellular hydrolysis of sucrose must precede absorption as reported by Saïd (**11**) has not been ascertained.

The growth response to sucrose was not increased by buffering the solution with phosphate at pH 4.6 or 5.7; it was reduced nearly to half at pH 6.6, and was completely abolished at pH 8.8. This result indicates also that sugar absorption was not limited by the phosphorus content of the leaves as may occur with P-deficient leaves according to Saïd (**11**). Addition of 0.1% or 1% Tween 20 to the unbuffered sucrose solution more than doubled its effectiveness.

Elongation of the stem thus may be taken as evidence that a supplied substance, or its conversion product, is transported from the leaf to the growing region. The amount and rate of growth may be influenced, of course, by factors such as ease of penetration into the plant, rate of transport, and intrinsic suitability of the supplied compound as a nutrient or its convertibility into such.

Growth of treated plants may be either increased or decreased relative to untreated controls, depending on whether small or large amounts of the exogenous growth-regulator are present in the growing region.

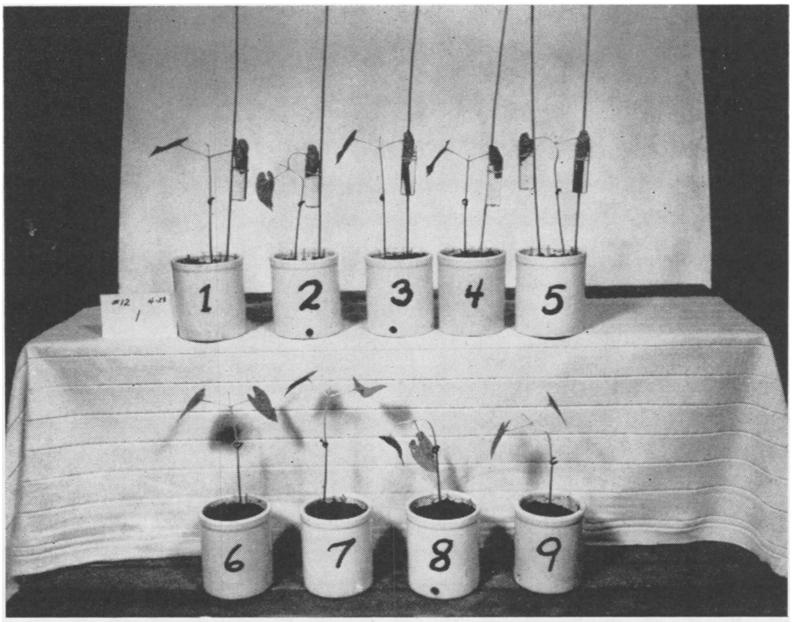

FIG. 2. Effect of sucrose on response to 2,4-dichlorophenoxyacetic acid. Unless otherwise specified, plants were depleted of carbohydrate by 24-hour dark period preceding treatments and maintained in darkness for additional 24 hours prior to photographing.

1—Leaf immersed in 1 M sucrose.
2—Leaf in 1 M sucrose plus 50 p.p.m. 2,4-D.
3—Leaf in water.
4—Leaf in 50 p.p.m. 2,4-D.
5—Right leaf in 1 M sucrose; left leaf in 1 M sucrose plus 50 p.p.m. 2,4-D. (Initial curvature towards right.)
6—Untreated.
7—10 micrograms 2,4-D applied as aqueous solution (0.01 ml.) to right leaf.
8—Treatment as in No. 7 without prior depletion of carbohydrate.
9—1 microgram 2,4-D applied unilaterally to first (epicotyledonary) internode.

SCHUFFELEN (**12**) has speculated that growth-regulators in high concentration may impede sugar transport by decreasing protoplasmic permeability. We incline to the view that the diminished growth observed with sugar plus growth-regulator in the present study is due primarily to an inhibitory effect of the latter upon some process more directly involved in growth, rather than to an interference with the sugar supply to the meristem.

Because small stimulations would be difficult to detect in these experiments it was more convenient to employ relatively high concentrations of growth-regulator so as to utilize inhibition as an indicator of its movement from the leaf. Further demonstrations of transport are furnished by curvature of the stem, which results from the unilateral distribution of growth-regulator in this organ following application to a single leaf, and by hypertrophy of petiole and stem which sometimes ensues.

As indicated by these criteria it has been demonstrated that, in the absence of light, exogenous growth-regulators are translocated from a starved leaf to which they are supplied only if nutrient (sugar) also is furnished to the same leaf. A typical experiment with sucrose and 2,4-dichlorophenoxyacetic acid is illustrated in figure 2. There was no evidence of movement of the growth-regulator from the leaf if it were applied alone, or if growth-regulator were applied to one leaf and sugar concurrently to the opposite leaf.

Similar results were obtained with several other growth-regulating chemicals representing a diversity of structural types: indole-3-acetic acid, beta-hydroxyethyl-4-chlorophenoxyacetate, p-chlorophenyl-2,4,5-trichlorophenoxyacetate, 4-chlorophenoxyacetamide, 2-bromo-3,5-dichlorobenzoic acid, N-(2,4-dichlorophenoxyacetyl)-urea, and the morpholine salt of 2,4-dichlorophenoxyacetic acid. Hence it appears that the effect of sugar upon transport of a growth-regulating compound is not dependent upon the presence in the latter of any particular molecular grouping.

In addition to sucrose, the following sugars have been found to facilitate movement of growth-regulators: glucose, fructose, maltose, lactose, and galactose. As shown by the shoot development, all of these sugars also serve as nutrients, although it is not established whether they are transported and utilized in the form supplied or first undergo some transformation. In table I are presented results from two experiments which show the comparative growth-promoting efficacy of several sugars. While these are typical of several other experiments, only the relative order of magnitude is to be regarded as significant. It is of interest that galactose is utilized to some extent inasmuch as this sugar has been found non-available for growth of other tissues of higher plants although respirable and convertible into starch (**1**, **2**, **10**, **14**).

The non-specificity with respect both to growth-regulator and to sugar suggests that transport of the stimulus is faciliated by the occurrence of food movement in general rather than by any specific combination with a particular metabolite. It may be postulated that movement of the stimulus from the leaves of bean seedlings is dependent upon the operation of the mechanism responsible for translocation of organic materials but that the growth-regulator itself is unable to initiate the transport process. Despite much work during the past several decades, the means by which organic materials are moved in plants remains one of the puzzling problems of plant physiology. Elucidation of the mechanism of movement of growth-regulators may require the solution of this more general problem. Insofar as the present results bear on this question they seem to point toward some type of mass conduction. It should, perhaps, be stated that these conclusions are not intended to apply to growth-regulator transport in specialized organs such as the grass coleoptile.

It would be of considerable interest to determine whether substances other than sugars can set the train of conduction in motion and so promote transport of growth-regulators. Conversely, the use of growth-regulators as indicators of movement of various supplied materials might constitute a helpful tool for determining which compounds are translocated in plants, a subject upon which there seems to be relatively little unequivocal information.

TABLE I

Elongation in darkness of second internode of Black Valentine bean seedlings supplied by leaf immersion with solutions of sugars and 2,4-dichlorophenoxyacetic acid. (All solutions contain 0.025% sulfanilamide. Ten replicates for each solution.)

Expt.	Sugar (0.25M)	Tween 20 (%)	Elongation (mm/plant)		
			2,4-D (p.p.m.)		
			0	10	25
1	None	0.1	1	1	......
	Sucrose	0.1	48	4	......
	Glucose	0.1	46	4	......
	Maltose	0.1	41	4	......
	Fructose	0.1	29	4	......
	Lactose	0.1	8	3	......
2	Lactose	......	4	......	2
	Galactose	......	6	......	2

Routes and mechanisms of growth-regulator transport

Various pathways and correspondingly diverse mechanisms of transport of the growth-regulatory stimulus in plants may be operative depending upon circumstances.

Phloem transport

Growth-regulators which enter the plant through the intact epidermis of stems or leaves may move either acropetally or basipetally, or in both directions concurrently.

If the amount of growth-regulator applied to the intact leaf is relatively small, downward transport appears to be restricted to living cells, presumably those of the phloem. This conclusion is based on the non-conduction basipetally through a killed region of petiole or stem. In such experiments tissue proliferation, and sometimes root formation, occur just above the killed zone, a phenomenon which may be taken as evidence that the stimulus has been transported via phloem elements and has accumulated at the site of the responses. Conduction of stimulus by the phloem of squash has been reported to approach a velocity of 20 cm. per hour (**13**).

The consensus of opinion at present appears to favor the phloem as the principal route of conduction of organic nutrient materials from the leaf. Accordingly, it may be inferred that this is the path principally involved in growth-stimulus movement of the type influenced by sugar. This view is supported by the results of experiments in which solutions were supplied, as described above, to intact leaves, the petioles of which had been flamed. Neither sugar nor growth-regulator, alone or in combination, appeared to traverse the killed zone under these conditions.

TABLE II

EFFECT OF FLAMING FIRST (EPICOTYLEDONARY) INTERNODE ON ACROPETAL TRANSLOCATION FROM PRIMARY LEAF OF BLACK VALENTINE BEAN SEEDLINGS

SUPPLIED TO UNIFOLIOLATE LEAF	FIRST INTERNODE	ELONGATION OF SECOND INTERNODE (MM)
Water	intact	1
	flamed	1
Sucrose	intact	23
	flamed	16
Sucrose + 2,4-D	intact	3
	flamed	8

The leaf-feeding technique has provided further information on the route of conduction from the primary leaf of the bean seedling. According to the anatomical studies of DOUTT (**3**), the three principal bundles supplying the unifoliolate leaf make direct connection with ascending major bundles in the stem, not at the level of the primary-leaf node, but near the level of the cotyledonary node. If this is correct, it might be expected that materials originating in the leaf and destined for the more apical regions of the shoot would move first downward at least as far as the cotyledonary node and then upward. Such a route would explain the curvatures in the internode below the primary leaf node which commonly result from application of growth-regulator to one of these leaves in seedlings at the stage described above.

The data in table II indicate that the amounts of sugar and of growth-regulatory stimulus transported from the primary leaf to the shoot apex are indeed diminished if a dead zone is present in the epicotyledonary internode, and thus are not inconsistent with the pathway described. However, inasmuch as conduction from leaf to apex is not prevented completely by the existence of the dead region, it is probable that an alternative route of movement is available.

XYLEM TRANSPORT

A quite different mechanism of transport is involved when growth-regulators find their way into the transpiration stream, as by absorption through the roots or by injection into the shoot. In such case, movement is controlled by those environmental factors which influence transpiration and may take place through a killed zone (**4, 5, 13**). Conduction in this manner, which in general is acropetal, is considerably more rapid than that in the phloem, velocities as great as 70 cm. per hr. having been noted (**13**).

Entry of 2,4-D and/or sugar into the plant via the xylem can be accomplished also by immersing the debladed end of a petiole into an appropriate solution. Separately or in combination these substances can pass through a flamed segment of a petiole, as shown by the previously described criteria. If a solution containing both 2,4-D and sugar is presented to a debladed petiole, stem curvature usually occurs while shoot elongation tends to be suppressed. The entry of these materials is probably due to a reversal of the normal transpiration stream, so that movement is away from rather than toward the leaf.

FIG. 3. Movement of 2,4-D stimulus through flamed region in second internode as indicated by stem proliferation. (Leaf blades and axillary shoots removed for photograph.)

Left—Flamed only. No growth-regulator.

Center—Both primary leaves below flamed zone painted with aqueous solution containing 1000 p.p.m. 2,4-D and 1% Tween-20.

Right—0.1 ml. aqueous solution containing 10 micrograms 2,4-D and 1% Tween-20 applied to first trifoliolate leaf above flamed region.

Application of large doses to the intact primary leaf may be followed by acropetal movement through a killed region of the stem as evidenced by the tissue responses (fig. 3). In this case movement from phloem to xylem appears to have occurred.

Summary and conclusions

Growth-regulating chemicals applied to the leaf of a bean seedling devoid of translocatable carbohydrates are not transported from the leaf to the stem. Movement of such growth-regulators can be brought about by supplying sugar to the leaf externally. A variety of sugars (sucrose, maltose, lactose, glucose, fructose, galactose) are able to induce translocation. Growth-regulators of diverse structural types (indole-3-acetic acid, β-hydroxyethyl-4-chlorophenoxyacetate, p-chlorophenyl-2,4,5-trichlorophenoxyacetate, 4-chlorophenoxyacetamide, 2-bromo-3,5-dichlorobenzoic acid, N-(2,4-dichlorophenoxyacetyl)-urea, 2,4-dichlorophenoxyacetic acid, and its morpholine salt) are transportable, no particular molecular grouping being requisite. The non-specificity with respect to both sugar and growth-regulator appears inconsistent with the occurrence of a definite combination between the two. Rather, movement of the growth-regulator seems to require only that the normal mechanism of transport of organic materials be set in operation by whatever means.

Growth-regulator translocation of the type facilitated by sugar takes place only through living cells, presumably of the phloem. A quite disparate mode of conduction occurs when the growth-regulator is introduced into the transpiration stream. The latter may be carried on through nonliving regions of stem or petiole and is independent of movement of sugars.

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