EFFECTS AND INTERACTIONS OF VARIOUS HORMONE AND LIGHT TREATMENTS ON THE TRANSLOCATION OF THREE PLANT GROWTH REGULATORS

By

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Bachelor of Science

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Lawton, Oklahoma

1976

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1980

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ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. Eddie Basler for his willing assistance and unlimited patience throughout the course of my graduate studies. I am also grateful to Dr. Jim Ownby, Dr. Paul Santelmann and Dr. Jim Stritzke for serving on my advisory committee.

I would like to thank Dr. Betty Hamilton and Alan Taylor for helpful suggestions and Denise Rex and Janice Green for valuable assistance.

I am especially thankful to Mike Bunning for tolerating numerous sacrifices and for making my time in graduate school more enjoyable.

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ABBREVIATIONS

ABA	abscisic acid
ancymidol	a-cyclopropyl-a-(4-methoxyphenyl)-4-pyrimidine-methanol
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
coumarin	1,2-benzopyrone
2,4-D	(2,4-dichlorophenoxy) acetic acid
DCCD	N,N'-dicyclohexylcarbodiimide
ethephon	2,chloroethyl-phosphonic acid
GA	gibberellin, A_3
2,4,5-T	(2,4,5-trichlorophenoxy) acetic acid

CHAPTER I

INTRODUCTION

The effectiveness of plant growth regulators is dependent on their ability to reach target areas from sites of synthesis or application. Changes in surrounding environmental conditions as well as variations in hormone concentrations and other intrinsic factors may influence the mechanisms which regulate hormone translocation. Interactions between external and internal factors may also have a significant influence on patterns of hormone distribution. In order to understand the movement of plant hormones and hormone-regulated plant processes it may be necessary to determine what effect different conditions have on the translocation of growth regulators.

The long-distance movement of most hormones appears to occur through phloem tissue but how their translocation relates to the movement of carbohydrates is not clearly understood. It is generally accepted that carbohydrates are transported, via phloem, from areas of synthesis or storage to sites of utilization as a result of hydrostatic pressure. Exogenous gibberellins (Zweig et al., 1961; Chin and Lockhart, 1965; Asakawa et al., 1974), auxins (Goldsmith et al., 1974) and abscisic acid (Bellandi and Dorffling, 1974; Milborrow, 1974) have rate and distribution profiles similar to assimilates moving in the phloem along source-to-sink gradients.

Prolonged periods of darkness tend to restrict the flow of sugars

by inhibiting carbohydrate production (Wardlaw, 1968). Auxin export from leaves of dark treated plants has been shown to be discontinued unless a source of carbohydrates is supplied, presumably because movement of auxin depends on the flow of assimilates (Rohrbaugh and Rice, 1951). Little has been reported about the effects of darkness on the translocation of gibberellins and ABA in intact plants.

Aside from providing the energy for the formation of the primary translocating substance, light may have other effects on translocation. At light intensities which caused no net uptake of carbon dioxide, basipetal translocation of sucrose was increased (Hartt, 1965). Exogeneous ATP can stimulate sucrose translocation by about 80 per cent, suggesting that an adequate supply may be necessary to maintain normal translocation rates (Solonick et al., 1974; Doman and Geiger, 1976). Following periods of darkness, ATP levels decreased because of the cessation of photophosphorylation (Troughton, 1977) and the activity of certain ATPase isoenzymes has been reported to be reduced during darkness (Raghavendra et al., 1976). Potassium appears to be essential for the translocation of sucrose (Hartt, 1969; Hartt, 1970) and its uptake may be enhanced by light (Rains, 1968).

Light conditions have frequently been observed to have differential effects on plant responses to gibberellins. Cellular processes leading to stem elongation are susceptible to light inhibition but gibberellic acid, which appears to compete with light for control of the process, can completely overcome the inhibition in some plants (Lockhart, 1958). In the dark, when shoot growth is significantly greater, stimulation by exogenous GA is not observed (Bardense and Lang, 1972). The effects of light and GA on stem elongation may involve sugar-starch

interconversions and consequential changes in osmotic potential (Katsumi and Kazama, 1978). The ability of gibberellic acid to substitute for the required light treatment in both red-light stimulated and red-light inhibited seed germination may also be mediated through increases in osmotic pressure (Thimann, 1977).

In isolated stem segments of dark grown plants, applied gibberellins accumulated in the apical region of the stem but light reduced this accumulation (Musgrave et al., 1969). Measurements of gibberellin effects on the translocation of dry matter revealed that light decreased the ratio of weight of the shoot to root but GA counteracted this influence and produced a distribution pattern similar to a dark treatment (Halevy et al., 1964; Currah and Thomas, 1979).

Gibberellins and darkness have both been shown to decrease the osmotic potential of some cells, thus increasing the capacity for water uptake (Katsumi and Kazama, 1978). Darkness inhibited chloroplast starch formation, resulting in increased levels of sucrose as osmoticum. Gibberellin's effect on osmotic potential may be attributed to stimulation of hydrolytic enzyme activity. GA-induced growth has been associated with increases in invertase (Kaufman et al., 1968) and α-amylase activity (Broughton and McComb, 1971).

Wood and Paleg (1972) reported that glucose transport across liposomes was increased in the presence of gibberellic acid. The chloride and potassium ion concentrations of chloroplasts and vacuoles have also been reported to increase after treatment with GA (Neumann and Janossy, 1977). As a result of changes in membrane fluidity, the in vivo activity of membrane-bound ATPases may be enhanced because of alterations in ion concentrations. Evidence does exist that GA

stimulates ATPase activity (Katsumi and Kazama, 1978) and the effects of DCCD, an inhibitor of membrane-bound ATPases, can be prevented by GA treatment (Katsumi, 1976; Corbett, 1977).

Although binding sites for gibberellins have not been characterized (Kende and Gardner, 1976), Katsumi (1976) suggests that GA may be competing with DCCD for a site on the ATPase.

The vectorial displacement of protons by membrane-bound ATPases establishes pH and charge separations across membranes (Mitchell, 1961). Giaquinta (1977) proposed that sugar-loading into sieve cells is facilitated by an ATPase-driven pH gradient. Auxin accumulation in cells may also depend upon a proton gradient (Goldsmith, 1977; Basler et al., 1977) established by membrane-associated ATPases which appear to be abundant in sieve elements and transfer cells (Gilder and Cronshaw, 1974). Within the cell, where the pH is higher, auxin molecules become ionized and cannot readily traverse the plasmalemma so they are essentially trapped. DCCD, which binds covalently to membrane-bound ATPases, inhibits elongation (Katsumi, 1976) and lends support to the chemiosmotic diffusion theory.

According to Goldsmith (1977), auxin has a limited ability to stimulate its own uptake into cells by increasing proton extrusion and thereby enhancing the pH gradient. This has been substantiated by Long and Basler's (1973) observation that, within a range of 1.0 to 5.0 micrograms, the acropetal translocation of 2,4,5-T to young shoots increased geometrically with an increase in concentration. Fusicoccin, known to increase proton secretion and potassium ion uptake, caused an 8-fold increase in 2,4-D absorption in tissue culture cells (Kurkdjian et al., 1979).

The effects of hormone concentrations on the uptake and translocation of the other phytohormones have not received a great deal of attention. Gibberellins, however, have been shown to influence the translocation of a variety of substances including several growth regulators. Gibberellic acid increased the transport of 2,4-D from leaves (Ashton, 1959; Basler, 1959) and enhanced the movement of 2,4,5-T to the young shoots (Basler, 1974). GA_3 was capable of reversing the inhibition of 2,4,5-T translocation by ABA and coumarin (Basler and McBride, 1977), DCCD (Corbett, 1977) and ethephon and ancymidol (Basler, 1977). GA also hastened the translocation of applied sucrose to the upper stem and leaves and reduced downward translocation to the lower stem and roots (Brian et al., 1954; Halevy et al., 1964). The enhancement of ^{14}C- and ^{32}P-labeled assimilate movement to the site of GA application is believed to be a localized action, possibly involving phloem unloading processes (Mulligan and Patrick, 1979).

Abscisic acid has been reported to inhibit auxin translocation (Basler and McBride, 1977), polar transport of auxin (Wodzicki et al., 1979) and its own transport in cotton petioles (Shindy et al., 1973). ABA also commonly counteracts GA-induced responses including growth, germination and senescence (Addicott and Lyon, 1969).

This study was designed to determine the effects of a twenty-one hour dark treatment on the translocation of GA_1, ABA and 2,4,5-T, and to measure the influence of gibberellic acid on their translocation during a normal photoperiod and after a dark treatment. The effects and interactions of abscisic acid and a darkness treatment on the movement of ABA and GA_1 were also determined as well as the effects of GA_1, GA_3 and a mixture of GA_4 and GA_7 on GA_1-^3H translocation.

CHAPTER II

MATERIALS AND METHODS

Bush bean (Phaseolus vulgaris L. cv. stringless greenpod) seeds were germinated in perlite moistened with half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) at 32 C under continuous fluorescent light of 5.5 Klux. After 5 days the seedlings were transferred to 500 ml amber glass bottles containing 400 ml of aerated half-strength Hoagland's solution and grown for 4 more days in a growth chamber with 14-hour, 33 C, 22 Klux days and 10-hour, 29 C nights. The plants were transferred to fresh half-strength Hoagland's solution approximately 24 hours before treatment. For the dark pretreatments, the lights in one growth chamber were turned off 17 hours before the plants were treated.

At the time of treatment, the cotyledons were removed and a 1 µl syringe was inserted into the pith of the stem at the cotyledonary node and extended to a point approximately 1 cm below the node. The treatments were as follows: 1) 5.6 ng GA_1-^{3}H (31 Ci/mmole); 2) 4 µg DL-cis,trans-ABA-2-^{14}C (6.1 mCi/mmole); or 3) 0.5 µg 2,4,5-T-1-^{14}C (54 mCi/mmole). These amounts were dissolved in 1 µl of 95% ethanol along with various concentrations of unlabeled gibberellins or abscisic acid. Dark pretreated plants were injected in a weak green light and remained in a darkened growth chamber during the 4 hours following treatment. Microscopic analysis has revealed no extensive damage to the xylem or

phloem tissue as a result of this stem-injection technique (Long and Basler, 1973).

Each treatment was replicated 8 times and plants were arranged within the growth chamber in a completely randomized pattern. Experiments testing the effects of darkness and hormone concentration were designed with a two-by-two factorial arrangement of treatments. Treatments were applied during the third or fourth hour of the light period. Four hours after treatment the plants were divided into the following parts: 1) the young shoots including all tissue above the primary leaf node; 2) primary leaves including petioles; 3) epicotyl; 4) treated area which included the stem tissue 0.5 cm above the cotyledonary node to a point approximately 2.5 cm below the node; 5) hypocotyl; and 6) roots. The plant parts were frozen, lyophilized, weighed and then homogenized in 5 ml of 95% ethanol (10 ml for leaves) with a Brinkmann Polytron homogenizer. An aliquot of 0.2 ml for the leaves and 0.5 ml for all other parts was transferred to 15 ml of Bray's solution or Beckman Readysolv counting solution and assayed for radioactivity with a Packard Tricarb 3320 or Beckman LS-100 liquid scintillation spectrometer. Five ml samples of the nutrient solutions were lyophilized and dissolved in 15 ml of counting solution and assayed for radioactivity. Adjustments for quenching were made by the use of standard quench curves.

An IBM 360, Model 168 computer was used to compile data for statistical analysis. Statistical measurements of interactions and total amounts of radioactivity recovered were determined using Statistical Analysis Systems 72 and 76. Standard F tests and Duncan's new multiple range tests were utilized to determine significant

differences among treatment means.

The 2,4,5-T-1-^{14}C and ABA-2-^{14}C were purchased from Amersham Corporation and GA_1-^{3}H was purchased from New England Nuclear. GA_3 and (±) cis,trans isomers of ABA were obtained from Sigma Chemical Company and were 90 and 95 per cent pure, respectively. The GA_1 was supplied by Monsanto Agricultural Chemical Company. Gibberellin A_4 and A_7 was obtained from Abbott Laboratories and purity was determined to be 100 per cent.

CHAPTER III

RESULTS

Interactions and Effects of ABA, GA_3 and Dark Treatment on ABA Translocation

Separately, dark treatment and GA_3 considerably reduced movement of ABA-^{14}C from the vicinity of the treated area but in combination their inhibitory effects were reversed and acropetal translocation increased (Table I).

After 17 hours of darkness, translocation of labeled ABA to the young shoots, primary leaves, roots and nutrient solution was restricted while the amount of radioactivity remaining in the epicotyl and treated area increased. The addition of 5 µg of GA_3 had essentially the same effect as a treatment of darkness except movement to the nutrient solution was not prevented.

When applied together, dark treatment and additional GA_3 did not restrict movement from the treated area but instead translocation to the young shoots was enhanced. Movement to the nutrient solution was still inhibited. The absence of ABA-^{14}C in the nutrient solution was not just a result of less ABA in the roots because it was measured in significant amounts in the nutrient solution even when there was less than a fifth as much ABA-^{14}C in the roots.

Thus GA_3 appears to inhibit ABA translocation to the young shoots

TABLE I

EFFECTS OF GA_3 AND DARK TREATMENT ON ABA TRANSLOCATION TO DIFFERENT PARTS OF THE PLANT*

	ng ABA-^{14}C/plant part			
	0 µg GA_3/plant		5 µg GA_3/plant	
Plant Part	Control	Dark Treatment	Control	Dark Treatment
Young Shoots[hli]	200.0b	100.0a	118.9a	324.0c
Primary Leaves	2151.0a	1972.5a	1587.0a	1794.7a
Epicotyl[i]	88.0a	406.3b	297.8b	109.4a
Treated Area[i]	730.2a	1531.2b	1200.0ab	885.1a
Hypocotyl	171.1a	144.0a	104.6a	154.3a
Roots[i]	158.8b	36.3a	20.2a	128.8b
Nutrient Solution[l]	37.6b	0.2a	27.4b	0.0a
Total	3536.7a	4190.5a	3355.9a	3396.3a

*Values for a single plant part followed by the same letter are not significantly different at the 5% level.

[h]Differences between the levels of hormone concentration are significant.

[l]Differences between the two light treatments are significant.

[i]Interaction between hormone concentration and light treatment are significant.

in light-treated plants and to promote translocation in dark-treated plants but GA_3 was not able to overcome the inhibitory effects of a dark treatment on the movement of ABA into the nutrient solution.

There was significant interaction between GA_3 concentration and light treatment in the young shoots, epicotyl, treated area and roots, indicating that plants responded differently to light treatments at different GA_3 concentrations. Light alone had a significant effect in the young shoots and nutrient solution and GA_3 concentration was a contributing factor in treatment differences seen in the young shoots.

The addition of 5 µg of ABA did not change the general distribution pattern of ABA-^{14}C but the combination of dark treatment and addition of ABA significantly decreased the amount of label accumulating in the primary leaves and increased the amount of ABA remaining in the treated area (Table II).

More of the total ^{14}C-ABA applied accumulated in the primary leaves of the control than the GA_1-^{3}H or 2,4,5-T-^{14}C used in other experiments. Dark treatment plus additional ABA reversed the ratio of radioactivity in the primary leaves to treated area compared to the control.

Measurements of transpiration rates (Basler, 1974) indicated that injected ABA enhanced stomatal closure. The level of ABA-^{14}C measured in the primary leaves appears to reflect the effects of darkness and ABA on transpiration.

There was no significant interaction between the two factors, ABA concentration and dark treatment, indicating that they were acting independently.

The effect of dark treatment was found to be significant in all

TABLE II

EFFECTS OF ABA AND DARK TREATMENT ON TRANSLOCATION OF ABA TO DIFFERENT PARTS OF THE PLANT*

	ng ABA-^{14}C/plant part			
	0 µg ABA/plant		5 µg ABA/plant	
Plant Part	Control	Dark Treatment	Control	Dark Treatment
Young Shoots[l]	343.0b	116.1a	298.1b	78.0a
Primary Leaves	1885.3c	1424.0b	1647.0bc	887.0a
Epicotyl[hl]	107.9a	278.4ab	281.3ab	410.7b
Treated Area[l]	895.1a	1480.5b	927.1a	2008.2c
Hypocotyl	207.3a	249.2a	165.0a	252.3a
Roots[l]	204.4b	79.2a	171.0b	60.2a
Nutrient Solution[l]	56.9c	0.0a	42.2b	0.0a
Total	3699.9a	3627.4a	3531.7a	3696.4a

*Values for a single plant part followed by the same letter are not significantly different at the 5% level.

[h]Differences between hormone concentrations are significant.

[l]Differences between light treatments are significant.

[i]Interaction between hormone concentration and light treatment are significant.

plant parts except the hypocotyl and ABA concentration was a causative factor in treatment differences seen in the primary leaves and epicotyl.

Effects and Interactions of a Dark Treatment and GA_3 on the Translocation of 2,4,5-T

A 21-hour dark treatment restricted the movement of 2,4,5-T-1-^{14}C but GA_3 caused little apparent change in the distribution of auxin (Table III).

In the young shoots and primary leaves a significant reduction in 2,4,5-T accumulation could be attributed to the darkness treatment but the change caused by additional GA_3 was not significant in either light treatment. The epicotyls of the dark-treated plants had appreciably higher levels of 2,4,5-T than the controls and the presence of GA_3 did not noticeably alter this effect.

Approximately twice as much labeled auxin remained in the treated areas of dark treated beans compared to those receiving light treatment. The amount of label in the treated areas of plants exposed to a dark pretreatment was significantly increased by GA_3.

Basipetal movement was restricted by the dark pretreatment and the level of radioactivity detected in the hypocotyls, roots and nutrient solutions was significantly less than that found in the same plant parts of the light-treated plants.

Measurements of the significant effects of the separate factors revealed that light was a significant factor in differences observed in all plant parts and interaction was significant in the hypocotyl.

TABLE III

EFFECTS OF GA_3 AND DARK TREATMENT ON THE TRANSLOCATION OF 2,4,5-T TO DIFFERENT PARTS OF THE PLANT*

	ng 2,4,5-T-1-^{14}C/plant part			
	0 µg GA_3/plant		5 µg GA_3/plant	
Plant Part	Control	Dark Treatment	Control	Dark Treatment
Young Shoots[l]	25.0b	0.4a	16.7b	0.5a
Primary Leaves[l]	76.2b	25.1a	74.9b	14.9a
Epicotyl[l]	91.5a	153.5b	111.7a	157.3b
Treated Area[l]	172.3a	343.5b	190.7a	409.7c
Hypocotyl[li]	108.1b	34.3a	139.9c	18.5a
Roots[l]	10.7b	0.7a	12.8b	0.3a
Nutrient Solution[l]	1.5b	0.1a	1.6b	0.2a
Total	485.3a	557.6b	548.3b	601.4b

*Values for a single plant part followed by the same letter are not significantly different at the 5% level.

[h]Differences between hormone concentrations are significant.

[l]Differences between light treatments are significant.

[i]Interaction between hormone concentration and light treatment are significant.

Interactions and Effects of Various Gibberellins, ABA and Dark Treatment on the Translocation of GA_1-^3H

Exposing plants to an extended period of darkness restricted the basipetal movement of GA_1-^3H while increasing the GA_3 concentration enhanced upward translocation. ABA did not appear to have any major effects on the translocation of GA_1 in this study (Tables IV and V).

Darkness treatment alone limited movement to the young shoots but this was reversed in the presence of ABA or GA_3.

The addition of 5 µg of GA_3 appeared to increase the accumulation of GA_1-^3H in the primary leaves but the total amount of radioactivity was higher in plants receiving additional GA_3 indicating a possible protective effect on GA_1 metabolism. The unlabeled GA_3 may have interfered with the metabolism of GA_1-^3H by the plant. The total amount of labeled GA_1 recovered was greater in treatments receiving additional GA_3.

The amount of GA_1-^3H detected in the epicotyl appears to reflect the restriction of downward movement. Treatment with GA_3 and darkness treatment, alone or with additional ABA or GA_3, restricted basipetal movement while epicotyl values increased.

Translocation of GA_1 to plant parts below the treated area was inhibited by GA_3 alone and by the darkness treatment, with and without additional ABA or GA_3, but not by ABA under a normal photoperiod.

Interaction between ABA concentration and light treatment was significant in the epicotyl, treated area and nutrient solution. Significant differences due to the light treatment were observed in the young shoots, epicotyl, hypocotyl, roots and nutrient solution.

TABLE IV

EFFECTS OF GA_3 AND DARK TREATMENT ON THE TRANSLOCATION OF GA_1 TO DIFFERENT PARTS OF THE PLANT*

	ng GA_1-^3H/plant part X 100			
	0 µg GA_3/plant		5 µg GA_3/plant	
Plant Part	Control	Dark Treatment	Control	Dark Treatment
Young Shoots	10.8a (2.2)	4.2a (1.1)	14.0a (2.0)	14.9a (2.2)
Primary Leaves[h]	172.1a (35.5)	189.6a (48.6)	440.7b (62.5)	445.2b (67.2)
Epicotyl[hl]	11.1a (2.3)	30.5c (7.8)	21.5b (3.0)	35.5d (5.4)
Treated Area	149.1a (30.8)	149.9a (38.4)	176.6a (25.0)	161.1a (24.3)
Hypocotyl[hli]	88.2d (18.2)	13.6b (3.5)	39.0c (5.5)	5.4a (0.1)
Roots[hli]	53.2c (11.0)	2.4a (0.1)	13.1b (1.9)	0.6a (0.0)
Nutrient Solution[li]	0.0a (0.0)	0.0a (0.0)	0.0a (0.0)	0.0a (0.0)
Total	484.5b	390.2a	704.9c	662.7c

*Values for a single plant part followed by the same letter are not significantly different at the 5% level. Values in parentheses represent percent of total radioactivity recovered.

[h]Differences between hormone concentrations are significant.

[l]Differences between light treatments are significant.

[i]Interaction between hormone concentration and light treatment are significant.

TABLE V

EFFECTS OF ABA AND DARK TREAMENT ON THE TRANSLOCATION OF GA_1 TO DIFFERENT PARTS OF THE PLANT*

	ng GA_1-^3H/plant part X 100			
	0 µg ABA/plant		5 µg ABA/plant	
Plant Part	Control	Dark Treatment	Control	Dark Treatment
Young Shoots[l]	9.6b (1.5)	2.3a (0.1)	10.3b (1.5)	7.4b (1.2)
Primary Leaves	222.4a (35.7)	165.1a (47.4)	195.3a (27.5)	229.8a (37.7)
Epicotyl[hli]	22.5a (3.6)	27.5a (7.9)	22.3a (3.1)	87.3b (14.3)
Treated Area[hi]	150.9a (24.2)	140.5a (40.3)	226.8b (32.0)	248.5b (40.8)
Hypocotyl[l]	137.2b (22.0)	9.3a (2.7)	160.5b (22.6)	32.6a (5.3)
Roots[l]	75.5b (12.1)	1.1a (0.3)	90.0b (12.7)	2.0a (0.3)
Nutrient Solution[hli]	4.8b (0.7)	2.7a (0.8)	2.9a (0.4)	2.1a (0.3)
Total	622.9b	348.5a	709.0b	609.7b

*Values for a single plant part followed by the same letter are not significantly different at the 5% level. Values in parentheses represent percent of total radioactivity recovered.

[h]Differences between hormone concentrations are significant.

[l]Differences between light treatments are significant.

[i]Interaction between hormone concentration and light treatment are significant.

The effects of GA_3 concentration and light treatment, as well as the interaction between them, were significant in the hypocotyl and roots. Hormone concentration was a significant factor in differences between treatments in the primary leaves and epicotyl and light was a contributing factor in the epicotyl.

The effects of 1.0 and 5.0 µg of GA_1, GA_3 and mixtures of GA_1 and GA_3 and GA_4 and GA_7 on the translocation of GA_1-^3H to different plant parts are given in Figures 1 through 4.

In general, all the gibberellin treatments seemed to enhance upward movement. Accumulation in the primary leaves increased with increasing concentration of gibberellin but there were not significant differences in the amount of GA_1-^3H translocated to the young shoots.

Only in the presence of GA_3 did accumulation in the epicotyl increase and the increase was independent of concentration but in the treated area only the low concentration of GA_4 and GA_7 and the high concentration of GA_3 were significantly different than the control.

Translocation of GA_1-^3H to the hypocotyl was reduced in the presence of all the gibberellins, probably as a result of increased upward movement. At the higher concentration, all of the gibberellin treatments were significantly less than the control. Significant differences were not observed in the roots or nutrient solution.

A survey of the total radioactivity in all plant parts indicated an increase with increasing amounts of GA_3. Significant differences seen in the epicotyl and treated area with GA_3 treatment may be the result of changes in translocation or differences in GA_1-^3H metabolism.

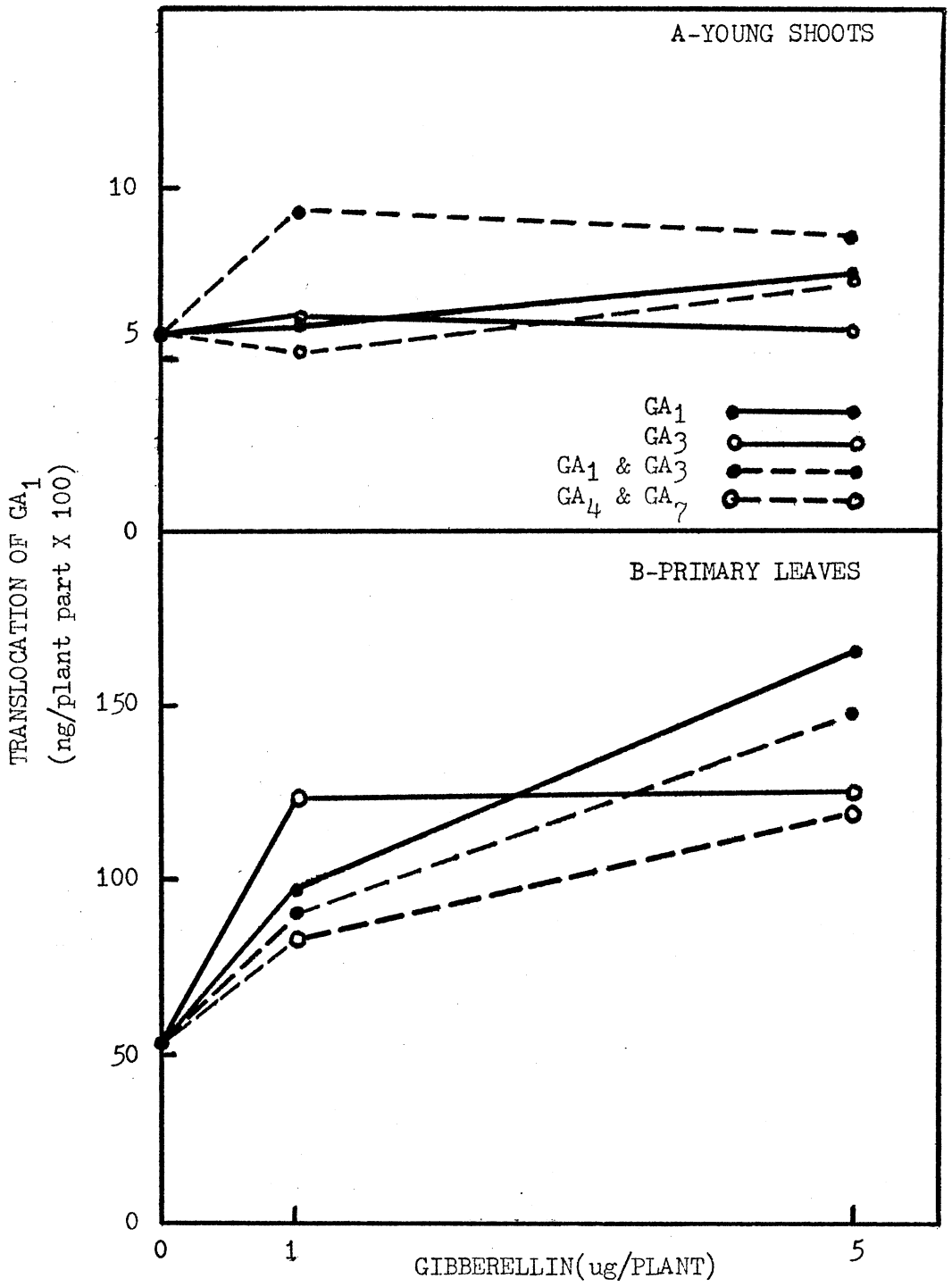

Figure 1. Influence of Gibberellin Concentration on GA_1 Translocation in (A) Young Shoots and (B) Primary Leaves. Each plant was treated with 5.6 ng GA_1-^3H.

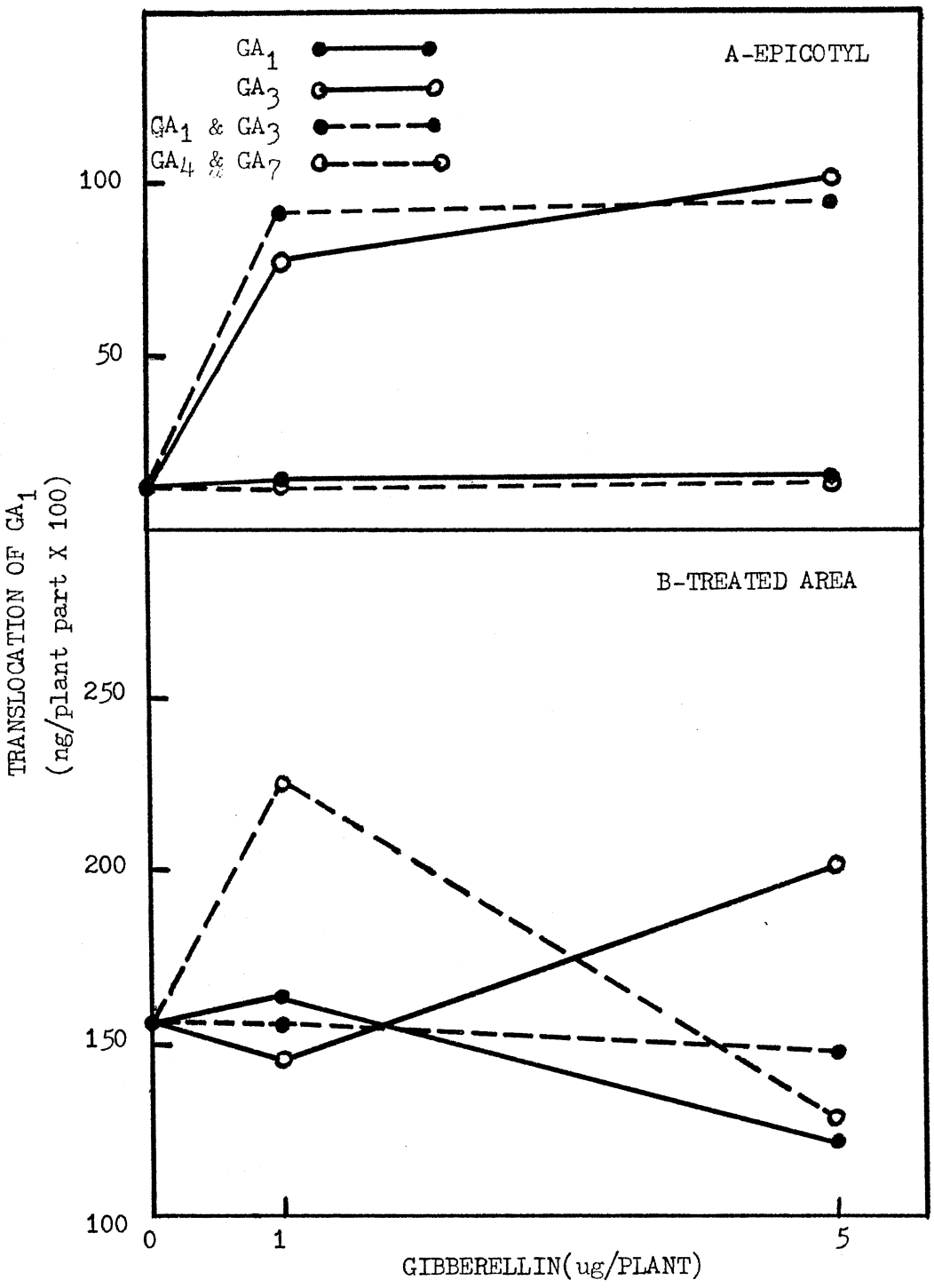

Figure 2. Influence of Gibberellin Concentration on GA_1 Translocation in (A) Epicotyl and (B) Treated Area. Each plant was treated with 5.6 ng GA_1-^3H.

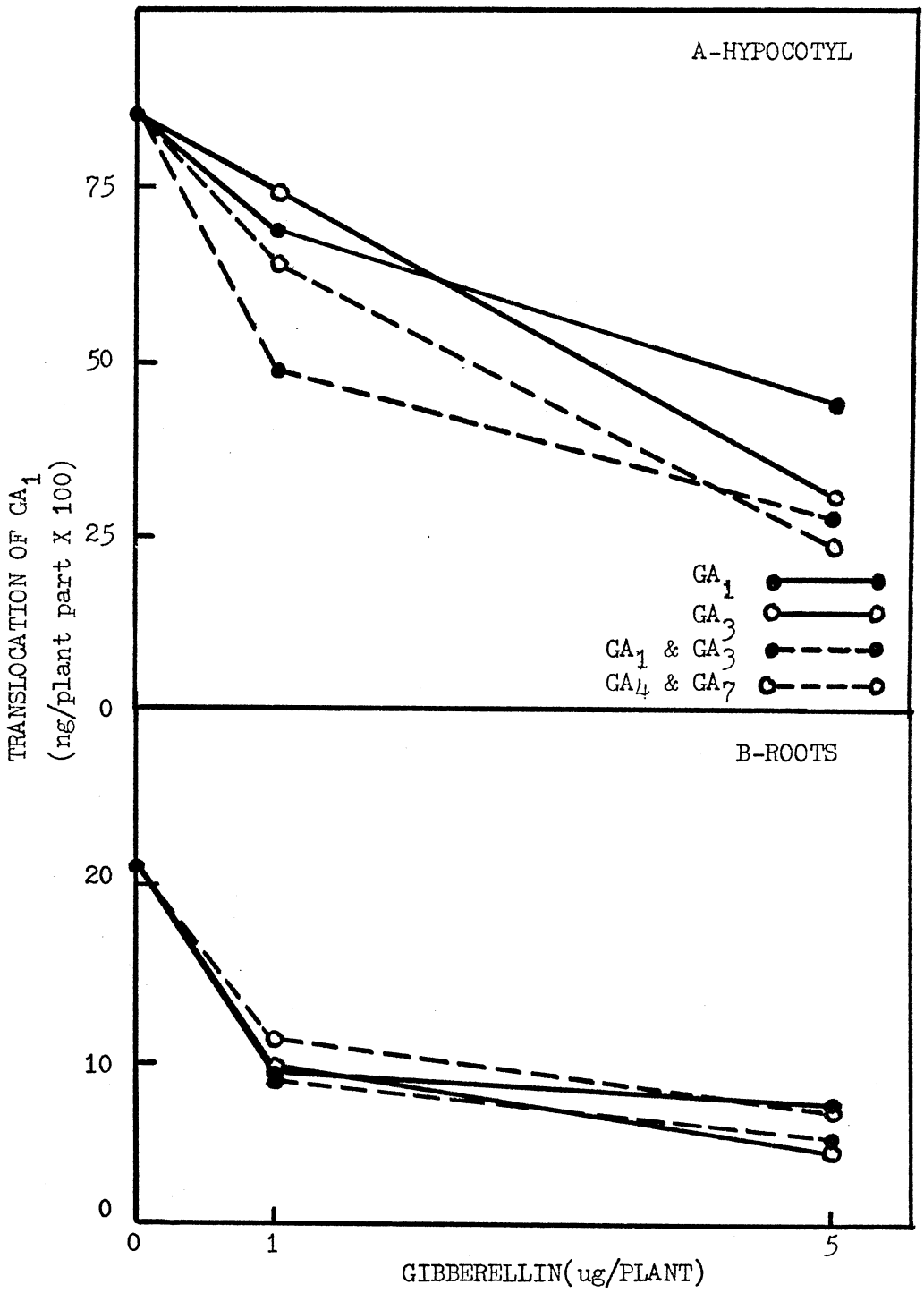

Figure 3. Influence of Gibberellin Concentration on GA_1 Translocation in (A) the Hypocotyl and (B) the Roots. Each plant was treated with 5.6 ng GA_1-^3H.

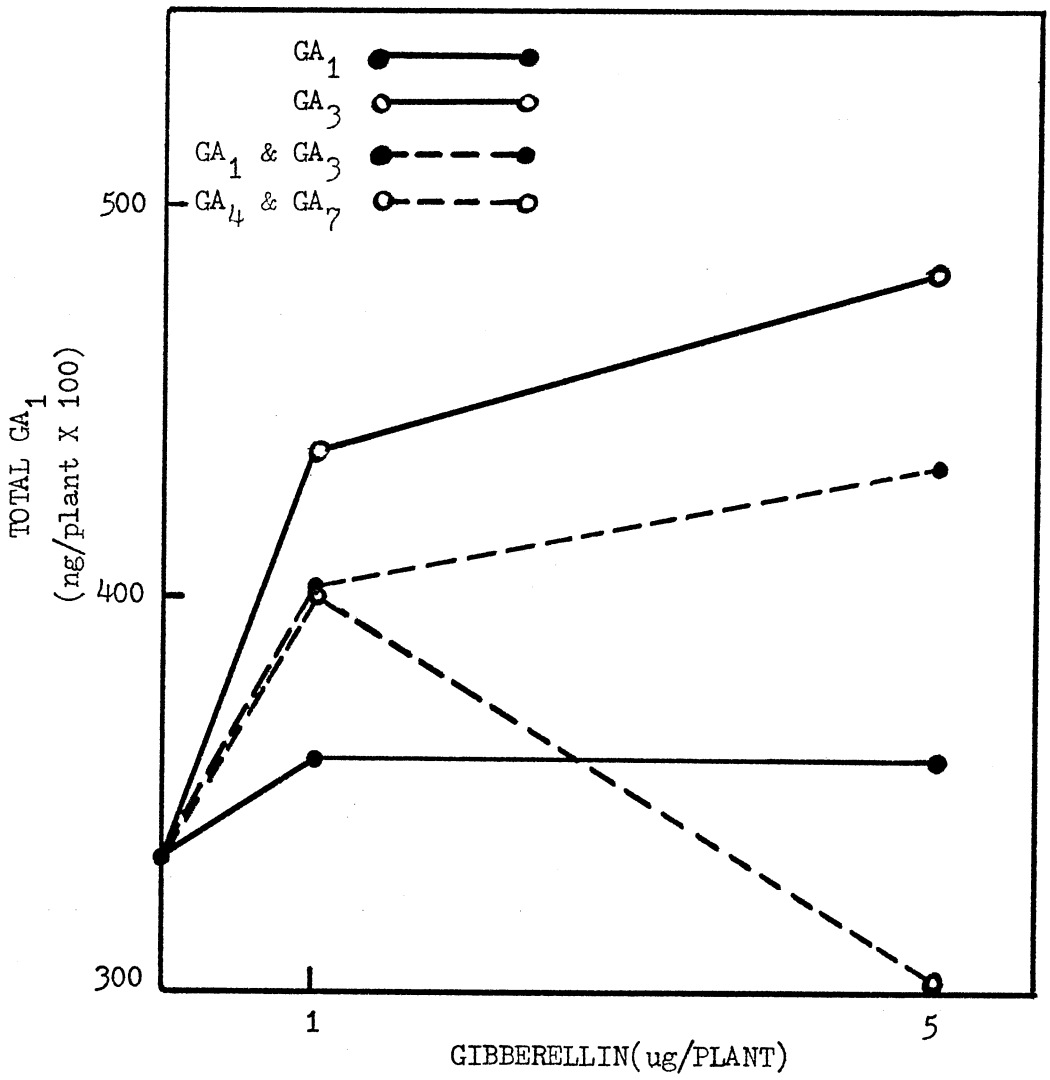

Figure 4. Influence of Gibberellin Concentration on the Total Amount of GA_1 Recovered. Each plant was treated with 5.6 ng GA_1-^3H.

CHAPTER IV

DISCUSSION

Differing responses to darkness treatment and hormone concentration indicate that different mechanisms may be involved in the translocation of these three growth regulators. The dissemination of the three growth substances to all parts of the plants within the four-hour treatment period implies that much of the movement was occurring in the vascular system. After seventeen hours of darkness, carbohydrate levels in the plant should be sufficiently reduced to restrict phloem movement (Wardlaw, 1968). If growth regulators are translocated primarily in phloem tissue with assimilates, a corresponding reduction of hormone movement would be expected. Plant hormone distribution may, however, be strongly influenced by the partitioning of the substances between xylem, phloem and nonvascular tissue with existing conditions having an influence on the pathway taken. Under conditions favoring transpiration, the amount moving in the xylem to mature leaves may increase. When sucrose translocation is proceeding at a maximum rate more may be carried in the phloem to roots and young shoots. The sequestering of growth substances by developing tissues may also have major effects on their distribution. Although mass flow is probably a primary factor in the long-distance translocation of plant hormones, the rate of uptake into cells and the ability to exchange with xylem and nonvascular tissue may have

significant effects on hormone distribution.

In addition to restricting the mass flow of assimilates, an extended period of darkness would be expected to reduce ATP synthesis and decrease transpiration by reducing stomatal openings. Decreases in transpiration would be reflected in movement to primary leaves.

From the pith of the stem, where the treatment solutions were deposited, the hormones may have moved along cell wall interfaces until reaching xylem tissue or crossed cell membranes and entered the symplast, with a portion eventually reaching phloem cells.

The transport of auxin across the plasmalemma may be dependent on a proton gradient established by membrane-bound ATPases (Goldsmith, 1977). In the presence of DCCD, which may prevent cellular uptake through ATPase inhibition, 2,4,5-T was not translocated to an appreciable extent in the assimilate or transpiration stream, suggesting that it must be loaded into living cells even before entering the xylem (Corbett, 1977).

Gibberellic acid has been shown to counteract the effects of DCCD (Corbett, 1977) and to promote acropetal movement of auxin (Basler, 1974), possibly through increased auxin uptake into cells by maintaining active ATPases. During the four-hour treatment in this study, GA_3 did not promote 2,4,5-T movement suggesting that a longer treatment time may be necessary to observe an enhancement of auxin translocation.

Darkness treatment proved to be very restrictive to 2,4,5-T translocation. More than 30-fold less 2,4,5-T-1-^{14}C was detected in the young shoots of dark treated plants compared to the controls. This effect could be due to a curtailment in mass flow or the inability of phloem cells to accumulate 2,4,5-T because of an ATP deficiency. If

the effects of darkness treatment were due strictly to changes in mass flow, similar reductions might be seen with all three growth regulators but maximums of 2-to-3-fold decreases in ABA and GA_1 movement to young shoots was observed after dark treatment.

Gibberellic acid was unable to overcome the inhibitory effects of darkness on 2,4,5-T translocation as it had DCCD effects. It may be possible that gibberellic acid can prevent the inhibition of ATPases by DCCD but it cannot compensate for the reduction in ATP caused by darkness treatment.

A pretreatment of darkness appeared to reduce the movement of ABA through decreases in transpiration and mass flow as movement to young shoots, roots and primary leaves was reduced. The release of ABA from the roots to the nutrient solution was completely inhibited by a dark treatment, suggesting that energy may be required to transfer ABA out of the root cells.

Although a general tendency for GA_3 to promote acropetal movement has been observed (Brian et al., 1954; Halevy et al.; 1964; Basler, 1974) it acted to decrease the movement of ABA in the light. Gibberellin may somehow affect the uptake and release of ABA from parenchyma cells of the stem through changes in ATPase activity, membrane permeability or osmotic potential.

After a darkness treatment, GA_3 reversed most of the inhibitory effects of darkness except the release of ABA to the nutrient solution. Energy may be required to release ABA from the roots and GA and ATP may be necessary to sequester ABA in the epicotyl and treated area of the plant.

ABA appeared to affect its own movement through changes in

transpiration. The effects of darkness treatment and ABA on stomatal resistance appeared to be reflected in the movement of ABA to the primary leaves.

The effects of various treatments on GA_1-^3H translocation are difficult to interpret because there were large differences among the treatments in the amount of labeled GA_1 recovered. These differences may be due to direct effects the treatments had on GA_1 metabolism or may be due to effects on patterns of GA_1 movement.

The synthesis of GA_3 by the plant may involve the conversion of GA_1 to GA_8 and then to GA_3 (Lang, 1970). In this process a double bond is formed and two hydrogens are released. If the plants converted GA_1-^3H to GA_3 the two labeled hydrogens would be lost. The addition of GA_3 may have 'spared' GA_1 from being metabolized as readily by providing enzymes with an alternate substrate.

In the presence of cellular enzymes, a more rapid rate of breakdown would be expected. The treatments may have affected metabolism by influencing uptake into cells and patterns of translocation in the apoplast and symplast.

After a period of darkness treatment there was significantly less GA_1 in parts below the treated area but the difference was approximately equal to differences in the total amount recovered. Darkness may have affected the enzymes involved in catabolism of GA_1 or the uptake and release of GA_1 from the symplast.

The total differences in GA_3 treatments could be attributed mostly to differences in primary leaves. GA_3 may have enhanced the movement of GA_1 in the transpiration stream and consequently protected GA_1 from metabolism or GA_3 may have interfered with gibberellin degradation by

'diluting' the GA_1-^3H.

Although ABA did not significantly change the amount of GA_1-^3H recovered in the light, it did appear to prevent the effect of a dark pretreatment on GA_1 metabolism but ABA did not affect the reduction in basipetal translocation observed after dark treatment. This might indicate that decreases in parts below the treated area after a dark treatment are the result of decreased translocation and not increased breakdown in those parts.

ABA and dark treatment did not decrease accumulation in primary leaves as was the case with ABA-^{14}C, so transpiration rate may not be as important to GA_1 translocation in the absence of additional GA.

The effects of various gibberellin compounds on GA_1 translocation support the idea that GAs enhance the accessibility of GA_1 to the xylem tissue since translocation to young leaves increased and basipetal translocation decreased. The primary difference between gibberellins seemed to be that GA_3 enhanced accumulation in the epicotyl. Since GA_3 treatment also significantly increased the amount of total GA_1-^3H recovered, it's effect in the epicotyl may be due to effects on GA_1 metabolism. GA_3 is one of the most common gibberellins isolated from plant material and it may have a greater affinity for enzymes involved in gibberellin degradation.

CHAPTER V

SUMMARY AND CONCLUSIONS

Carbohydrate synthesis is essential for the maintenance of assimilate mass flow, which appears to be a major mechanism involved in the distribution of plant hormones and applied growth regulators. The prohibition of carbohydrate synthesis by preventing light exposure restricts mass flow of assimilates and possibly hormone translocation.

In this study the translocation of ABA, GA_1 and 2,4,5-T in dark treated plants was examined along with the effects of various hormone treatments on their translocation in normal and dark treated plants.

Darkness treatment proved to be quite restrictive to the translocation of the growth substances, particularly 2,4,5-T's translocation to the young shoots and roots. GA_3 was able to reverse most of the inhibitory effects of darkness pretreatment on ABA translocation but not on 2,4,5-T or GA_1. ABA acted with dark treatment to decrease the movement of ABA to leaves, possibly through increases in stomatal resistance.

Treatment with 5 µg of GA_3 reduced acropetal and basipetal movement of ABA but GA_3 increased the accumulation of GA_1 in primary leaves. The application of various concentrations of GA_1, GA_4 and GA_7 also increased translocation of GA_1 to the mature leaves possibly by increasing accumulation in the xylem.

The total amount of labeled GA_1 recovered after four hours

decreased after a dark pretreatment but increased after GA_3 treatment and this may be due to changes in patterns of translocation or effects of hormone and light treatment on the metabolism of GA_1.

There appears to be more involved in hormone translocation than passive movement in the assimilate stream. Apparently ABA, GA and auxin are not traveling along identical pathways in the plant. Movement from the treatment site may differ because of differences in cellular uptake and varying affinities for xylem and phloem tissue.

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