THE EFFECT OF ABA AND GA ON THE TRANSLOCATION OF AUXIN IN TREE SEEDLINGS

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CHAPTER I

INTRODUCTION

Classical definitions of a plant hormone state that a plant hormone is a substance manufactured in one part of the plant which moves to other parts of the plant where it produces physiological effects at very low concentrations. An integral part of these definitions is that a plant hormone must move from its site of synthesis to the site(s) where it has its physiological effect(s). There are two different pathways by which a plant hormone can move in a plant. Movement of a phytohormone or other substance from cell to cell has been termed transport. Movement of a hormone or other substance through the vascular tissues over much longer distances than with transport has been termed translocation.

Most, if not all, plant researchers believe that plant growth and development is controlled by hormones. These plant hormones have many complex interactions with each other. Many workers in this area also believe that the relative level of each hormone present in a cell, tissue, or organ control the metabolic processes, especially those of growth and development in that cell, tissue, or organ.

At least three factors play a role in determining the level of each hormone in a cell, tissue, or organ. First, the rate of synthesis of a given hormone is critical. Secondly, the rate of movement into or from a cell, tissue, or organ whether by translocation or transport

will affect the level of each hormone in that plant part. And, finally, the rate of destruction or inactivation of a particular plant hormone in a cell, tissue, or organ will affect the level of that hormone in a given plant part. These factors which control the level of each hormone can be, and probably are, different for each phytohormone even in the same tissue or organ.

One of the ways a specific plant hormone can interact with a second hormone is to affect any of the aforementioned factors. There are numerous instances in the literature in which one hormone affects the level of another hormone in a given tissue or organ. In this way a given hormone can influence the processes of plant growth and development by altering the levels of other hormones. This is in addition to its own direct physiological effects on growth and development. The effects of a hormone on the rate of synthesis, destruction or inactivation of another hormone in a particular tissue or organ have also been investigated many times. Much less work has been done on the effects of one hormone on the movement of another hormone. Most of this work has involved the effects of various hormones on indoleacetic acid (IAA) transport in excised tissues of herbaceous plants, usually *Avena* coleoptiles. Relatively little work has been done on the effects of various hormones on IAA transport in intact plants and far less work has been done in auxin translocation. In woody species even less work has been done on auxin transport and essentially no work has been done on auxin translocation.

A recent review by Wareing and Saunders (98) advanced the theory that plant dormancy is under hormonal control. The chief hormones in woody dormancy appear to be abscisic acid (ABA), gibberellic acid (GA),

and IAA. High levels of ABA are associated with the onset and maintenance of dormancy while high levels of GA and IAA are associated with active growth and the breaking of dormancy. Basler (5) has shown that ABA inhibits the movement of auxin into the growing point while GA promotes translocation into the young shoots. In light of their effects on dormancy, one could postulate that ABA and GA regulate plant growth by altering the pattern of auxin translocation to the growing point.

The phytohormone ABA has been shown to be involved with stress. Under conditions of stress, such as drought (33, 68, 103, 104) or salt stress (64), ABA levels rapidly increase and may play a role in causing a temporary cessation of growth. High ABA levels also result in stomatal closure (33). It has been reported that actively growing plants are more readily killed by systemic herbicides than severely stressed plants. For optimum effect the systemic herbicides must be translocated from the site of application to the site where they exert their phytotoxic effects. Since ABA has been shown to increase in stressed plants, the resistance of stressed plants to herbicides could be due, in part, to altered uptake or patterns of herbicides translocation caused by ABA. This could result in the herbicides not reaching its site of phytotoxicity.

This research had several goals. One of these goals was to study the patterns of auxin translocation in tree seedlings. Essentially no work has been done on auxin translocation in trees. A second goal was to study the effects of ABA and GA on auxin translocation in tree seedlings. A third goal was to test the feasibility of ABA and GA exerting their effects on plant growth and development by altering auxin translocation to the growing point and other portions of the

plant. Finally, it was hoped that this study might shed some light on the reasons why stressed plants are relatively insensitive to several systemic herbicides.

CHAPTER II

LITERATURE REVIEW

Auxin Movement

The movement of the naturally occurring auxin, IAA, can occur via two pathways. Auxin transport refers to auxin movement from cell to cell but does not involve the vascular tissues. Since the discovery of auxin, this type of auxin movement has been studied extensively using excised tissues. The second type of auxin movement that occurs only in intact plants is auxin movement through the vascular tissues. This type of auxin movement has been studied by Morris and coworkers (65, 66, 67) in *Pisum sativum* (pea) seedlings, by Basler and coworkers (2, 4, 5, 6, 56) using *Phaseolus vulgaris* (bean) seedlings and by Goldsmith and coworkers (27) using *Coleus* plants.

The classical view of auxin transport is that auxin moves in a basipetal direction from its site of synthesis. This basipetal movement of auxin is termed polar auxin movement. The polar nature of auxin movement is generally thought to be an active process because it fulfills the requirements proposed by Leopold (52). These requirements are: a requirement for metabolic energy (22, 24, 30, 79), ability to "pump" against a concentration gradient (15, 23, 52, 99), selectivity (23, 30, 31, 52, 58), saturable kinetics (22, 30) and a velocity faster than diffusion (22, 24, 26, 44, 99).

Several hypotheses to explain polar auxin transport have been developed. Hertel and Leopold (30) have proposed that the active step involved in auxin transport is the secretion of auxin from cells. They feel that the site of this auxin pump would appear to be located at the plasmalemma. Rubery and Sheldrake (82) have proposed a model for auxin transport. The efflux or influx of auxin is controlled by pH but the movement of auxin is passive. Metabolic energy is used to maintain the pH gradient.

The classical concept of strict polarity in auxin transport has been modified. Many laboratories including Jacobs (39), Leopold and Gurnsey (50), Goldsmith and Wilkins (23) and Leopold and Lam (51) have shown that there is a gradient associated with polarity and that polarity decreases with the age of the cell. Dela Fuente and Leopold (15) showed that excised tissues of increasing length have increasing polarity and this polarity is the result of cell number. A mathematical model developed by Leopold and Hall (54) showed that a ratio of 1.1 auxin molecules moving out of the base of the cell per 1.0 molecule moving into the apical end could account for polar auxin movement. Thus, the polar nature of auxin transport could be the result of a series of small amplifications of transport by each cell.

The effect of other phytohormones on auxin transport in excised segments has been studied. Pilet (77) showed that gibberellic acid enhanced the uptake and rate of movement of auxin applied in donor blocks in stem section of *Lens culinaris* (lentil). Using bean petiole segments, McCready (59) found that GA inhibited basipetal auxin transport and enhanced acropetal auxin movement. He also found that kinetin enhanced basipetal movement and inhibited acropetal auxin movement in

bean petioles. Using lentil epicotyl sections, Pilet (78) found that ABA inhibited the uptake of auxin from donor blocks and inhibited the transport of IAA. ABA pretreatment also inhibited the rate of auxin transport. These data suggest that the other phytohormones have effects on auxin transport in excised tissue segments. However, the use of excised tissue segments has the disadvantage that the vascular system is inoperative in these short segments. The findings of Hoad (36), Hoad and Bowen (37), Maxwell and Painter (57), and Leep and Peel (47) all indicate that phytohormones, including auxin, do occur in the phloem of intact plants. Studies of auxin movement using only excised tissues in which vascular transport is inoperative could lead to erroneous conclusions about the nature of auxin movement in intact plants.

In work done with auxin translocation which occurs in vascular tissues of intact plants, two different responses have been observed. These responses are either a rapid translocation of exogenously applied auxin or a much slower movement that appears to be very similar to polar auxin transport in excised tissues.

The rapid translocation of auxin was first observed with the phenoxy herbicides. When these herbicides were foliarly applied, rapid translocation of these chemicals throughout the plant resulted. Using the synthetic auxin (2,4-dichlorophenoxy)acetic acid (2,4-D), Rohrbaugh and Rice (81) showed that the movement of foliarly applied auxin is associated with export of carbohydrates from the leaf. Eschrich (20) showed that ^{14}C-labeled IAA applied to the primary leaves of young *Vica faba* (broad bean) plants moved out of the leaf and was distributed both acropetally and basipetally in the stem.

Metabolites of IAA formed in the stem appeared to be immobile. Basler, Slife, and Long (4) showed that both the direction and amount of movement of the auxins 2,4-D and (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) injected into the cotylendonary node of young bean seedlings were affected by humidity. High humidity enhanced downward translocation of these auxins while low humidity enhanced acropetal translocation of the auxins. All these data indicated that exogenously applied or injected auxins appeared to move with the assimilation stream in the phloem. The velocity of auxin transport in these experiments appeared to be 10 to 24 cm per hour.

In contrast with these experiments, apically applied auxin is translocated in a much slower, polar manner. Morris, Bryant and Thompson (66) found in pea seedlings that part of the apically applied ^{14}C-labeled IAA was transported unchanged to the root system where it accumulated in the developing lateral root primordia. The velocity of transport was approximately 1.1 cm per hour. A large part of the applied IAA was converted to indole-3-acetyl-aspartate which was not transported. A small part of the applied IAA apparently complexed with protein to form an IAA-protein complex in the shoot apex and in the roots. In the underground portions of these plants, large quantities of the applied IAA were decarboxylated to form indole-3-aldehyde. Hollis and Tepper (38) found that actively growing *Fraxinus americana* (white ash) shoots translocated exogenous IAA applied to the apical bud with a velocity of about 1.3 cm per hour while dormant buds transported the IAA with a velocity of about 0.3 cm per hour. These workers found that only five per cent of the total applied label was mobile. Their data also suggested that IAA was the substance transported.

The discrepancy between apically applied exogenous auxin and other methods of application was resolved by Morris and Kadir (65). They presented convincing evidence that there are two pathways of auxin transport in intact plants. One pathway involves the phloem. It is in this pathway that foliar applications of exogenously applied auxins move. The other pathway is a polar transport mechanism that doesn't involve movement in sieve tubes and in which exogenously applied auxins move at velocities similar to polar, basipetal auxin movement in excised tissues. In a later paper (67) they and Barry also showed that 2,3,5-triiodobenzoic acid (TIBA) also blocked this slow movement of apically applied auxin but had no effect on foliarly applied auxins. Their data suggested that the mechanism of the slow movement of apically applied auxin is the same as the mechanism of polar auxin movement in excised tissues. Goldsmith et al. (27) also showed that stem applied TIBA pretreatment had no effect on the rapid phloem transport system for auxin in *Coleus*. They also showed that TIBA pretreatment to the leaf, significantly decreased the entry of IAA into the midrib vein from a lateral vein in which ^{3}H-labeled IAA was fed. They suggested that the entry of IAA into the phloem is under metabolic control. Using microautoradiography, they also found that IAA is concentrated in the phloem of *Coleus* leaves that were producing and exporting endogenous auxin. They concluded that a special mechanism of physiological importance secretes auxin into the phloem and this entry into the phloem was not a passive process. Pate and Gunning (73) have noted that there is a cell type (transfer cells) which apparently functions in solute loading and unloading from the vascular tissues. They also theorized that these processes of loading and unloading

which are important in the growth and development of plants, are under metabolic control.

The movement of auxin in the translocation system is altered by other plant hormones, particularly gibberellic acid and abscisic acid. Basler (5) showed that ABA enhanced the basipetal translocation of the auxin 2,4,5-T acid and inhibited movement of the auxin to the growing point and primary leaves in bean seedlings. GA enhanced auxin movement to the epicotyl and growing point and inhibited basipetal movement of auxin to the roots and nutrient solution. Experiments using sucrose and glycine suggested that the effects of ABA and GA were relatively specific for auxin since these hormones had little effect on the translocation patterns of sucrose and glycine. The effects of the ABA and GA on auxin translocation were different from their effects on metabolic processes or on altering the assimilate stream. One could speculate that translocation may play a role in plant growth because high ABA and low IAA levels are associated with a cessation of growth while high GA and IAA levels are associated with active growth. While the causes of changes in IAA content in the shoot apex are not known, Basler (5) suggests that the levels of ABA and GA could regulate the growth status of the apex by altering the translocation of auxin into or out of the bud. The possibility that ABA and GA could exert their effects on growth and development in woody plants by altering the translocation pattern of auxin was tested in this research.

Plant Hormones and Growth and Development of Woody Perennials

The relationship between the levels of plant hormones and the growth of woody perennials is not clearly defined and is often contradictory. Since knowledge in this area is very incomplete, a plethora of theories has resulted but none of these offer a satisfactory explanation. Almost all of the work on the effect of plant hormones on the growth and development of woody species has been to determine the hormonal basis for bud dormancy. As a result it is necessary to discuss dormancy to review the available literature on plant hormones and their effect on the growth and development of woody perennials.

One of the earliest theories for woody plant dormancy implicated inhibitory concentrations of auxins. This idea was first advanced by van Overbeek (71) and was supported by Michener (61). Eggert (18) also concluded that supra-optimal concentrations of auxins caused bud dormancy. However, Zimmerman (106) had previously concluded that it was a lack of auxin that caused bud dormancy as did Skoog (88). Samish (83) also concluded that supra-optimal auxin contents didn't occur and rules out this mechanism of dormancy. An alternative theory to explain bud dormancy was proposed by Bennett and Skoog (7) using *Pyrus communis* (pear). These workers found that auxin content in dormant buds increased as the degree of dormancy decreased. They suggested that a lack of auxin was the cause of dormancy. This idea was supported by Kassem (41) who showed dormant pear buds were low in free auxin which increased as spring approached. Evidence from other species indicates that low auxin levels do occur in dormant buds of many, but not all, species and that limiting auxin levels provide a general mechanism for

dormancy. Hemberg (29) found that the level of auxin in dormant and actively growing potato buds remain constant. The data of Bennett and Skoog (7) also do not suggest why auxin levels increase in the spring or decrease in the fall. In addition this idea does not explain the role of other phytohormones which we now know are involved.

Hemberg (29) was the first to propose that there was an inhibitor that caused dormancy. This idea was strengthened by data of Steward and Caplin (86) who also found an inhibitory substance present in dormant buds which decreased markedly by the end of the dormant season but did not characterize it. Bonde (9) also presented evidence in support of Hemberg's hypothesis.

Work by Wareing's group also provided strong evidence supporting the inhibitor concept. Wareing's interest in an inhibitory substance came from some work on photoperiodism (21) and the induction of dormancy. In *Acer pseudoplatanus* (sycamore) and *Robinia pseudacacia* (black locust) he (95) found that the leaves when exposed to long days caused the buds to go dormant. He felt that this meant that there was an inhibitor produced in the leaves which was translocated to the shoot apex inducing morphological changes and inducing dormancy. Wareing and coworkers (12, 17, 74, 75, 95) proceeded to study this inhibitor (abscisic acid), its relationship to dormancy, and to isolate and characterize it.

Phillips and Wareing (74) studied the changes in inhibitor content for a one year period. They found the concentration of the inhibitor in the tissues showed a positive correlation with the state of dormancy in sycamore tissues. They concluded that the mature leaves produced the inhibitor which was translocated to the shoot apex where it

accumulated. The level of the inhibitor declined during the winter but low levels of it were present even during active growth. They postulated that growth in sycamore could be controlled by a balance between the inhibitor and an unknown auxin or some other growth promoting substance not detected by the wheat coleoptile section assay. In a later paper (75) they were able to show that two cycles of short days caused an increase in the inhibitor content of leaves before any obvious cessation of growth occurred. Another worker, Kawase (42), showed that there was a decrease in the inhibitor β which paralleled emergence from dormancy for *Diospyros virginiana* (eastern persimmon), *Pyrus malus* (apple), *Prunus persica* (peach), and *Ulmus americana* (american elm).

The inhibitor theory proposed by Hemberg and supported, in part at least, by Wareing's group was challenged on several grounds. The *Avena* coleoptile test, which was the bioassay used in many of these experiments, is not specific for dormancy regulating substances. It is very possible that numerous substances could inhibit extension growth of coleoptile sections and not play a role in the regulation of dormancy. Wareing and Saunders (98) have also mentioned this possibility. The inhibitory substances could have risen as a consequence of dormancy rather than having a causal role in dormancy. Burton (11) showed that sprouting of *Solanum tuberosum* (potato) did not necessarily coincide with the disappearance of the inhibitor β which was present in rapidly expanding tissues as well as dormant tissues. These results suggest that perhaps growth promoting substances also play a role in regulating bud dormancy. Kawase and Nitsch (43) presented evidence that supported a role for growth

promoting substances. These workers demonstrated that short days caused an increase of inhibitory activity in *Betula pubescens* (birch) grown in short day conditions. The increase of inhibitory substances could be prevented by a light break or by GA_3 treatment. They also showed that the onset of dormancy could be prevented by a light break or GA_3. Their conclusions were supported by data from Wareing's laboratory.

Eagles and Wareing (17) tested the hypothesis that a growth promotor had a role in dormancy. They showed that application of the inhibitor to young leaves caused production of dormant buds. They found that 1 µg of GA_3 broke the dormancy of birch seedlings even though they were grown under short day conditions. They demonstrated that GA_3 enhanced bud break of buds collected in March and that application of the inhibitor delayed bud break. These workers also showed that high levels of GA_3 overcame the effect of the inhibitor. Eagles and Wareing suggested that in some species a decline in the inhibitors resulted in emergence from dormancy. In other species, they felt that the degree of dormancy was due to a balance between endogenous promotors and inhibitors. They proposed the name dormin for the inhibitors that function in regulation of dormancy.

Wareing's group (12) isolated one compound and showed that dormin was identical to "abscisin II". Abscisin II is a compound which hastened abscission in *Gossypium hirsutum* (cotton) explants (stem sections) and was isolated from cotton by Okhuma et al. (69). The chemical structure was established by Okhuma et al. (70) and confirmed by synthesis by Cornforth et al. (13). The accepted nomenclature for abscisin II or dormin is abscisic acid frequently shortened to ABA.

El-Antably et al. (19) using synthetic abscisic acid found that it produced dormant buds in birch, *Ribes nigrum* (blackcurrant), and sycamore even when these woody plants were growing under long day conditions. Simply spraying the leaves with ABA solutions produced dormant buds in blackcurrant but not in birch and sycamore. Their data suggested that ABA has low rates of foliar penetration when applied to the leaf surfaces. Blummenfeld and Bukovac (8) showed that 6 to 12 times less ABA penetrated isolated leaf cuticles than naphthalene acetic acid (NAA) or 2,4-D. They concluded that penetration could be a limiting factor for foliarily applied ABA.

El-Antably et al. (19) also studied other physiological responses to ABA. They found that ABA inhibited sprout growth of potato buds. ABA enhanced the senescence of leaf discs of a wide variety of species but is ineffective in bringing about the senescence of intact attached leaves. Abscisic acid also inhibited flowering of the long day plant *Lolium temulentum* (darnel). In short day plants ABA was shown to stimulate flowering. In his review, Milborrow (63) concluded that ABA has a weak but consistent promoting effect on flower growth in short-day plants. They concluded that ABA was the plant hormone causing dormancy in many plants.

There are several lines of evidence for this conclusion. Exogenously applied ABA can cause formation of dormant buds in many woody species. Using bioassay techniques it has been shown that the content of an inhibitor increases as plants enter dormancy. Bowen and Hoad (10), and Hoad (36) using the *Triticum aestivum* (wheat) coleoptile test, have shown in *Salix viminalis* (willow), that inhibitor levels in the phloem and xylem sap increase as plants enter dormancy. Work

by Stewart (87) showed that ABA induced turion (a specialized dormant frond or leaf) formation in the Lemnaceae. Turion formation is important for the overwintering in this group.

There is also a growth promoter involved with the breaking of dormancy. This promoter is one or more gibberellin-like substances. Work by Kawase and Nitsch (43) and by Eagles and Wareing (17) showed that GA_3 was effective in preventing dormancy of birch plants grown under short days. Gibberellic acid and cytokinins are frequently effective in overcoming dormancy of buds and other resting structures as has been pointed out in a review by Wareing.

On the basis of these experimental conclusions, Wareing and Saunders (98) suggested that bud dormancy was the result of an interaction between growth inhibiting substances (mainly ABA) and growth promoting hormones, probably gibberellic acid(s). They further suggested that ABA promotes the repression of deoxyribonucleic acid (DNA) during the induction of dormancy and that gibberellins reversed this effect in breaking dormancy. They based this conclusion on work by Villiers (94) and by Varner and Chandra (93). Villiers concluded that ABA maintained dormancy by blocking production of specific messenger ribonucleic acids (mRNA's). Varner and Chandra (93) demonstrated that GA_3 stimulated α-amylase induction in barley endosperm which was blocked by ABA. Ho and Varner (34, 35) also showed that ABA caused the production of a specific mRNA which was involved. Wareing and Saunders (98) further point out that the balance between ABA and GA is not the only factor involved in dormancy because gibberellins are not able to overcome bud or embryo dormancy in all species. It should be pointed out that most

of their conclusions were based on the use of bioassays and not based on the quantitative measurement of ABA.

Quantitative methods of measuring ABA were developed at about this time. Milborrow (62) had developed a spectropolarimetric technique which had been used to estimate the ABA levels of aphid honeydew. Preliminary results cited by Wareing and Saunders (98) showed an increase in ABA levels when willow plants were transferred from long days to short days. Lenton et al. (48) have developed a second technique utilizing gas liquid chromatography. Using this technique Lenton et al. (49) measured the ABA content of long day and short day plants of sycamore, birch, and *Acer rubum* (red maple). These workers found that all these species of plants under short day conditions had approximately the same ABA content as long day plants. They also assayed for gibberellin-like activity using the *Lactuca sativa* (lettuce) hypocotyl bioassay and showed that there was significantly less gibberellin-like activity in short day plants than in long day plants. They also showed that there was a decrease in gibberellin-like activity when plants were transferred from long day to short day conditions. Lenton et al. (49) discounted the possibility of ABA not being involved in dormancy. They suggested that ABA synthesized in the leaves is transported to the shoot apex where it inhibits growth and induces dormancy unless it is accompanied by compounds that can overcome the inhibitory effects of ABA. These compounds could be gibberellins. They concluded that the balance between growth promoters and growth inhibitors was altered to favor inhibition by a decrease in growth promoters. Their results were supported by data from Zeevart's laboratory. Zeevart (104, 105)

showed there was an increase in ABA content when *Spinacia oleracea* (spinach) plants were transferred to long days from short days. He concluded that the stimulatory effect on growth by long day conditions could be explained by increased rate of gibberellin synthesis and increased sensitivity to gibberellins. Harrison and Saunders (28) have shown that in *Betula verrucora* (birch) the proportion of esterified ABA in buds underwent a progressive increase that could be associated with emergence from dormancy.

Wareing, Good and Manuel (96) also showed that exogenous ABA reduced the levels of endogenous gibberellins in *Zea mays* (corn) and inhibited the increase of endogenous gibberellins in spinach that occurred when plants were transferred from short days to long days. Using long day *Trifolium pratense* (red clover), Stoddart and Lang (89) found more gibberellins were produced under short day conditions than under long day conditions.

In addition to inhibitors and gibberellins being involved in dormancy, it has recently become apparent that cytokinins also play a role in plant dormancy. Pieniazek (76) found that kinetin was able to cause bud break in apple and suggested that cytokinins are the growth regulators involved in breaking dormancy in apple. Reid and Burrows (80) showed that cytokinin activity increased in spring sap prior to bud break. Hewett and Wareing (32) followed cytokinin levels in *Populus* x *robusta* over the year. They found that from nondetectable levels in December and January, levels of cytokinins increased in both the sap and buds. The maximum level in the sap occurred prior to the maximum in the buds. Tucker and Mansfield (90, 91) showed that ABA levels in lateral dormant buds were 50 to 250 times that of released

lateral buds of *Xanthium strumarium* (cocklebur). The levels of endogenous cytokinins were much higher in dormant buds than in actively growing buds. These workers suggested that the high concentration of ABA in the dormant buds of cocklebur kept these buds from responding to the high endogenous levels of cytokinins.

Although the concept of ABA being a major hormone in plant dormancy has been challenged, the evidence for involvement of ABA with dormancy is convincing. It should be pointed out that in a recent publication by Lesham, Philosoph, and Wurzberger (55) it was concluded that there was a decrease in the amount of free trans-ABA while the total amount of ABA remained constant during emergence from dormancy.

From the results above, it can be concluded that there is a hormonal basis for the control of growth. The control mechanisms involve interactions between various plant hormones. These interactions are complex depending on both the hormones present and the quantities of each hormone relative to each other. The control mechanisms may differ from species to species. Khan (45) has proposed a model for hormonal activity in which hormones play different roles. He characterizes these roles as primary, preventive, and permissive. If the primary hormone is present, a preventive hormone's presence would keep the primary hormone from being active. A permissive hormone would allow the primary hormone to exert a physiological effect. The effect of the preventive hormone can be overcome by high levels of the permissive hormone or by application of exogenous permissive hormone. If the primary hormone is absent then the presence of the permissive hormone would have no effect. His concept

is attractive because it can explain many of the effects of exogenously applied hormones to plant systems. Further work is needed to verify his ideas.

Physiological Implications for Herbicide Susceptibility

Several weed scientists (60, 92) have noted that a plant must be healthy and actively growing for the phenoxy herbicides to be effective. Plants that are quiescent or dormant may have markedly lower susceptibility to herbicides. It is also generally accepted that for systemic herbicides to be effective they must enter the plant and be transported to the site where they exert their phytotoxic effects. The levels of ABA rapidly increase in stressed plants. It is surprising that no work has been done that attempts to link ABA and the decreased susceptibility of stressed plants to herbicides.

Several papers (16, 33, 64, 104) have shown that stressed or dormant plants have considerably higher ABA levels. The relationships between dormancy and ABA levels have been discussed above. Wright (103) showed that wilting caused a marked increase in the inhibitor β content of detached, wilted, wheat leaves and that there was an increase in the ABA content of the detached wheat leaves. Kriedemann et al. (46) have shown that ABA is involved in stomatal regulation. Using bean seedlings, Hiron and Wright (33) suggested that ABA caused stomatal closure. They also found upon rewatering that free ABA levels rapidly declined but there was an increase in a bound form of ABA that appeared to be the glucose ester. Dorffling et al. (16) also found a rapid decrease in ABA levels in water stressed pea seedlings but there was

no formation of a glucose ester in seedlings recovering from water stress. They suggested that the decline in ABA levels was due to metabolic alteration of the ABA molecule to an inactive form. Salt stress (64) has also been shown to increase ABA levels.

Several papers have linked stress and translocation of phenoxy herbicides. Pallas (72) concluded that translocation of 2,4-D was greatly reduced in plants grown in soil near the permanent wilting point. Basler, Todd, and Meyer (2) showed that drought stress decreased 2,4-D translocation in bean seedlings. Plants, with relative turgidities below 80 per cent, translocated only trace amounts of 2,4-D. They found that as soil moisture declined, the translocation of 2,4-D also declined, but drought stress did not appear to alter the absorption of foliarly applied 2,4-D. Plants first subjected to drought stress and then rewatered rapidly recovered turgidity, but the ability to translocate 2,4-D was recovered at a much slower rate. This pattern of translocation recovery is very similar to the decrease in ABA levels in the experiments by Hiron and Wright (33). Merkle and David (60) found similar results. They found that foliar absorption of 2,4,5-T and 4-amino-3,5,6-trichloropicolinic acid (picloram) by bean seedlings was unaffected by severe moisture stress, but translocation of 2,4,5-T and picloram was markedly reduced. Moderate water stress decreased 2,4,5-T translocation but had no effect on picloram movement. Badiei, Basler, and Santelmann (1) showed that absorption and translocation of 2,4,5-T were inversely related to soil moisture in *Quercus marilandica* (blackjack oak). A decrease in soil moisture caused a moderate decline in absorption but markedly reduced translocation of 2,4,5-T. Wills and Basler (101) showed that variations in soil moisture

had little effect on absorption of 2,4,5-T, but a decrease in soil moisture reduced translocation of 2,4,5-T in *Ulmus alata* (winged elm). Basler and Slife (6) showed hydroponically grown bean plants treated with various salts in the nutrient solution, in general, translocated 2,4,5-T in much of the same way as plants treated with ABA. Since ABA has been shown to increase in plants under a salt stress (64), it seems possible that the ABA production induced by salt treatment could have been the cause of the decreased acropetal translocation and the increased basipetal translocation of 2,4,5-T.

The observations presented above indicate that treatments that increased ABA levels decreased the translocation of the phenoxy herbicides in stressed plants. This decreased translocation of the phenoxy herbicides might be the physiological basis as to why stressed plants are less affected by these compounds than unstressed plants.

CHAPTER III

MATERIALS AND METHODS

The first stage of this work was to find a suitable species to conduct detailed studies of auxin translocation and the effects of ABA and GA_3 on auxin translocation. This consisted of doing a series of experiments with various tree species. The species used in these studies were *Quercus marilandica* Muench. (blackjack oak), *Quercus macrocarpa*, Michx. (bur oak), *Juglans nigra*, L. (black walnut), and *Diospyros virginiana*, L. (eastern persimmon). The experimental methods used in conjunction with each of these species is given below. Black walnut and eastern persimmon were selected for more detailed studies and the techniques used in these studies are also given below.

The auxin chosen for use in this study was 2,4,5-T, a synthetic auxin (58). This auxin has several advantages over IAA for studies involving auxin movement. McCready (58) has shown that 2,4,5-T moved like IAA in polar transport and that large quantities are translocated. Basler (3) has shown that isolated leaf discs of various tree species had low rates of breakdown of the phenoxy herbicides. On the other hand, exogenous IAA when applied to plants is broken down and/or complexed to other chemicals. These chemical reactions function to remove auxin from the transport system. In addition, IAA is subject to photo-oxidation.

Blackjack Oak

Blackjack oak acorns were collected near Stillwater, Oklahoma, stratified at 4 C for at least three months and stored at 4 C in moist perlite until used for experimental purposes. The acorns were planted in perlite in plastic dishpans and watered with 1/2 strength Hoagland's solution. Distilled water was added as necessary to keep the perlite moist. The acorns were germinated under constant illumination of about 8 Klux supplied by cool white fluorescent lights at 33 C. Eleven days after planting, the seedlings with their attached cotyledons were transferred to liquid culture.

The seedlings were washed to remove perlite and transferred to amber glass bottles containing 480 ml of continuously aerated 1/2 strength Hoagland's solution containing 10 ppm Fe as the Fe-EDTA chelate. The seedlings were placed in a growth chamber with 14 hours, 33 C day and 10 hour, 29 C night. The light intensity was about 20 Klux and the relative humidity ranged from 20 to 30% during the course of the experiments. Three days later the plants were transferred to fresh 1/2 strength Hoagland's solution and returned to the growth chamber.

The plants were treated three days later by injecting 0.5 µg of carboxyl ^{14}C-labeled 2,4,5-T (2,4,5-T-1-^{14}C) in 1 µl of ethanol or 1 µl of ethanol containing both 0.5 µg of the 2,4,5-T-1-^{14}C and 10 µg ABA into one of the cotyledons using a 1 µl syringe. The 2,4,5-T-1-^{14}C had a specific activity of 21 mCi/mmole and the ABA was a 90% mixture of synthetic isomers obtained from Sigma Chemical Company. A completely randomized experimental design was used. Twenty-four hours after treatment the plants were harvested.

The root system of each plant was rinsed to remove any surface activity from the nutrient solution, blotted dry, and the plant was divided into seven parts (Figure 1) for analysis. These parts were the growing point which consisted of the shoot apex and expanding leaves, the leaves, upper stem which consisted of all stem tissue below the growing point and above the lower stem, lower stem which was a 4 cm section from the cotyledonary node up to the upper stem, treated cotyledon, untreated cotyledon, and roots which were all portions of the seedling below the cotyledonary node. The plant parts were frozen immediately after harvest at -20 C and later lypohilized. After the nutrient solutions were adjusted to 480 ml by addition of distilled water, a 5 ml aliquot was frozen and later lyophilized and assayed for ^{14}C activity by liquid scintillation counting. The lyophilized plant parts were weighed and then homogenized in 10 ml of 95% ethanol using a Virtis homogenizer. Aliquots of the homogenized tissue were taken and assayed for ^{14}C activity by liquid scintillation counting.

Bur Oak

Bur oak acorns were collected near Stillwater, Oklahoma and stratified at 4 C for 3 months and stored at 4 C in moist perlite until used for experimental purposes.

The acorns were germinated and the seedlings transferred after nineteen days to liquid culture as described for blackjack oak. The light intensity was about 23 Klux and the relative humidity ranged from 20 to 40% during the course of the experiments. Six days later the seedlings were transferred to fresh 1/2 strength Hoagland's

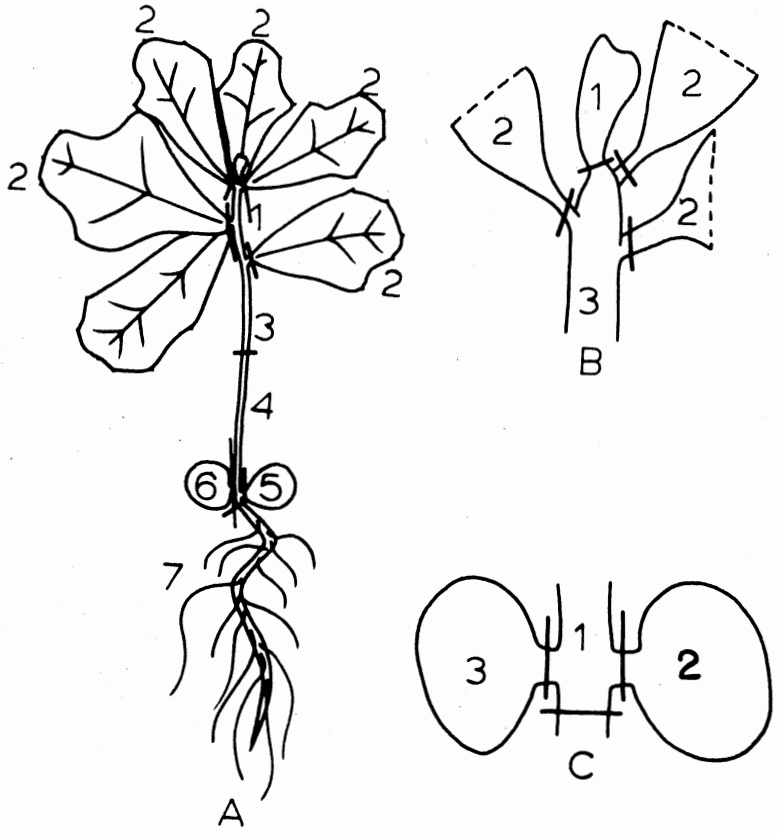

Figure 1. A. Diagram of Blackjack Oak Seedling:
1. Growing Point 2. Leaves
3. Upper Stem 4. Lower Stem
5. Treated Cotyledon 6. Untreated Cotyledon 7. Roots.
B. Detail of the Growing Point: 1. Growing Point 2. Leaves 3. Upper Stem
C. Detail of the Cotyledonary Node:
1. Lower Stem 2. Treated Cotyledon
3. Untreated Cotyledon

solution. The seedlings were treated the next day.

There were three treatments. Plants were treated by injecting 1 µl of ethanol containing 0.5 µg of 2,4,5-T-1-^{14}C, or by injecting 1 µl of ethanol containing both the 2,4,5,-T-1-^{14}C and 10 µg of ABA into the cotyledon, or in the third group of seedlings 10 µg of GA_3 were substituted for the ABA. The 2,4,5-T-1-^{14}C had a specific activity of 31 mCi/mmole. The ABA and GA_3 were obtained from the Sigma Chemical Company and had a purity of 90%. A completely randomized design was used in these experiments. Twenty-four hours after treatment the plants were harvested. The root system of each plant was rinsed to remove any residual activity from the nutrient solution, blotted dry, and the plant was divided into seven parts (Figure 2) for ^{14}C analysis. The parts consisted of the growing point, leaves, upper stem which consisted of all stem tissue from the growing point to the lower stem, lower stem which was an 8 cm section from the cotyledonary node up to the upper stem, treated cotyledon, untreated cotyledon and the roots which consisted of all portions of the plant below the cotyledonary node. The plant parts were frozen immediately after harvest at -25 C and then lyophilized. The lyophilized plant parts and nutrient solutions were treated as described for blackjack oak except a Polytron homogenizer was used instead of the Virtis.

Black Walnut

Hulled black walnut seeds collected near Stillwater, Oklahoma were stratified at 3 C for five months and stored at 3 C in moist perlite. Some experiments were conducted with walnuts planted in metal flats using a 1 to 1 mixture of sandy soil and vermiculite. The flats were

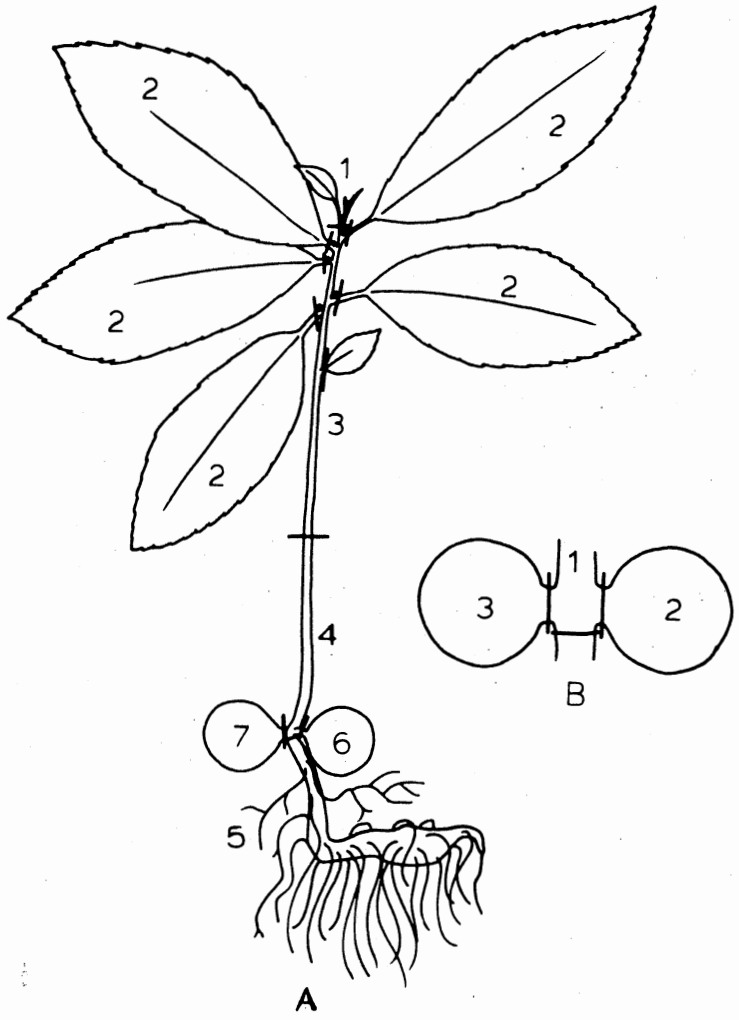

Figure 2. A. Diagram of Bur Oak Seedling: 1. Growing Point 2. Leaves 3. Upper Stem 4. Lower Stem 5. Roots 6. Treated Cotyledon 7. Untreated Cotyledon
B. Detail of Cotyledonary Node: 1. Lower Stem 2. Treated Cotyledon 3. Untreated Cotyledon

placed in a greenhouse and the walnuts were allowed to germinate. The temperature ranged between 20 C and 33 C. Supplemental light was supplied by cool white fluorescent bulbs to maintain a 14-hour day photoperiod. The flats were watered as needed with tap water. After two weeks the flats were watered one time a week with 1/2 strength Hoagland's solution to supply nutrients. Three weeks after planting the seedlings were thinned and transferred to a large walk-in growth chamber. The plants were maintained under a 14-hour, 33 C day and a 10-hour, 29 C night in the chamber. A mixture of cool-white fluorescent lampsand incandescent lamps supplied light with a total intensity of about 30 Klux. The relative humidity ranged between 30 and 60%. The plants were treated 4 weeks after planting.

Several different kinds of experiments were conducted with walnut seedlings grown in this manner. In one experiment the plants were treated by injecting 1 µl of ethanol containing plant hormones into the stem at soil level using a 1 µl syringe. The stem of these seedlings had a central core of pith surrounded by a relatively thin layer of xylem. In the first experiment of this series the 1 µl of ethanol contained either 0.5 µg of 2,4,5-T-1-^{14}C (31 mCi/mmole) or 0.5 µg of 2,4,5-T-1-^{14}C and 25 µg of ABA. The seedlings were harvested 24 hours after they were treated.

During the harvesting process, the seedlings and root system were removed from the soil and the lower portion of each plant was rinsed to remove any adhering soil and vermiculite. Then each seedling was divided into six parts (Figure 3). These parts were the growing point with the expanding leaves, all other leaves, upper stem, treated area which was 6 cm long and extended from the cotyledonary node to

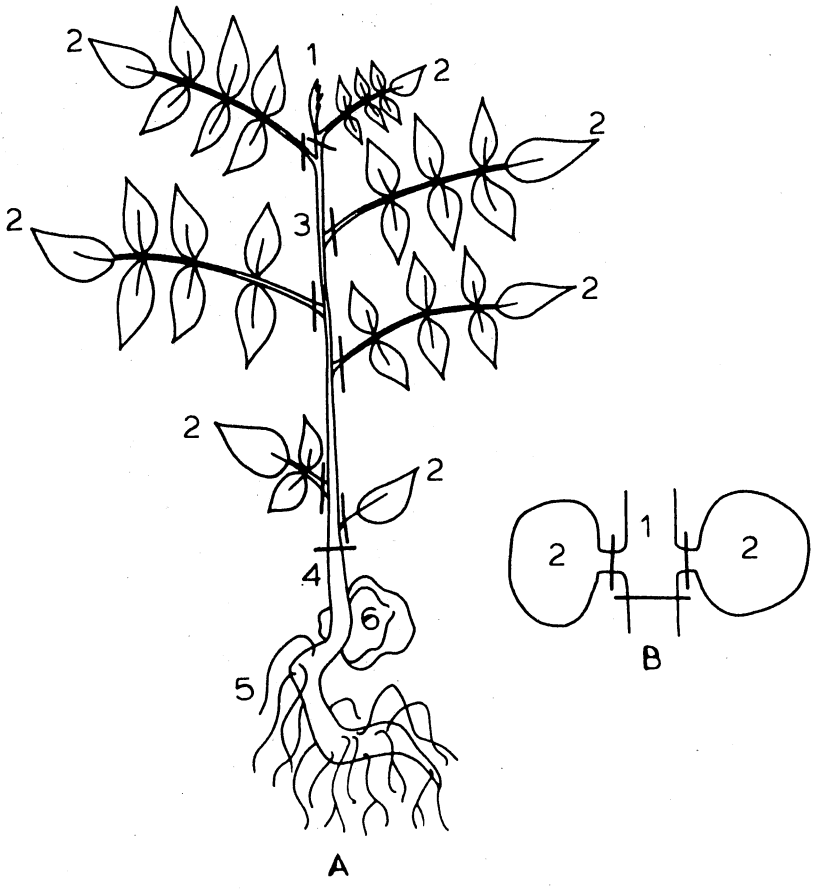

Figure 3. A. Diagram of a Stem Injected Walnut Seedling: 1. Growing Point 2. Leaves 3. Upper Stem 4. Treated Area 5. Roots 6. Cotyledon
B. Detail of Cotyledonary Node: 1. Treated Area 2. Cotyledons

the upper stem, roots and the cotyledons. The plant parts were frozen at -25 C and lyophilized. The nutmeats from the cotyledons were removed from the shell for weighing and the outer shell discarded. The dried plant parts were handled as described for bur oak.

Two other experiments were performed using this stem injection technique. In one of these experiments, black walnut seedlings were pretreated by injecting plant hormones into the stem. Three groups of plants were used in this experiment. Each of the control groups was injected with 1 µl of ethanol. Another group was injected with 1 µl of ethanol containing 10 µg of ABA while the final group was injected with 1 µl of ethanol containing 10 µg of GA_3. Twenty-four hours later each plant was injected with 1 µl of ethanol containing 0.5 µg of 2,4,5-T-1-^{14}C in the same place that the pretreatment solution was injected. The plants were harvested at the end of 24 hours as described earlier.

The technique of stem injecting the plant hormones had several disadvantages. It was very difficult to penetrate the xylem tissues with the syringe needle. It was difficult to remove adhering soil and vermiculite from roots without damaging them. Some of the studies had indicated that there was a substance or substances enhancing growth of the seedlings produced by the cotyledons. It was felt that the removal of the cotyledons would simplify the system and eliminate this growth promoter from antagonizing the effects of ABA. In addition, treating the plants while they were in liquid culture would permit determination of the activity exuded by the roots.

The walnuts used in these later experiments were planted in individual 1 liter plastic containers using sand as a potting medium.

The walnuts were germinated as described above. The containers were watered as needed for 2 weeks. Then, once a week, they were watered with 1/2 strength Hoagland's solution. The other waterings during the week were tap water. When the walnut seedlings were approximately 4 weeks old they were transferred to a large walk-in growth chamber under the conditions previously described. Approximately 5 weeks after planting the seedlings were transferred to liquid culture. The walnut seedlings were rinsed with water to free the root system of sand and transferred to amber glass bottles containing 260 ml of continuously aerated 1/2 strength Hoagland's solution with 5 ppm Fe as the Fe-EDTA chelate. Distilled water was added to the bottles every second day to maintain the volume. The conditions in the chamber were the same as before. After 6 days the seedlings were transferred to fresh 1/2 strength Hoagland's solution and returned to the growth chamber. The plants were treated on the seventh day.

The technique used in these experiments was to allow the plant to absorb the hormones through a petiole stump. The third fully expanded leaf of each walnut seedling was excised leaving a petiole stump approximately 2 cm long. A piece of 0.318 cm tygon tubing was placed on the stump as shown in the inset of Figure 4. The treatment solutions containing plant hormones were injected into the tubing using a 10 µl syringe. The tygon tubing was capped and solutions were taken up by the plant within 5 minutes.

Treatment solutions were prepared by reacting each of the plant hormones used in these experiments with equimolar amounts of $KHCO_3$ to form the potassium salts. Each 10 µl of the treatment solution contained 0.5 µg of 2,4,5-T-1-^{14}C as the potassium salt and some

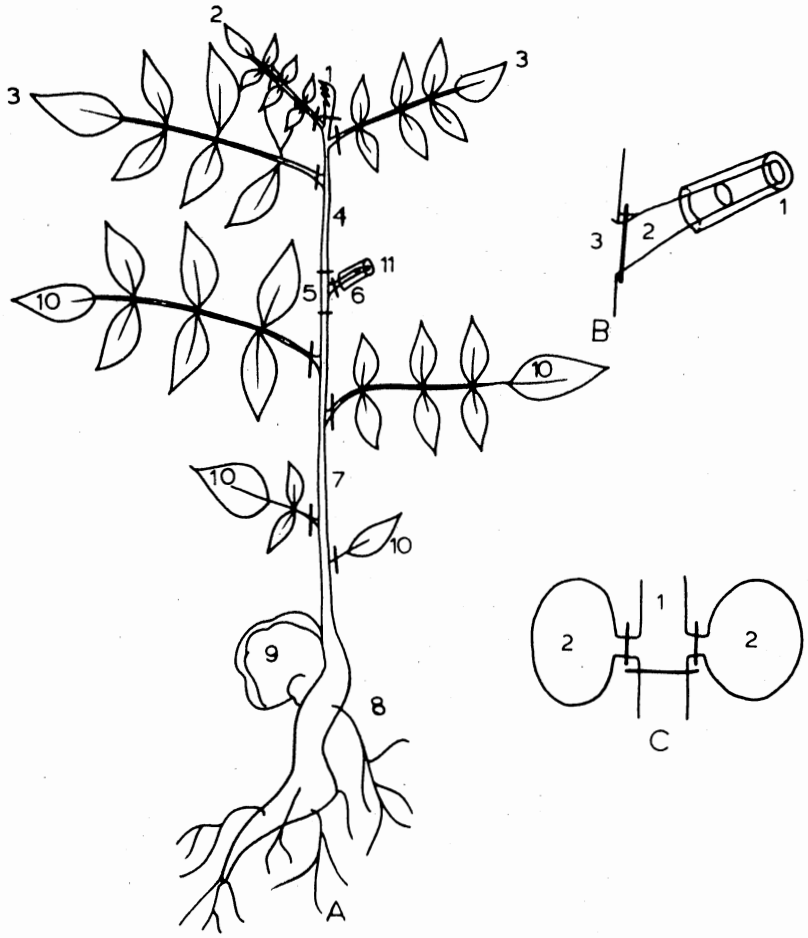

Figure 4. A. Diagram of a Treated Petiole Black Walnut Seedling: 1. Growing Point 2. Expanding Leaf 3. Upper Leaves 4. Upper Stem 5. Treated Area 6. Treated Petiole 7. Lower Stem 8. Roots 9. Cotyledon 10. Lower Leaves 11. Tubing
B. Detail of Petiole Area: 1. Tubing 2. Treated Petiole 3. Treated Area
C. Detail of Cotyledonary Node: 1. Lower Stem 2. Cotyledon

solutions also contained 10 µg of ABA or 10 µg of GA_3.

Plants were harvested at various periods of time after treatment depending upon the experiment. During harvesting, the root system of each plant was rinsed and blotted to remove any surface ^{14}C contamination from the nutrient solution. Each seedling was divided into eleven parts (Figure 4) for ^{14}C analysis. These parts were growing point, expanding leaf, upper leaves which were all leaves above the treated area, lower leaves, upper stem which was all stem tissue above the treated area and below the growing point, treated petiole, treated area which was a 2 cm stem section 1 cm on each side of the treated petiole, lower stem, roots and cotyledons, if present, which were treated as in the earlier experiments. The plant parts were frozen at -25 C and then lyophilized. The dried tissues were treated as described for bur oak.

Eastern Persimmon

Locally collected seeds of eastern persimmon were stratified at 3 C for 5 months and stored at 3 C until used for experimental purposes. The seeds were planted in metal flats using sand as a planting medium and were watered with 1/2 strength Hoagland's solution. Distilled water was added as necessary to keep the sand moist. The seeds were germinated as described for blackjack oak. Approximately 17 days after planting when the first two true leaves were expanding the seedlings were transferred to liquid culture as described for blackjack oak. The light intensity at canopy level was about 20 Klux and the relative humidity ranged from 30 to 50%. After 10 days the seedlings were transferred to fresh 1/2 strength Hoagland's solution.

Plants were treated on the eleventh day between 9:00 a.m. and 11:00 a.m. by injecting 1 µl of ethanol containing plant hormones into the center of the hypocotyl swelling near the base of the stem. The experimental design was a completely randomized design. Plants were treated with ABA or GA_3 at concentrations of 0, 1, 5 or 10 µg along with 2,4,5-T-1-^{14}C at 0.5 µg per plant. The plants were harvested at various times after treatment depending upon the experiment.

During harvesting, the root system of each plant was rinsed to remove any ^{14}C from the nutrient solution and blotted dry. Each plant was divided into seven parts (Figure 5). The parts were the growing point, expanding leaf, leaves, cotyledons, stem, treated area, which was a 3 cm section at the base of the stem, and the roots. The plant parts were frozen at -25 C immediately after harvest and treated as described for bur oak.

Isolation and Bioassay of Black Walnut Cotyledonary Growth Factor

Preliminary studies had indicated that the walnut cotyledons were producing a growth promoting substance(s). It was thought that this substance could have been dihydroconiferyl alcohol which had been recently isolated by Shibata, Kubota, and Kamisaka (85). Using their methods, an attempt was made to isolate dihydroconiferyl alcohol from the nutmeats (cotyledonary material) of black walnuts.

Approximately 500 gm of the nutmeats from 5 week old black walnut seedlings were ground in 1 liter of hot water using a Waring blender. The homogenate was filtered and the residue was extracted with a second liter of distilled hot water. The filtrates were combined and

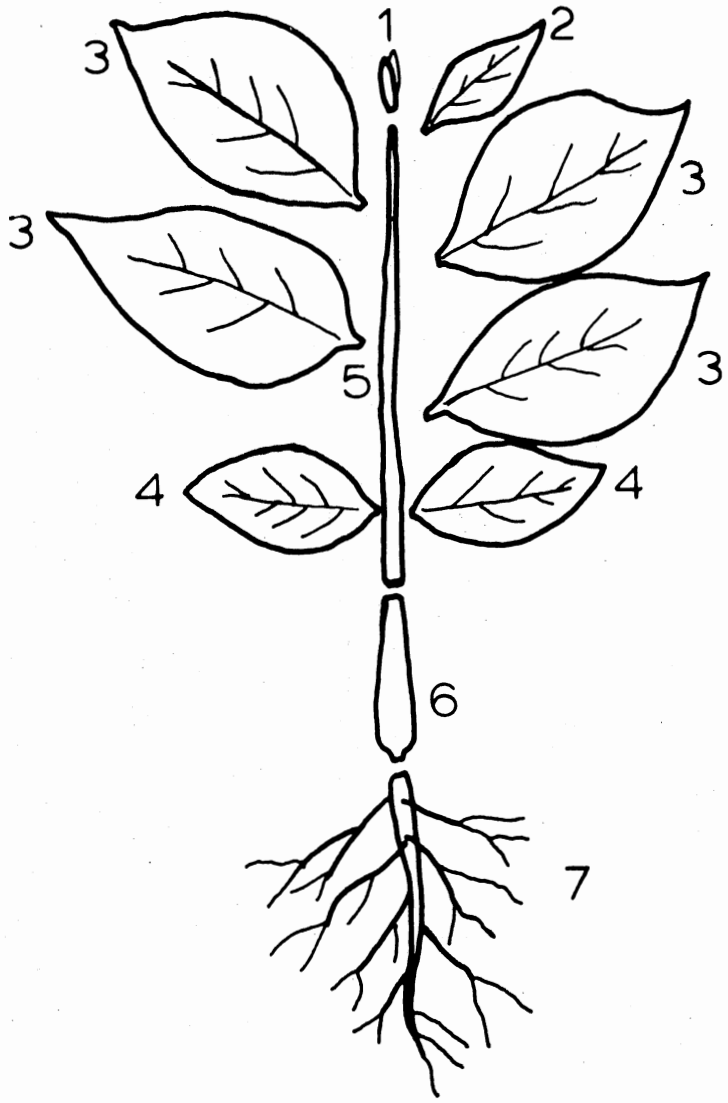

Figure 5. Diagram of Young Persimmon Seedling:
1. Growing Point 2. Expanding
Leaves 3. Leaves 4. Cotyledon
5. Upper Stem 6. Treated Area
7. Roots.

reduced to 250 ml by using a rotary evaporator, and 125 ml of methanol was added to the extract and the mixture was filtered again. The filtrate was washed 3 times with 250 ml of ethyl acetate. The ethyl acetate was evaporated using a rotary evaporator. The residue was extracted with 250 ml of chloroform. The chloroform was removed using a rotary evaporator leaving an oily red-brown liquid. This crude extract was used in bioassay studies of auxin translocation described below.

The methods used in bioassay for effects on auxin translocation of the cotyledonary growth factor was that of Basler (4, 5). Bush bean seeds (*Phaseolus vulgaris* L.) cv "Stringless Greenpod" were germinated in perlite moistened with 1/2 strength Hoagland's nutrient solution for 5 days at 33 C under continuous light of 55 Klux intensity. The seedlings were then transplanted to 500 ml amber glass bottles containing 400 ml of continuously aerated 1/2 strength Hoagland's nutrient solution and placed in a growth chamber. The plants were maintained at 33 C, 14-hour day and 29 C, 10-hour night. The light supplied by a mixture of cool white fluorescent and incandescent bulbs had an intensity of 23 Klux. The seedlings were transferred to fresh 1/2 strength Hoagland's solution after 3 days and the plants were treated the next morning.

The seedlings were treated by injecting 1 µl of ethanol containing 0.5 µg of 2,4,5-T-1-^{14}C and various concentrations of the crude extract into the cotyledonary node with a 1-µl syringe. The concentrations of crude extract were 1/2, 1/10, 1/20, 1/100, 1/500, and 1/1000 of full strength, and control which had no crude extract.

Twenty-four hours after treatment the plants were harvested and divided into young shoots including all tissues above the primary leaves, primary leaves, epicotyl which was all stem tissue 0.5 cm above the cotyledonary node to the young shoot, treated area which was a 3 cm section from 0.5 cm above the cotyledonary node to 2.5 cm below the cotyledonary node, hypocotyl which was all stem tissue below the treated area and above the roots and the roots. The plant parts were frozen at -25 C and later lypohilized for dry weight determinations. The nutrient solutions were refilled to a volume of 400 ml by the addition of distilled water and a 5 ml aliquot was taken for ^{14}C analysis by liquid scintillation counting. The plant parts were homogenized in 95% ethanol using a Polytron tissue homogenizer and aliquots were taken for ^{14}C analysis by liquid scintillation analysis.

Statistical Analysis

Data from liquid scintillation analysis was converted to disintegrations per minute (DPM) by correcting for quenching and background. All statistical calculations are based on DPM and various programs were used to analyze the data. A standard F test and/or Least Significant Difference test were used for statistical testing at the 0.5 level of significant differences within an experiment. Each experiment was conducted using a completely randomized design. Replication ranged from 8 to 20 individual seedlings depending on the experiment. The number of replicates is listed in the tables or in the figure legends.

CHAPTER IV

RESULTS

Auxin Translocation in Blackjack Oak and Bur Oak

A series of survey experiments were conducted with blackjack oak, bur oak, black walnut, and eastern persimmon. Results with blackjack oak are shown in Table I. Twenty-four hours after treatment, the auxin, 2,4,5-T, had translocated in both the acropetal and basipetal direction. Five to ten times more auxin was translocated into the leaves, upper stem, lower stem, and roots than into growing point, untreated cotyledon or nutrient solution. However the concentration of auxin per gram dry weight in the growing point was higher than in the leaves, roots, and untreated cotyledon. Simultaneously injected ABA inhibited movement of auxin to the lower stems and to the untreated cotyledon as compared to the control. ABA appeared to inhibit translocation to the leaves although the differences were not statistically significant, and had no effect on movement of auxin to the growing point, upper stem, treated cotyledon, roots or nutrient solution. Further study was precluded because seed acorns could not be collected to supply seedlings for further study.

The results of ABA or GA_3 treatment on the translocation of 2,4,5-T in bur oak are summarized in Tables II and III. There was very little translocation of auxin in this species. A large amount

TABLE I

THE EFFECT OF ABA ON THE TRANSLOCATION OF 0.5 µg OF 2,4,5-T-1-^{14}C IN BLACKJACK OAK AT 24 HR AFTER TREATMENT

	% of Total 2,4,5-T-1-^{14}C Injected		ng 2,4,5-T/gm dry wt.	
Plant Part	Control	10.0 µg ABA/Plant	Control	10.0 µg ABA/Plant
Growing point	0.5	0.4	147.1	121.3
Leaves	4.2	2.1	33.3	17.7
Upper stem	3.7	3.1	268.4	227.1
Lower stem	3.5 b	2.3 a	241.4 b	164.9 a
Treated cotyledon	88.1	59.5	533.2	731.1
Untreated cotyledon	0.7 b	0.0 a	6.3 b	0.9 a
Roots	3.0	2.0	26.0	18.0
Nutrient Solution	0.5	0.4	2.5	1.8

The values are the averages of eight determinations. Values followed by a different letter for a single plant part are significantly differently at the 0.05 level. Values for a single plant part without a letter are not significantly different.

TABLE II

THE EFFECT OF ABA ON THE TRANSLOCATION OF 0.5 µg OF 2,4,5-T-1-^{14}C IN BUR OAK AT 24 HR AFTER TREATMENT

	% of Total 2,4,5-T-1-^{14}C Injected		ng 2,4,5-T/gm dry wt.	
Plant Part	Control	10.0 µg ABA/Plant	Control	10.0 µg ABA/Plant
Growing point	0.00	0.00	0.50	0.40
Leaves	0.60	0.60	6.00	5.20
Upper stem	0.50	0.50	25.50	15.70
Lower stem	1.60	1.00	66.40	39.80
Roots	3.40 b	2.20 a	45.70 b	27.60 a
Treated cotyledon	50.70	48.60	295.00	338.20
Untreated cotyledon	0.50	0.40	6.00	4.90
Nutrient solution	0.60	0.40	6.10	4.30

The values are the average of 18 determinations. Values followed by a different letter for a single plant part are significantly different at the 0.05 level. Values for a single plant part without a letter are not significantly different.

TABLE III

THE EFFECT OF GA_3 ON THE TRANSLOCATION OF 0.5 µg OF 2,4,5-T-1-^{14}C IN BUR OAK AT 24 HR AFTER TREATMENT

	% of Total 2,4,5-T-1-^{14}C Injected		ng 2,4,5-T/gm dry wt.	
Plant Part	Control	10.0 µg GA_3/Plant	Control	10.0 µg GA_3/Plant
Growing point	0.00	0.00	0.50	0.40
Leaves	0.60	0.50	6.10	6.00
Upper stem	0.10	0.30	2.66	8.09
Lower stem	0.40	0.30	7.30	6.90
Roots	1.30	1.20	9.00	9.50
Treated cotyledon	51.00	47.60	227.60	244.50
Untreated cotyledon	0.30	0.30	1.47	1.73
Nutrient solution	0.50	0.50	---	---

The values are averages of 17 determinations. Values followed by the same letter for a single plant part are not significantly different at the 0.05 level. Values for a single plant part without a letter are not significantly different.

of radioactivity injected into the plants was lost by the end of the 24-hour treatment period suggesting that this species is probably able to decarboxylate 2,4,5-T. The only effect of ABA was to decrease translocation of auxin to the roots. GA_3 had no significant effect on auxin movement in bur oak. An almost threefold difference in the amount of 2,4,5-T in the upper stem may have been due to a large amount of variability. In general this species translocated very little 2,4,5-T and in addition these seedlings were extremely difficult to work with because of their large size and hard woody tissues which made for great difficulty in homogenization. For these reasons no further work was done with bur oak.

Auxin Translocation in Black Walnut

Data for black walnut treated with ABA and harvested 24 hours after treatment are shown in Table IV. The initial experiments were conducted by injecting 2,4,5-T directly into the stem. Stem injected 2,4,5-T was readily translocated in basipetal and acropetal directions. ABA enhanced movement of auxin into the leaves and growing point although it had no effect on auxin translocation to other plant parts. This enhancement of auxin translocation by ABA into the leaves was contrary to the expected results since previous work had reported an inhibition of auxin translocation to leaf tissue.

The following experiment was designed to determine whether pretreatment of the seedlings with either ABA or GA_3 would alter their effect on 2,4,5-T translocation. Work by Wareing's group (17) had indicated that several weeks of ABA treatment were necessary before the plant became dormant. A 24-hour pretreatment with ABA should allow

TABLE IV

EFFECTS OF ABA ON THE TRANSLOCATION OF 0.5 µg OF INJECTED 2,4,5-T-1-^{14}C IN WALNUT AT 24 HR AFTER TREATMENT

	% of Total 2,4,5-T-1-^{14}C Injected		ng 2,4,5-T/gm dry wt.	
Plant Parts	Control	25.0 µg ABA/Plant	Control	25.0 µg ABA/Plant
Growing point	1.1	1.8	124.8 a	213.4 b
Leaves	6.4 a	11.2 b	110.6	156.1
Upper stem	34.6	32.7	1388.9	1241.5
Treated area	50.1	49.6	2207.7	2159.8
Roots	1.1	2.3	20.1	38.1
Cotyledons	0.1	0.1	0.8	0.5

The values are averages of 18 determinations. Values followed by a different letter for a single plant part are significantly different at the 0.05 level. Values for a single plant part without a letter are not significantly different.

the hormone to be translocated through the plant where it could begin to exert its physiological effects. An experiment was done with plants that were pretreated by stem injecting ABA or GA_3. Twenty-four hours later these plants were injected with 2,4,5-T-1-^{14}C and the experiment was terminated 24 hours after auxin treatment. The translocation of 2,4,5-T to the growing point was decreased by ABA pretreatment but GA_3 pretreatment had no apparent effect on auxin movement to the growing point (Table V). Pretreatment with ABA in this experiment did not enhance auxin movement to the growing point as previously observed with simultaneous injection of ABA (Table IV). Both ABA and GA_3 pretreatment enhanced movement of auxin to the leaves similar to that observed when ABA was injected simultaneously with the auxin. In both cases, there was approximately twice as much auxin in the leaves of plants treated with ABA as compared to the controls. Neither GA_3 or ABA had an effect on auxin translocation into the other plant parts. However, there was a significant difference in the weights of the growing points of plants pretreated with ABA and the plants pretreated with GA_3. The plants pretreated with ABA had growing points weighing 33% less than those of the plants pretreated with GA_3 and this effect may be responsible for the lack of a significant difference between treatments in young shoots when the data were expressed on the basis of dry weight. Also when the amount of 2,4,5-T is expressed in terms of ng 2,4,5-T/gm dry weight the GA_3 pretreatment apparently immobilized the auxin in the treated area as compared to pretreatment with ABA.

The method of treating black walnut seedlings used above had several disadvantages. The seedlings were in soil and this made it

TABLE V

EFFECTS OF ABA AND GA_3 24 HR PRETREATMENT ON THE TRANSLOCATION OF 0.5 µg OF STEM INJECTED 2,4,5-T-1-^{14}C IN WALNUTS AT 24 HR AFTER TREATMENT

	% of Total 2,4,5-T Injected			ng 2,4,5-T/gm dry weight		
Plant Part	Control	10.0 µg ABA	10.0 µg GA_3	Control	10.0 µg ABA	10.0 µg GA_3
Growing point	0.5 b	0.3 a	0.5 b	321.7	200.3	216.2
Leaves	9.3 a	23.1 b	19.5 b	292.7 a	516.3 b	377.6 ab
Upper stem	6.4	5.6	6.0	802.2	612.5	908.7
Treated area	27.2	21.6	23.7	2311.9 ab	2029.9 a	2935.6 b
Roots	2.9	2.6	2.3	125.6	152.8	115.2
Cotyledons	1.5	1.5	1.5	16.6	15.9	15.7

Each value is the average of 18 determinations. Values followed by a different letter for a single plant part are significantly different at the 0.05 level. Values for a single plant part without a letter are not significantly different. ABA and GA were injected at 24 hr prior to 2,4,5-T injection.

difficult to monitor root exudation of ^{14}C activity. The differences in weight of certain plant parts were not reflected by gains in other plant parts. One explanation of this could have been that the roots exuded organic compounds. This could be monitored if liquid culture was used. The auxin and other plant hormones were administered by injection and it was very difficult at times to force the syringe needle through the hard woody tissues. For these reasons, the use of petiole application of plant hormones was tried.

The experiments utilizing stem injection of 2,4,5-T in black walnut seedlings had established that ABA apparently was enhancing auxin movement to the upper portions of the plant. Using the petiole application technique with plants in liquid culture, a series of experiments were run to determine if a time element was involved in this effect. All black walnut seedlings were treated with 0.5 µg 2,4,5-T-1-^{14}C. One third of these plants were treated with 10 µg ABA per plant and one third with 10 µg GA_3 per plant. The ABA and GA_3 were administered simultaneously with the auxin. Seedlings were harvested at 4 hours, 24 hours, and 48 hours after treatment.

The data for the plants harvested 4 hours after the treatment are summarized in Table VI. The 2,4,5-T was translocated readily throughout the plant although acropetal translocation was more extensive with the petiole application technique. ABA and GA_3 enhanced auxin translocation out of the treated area and ABA enhanced auxin translocation to the roots but inhibited 2,4,5-T translocation to the lower leaves. GA_3 slightly inhibited auxin transport to the lower stem. ABA and GA_3 did not have extensive effects on translocation of 2,4,5-T. There were no significant differences in weight of the

TABLE VI

EFFECTS OF ABA AND GA_3 TREATMENT ON TRANSLOCATION OF 0.5 µg OF 2,4,5-T-1-^{14}C APPLIED THROUGH A PETIOLE IN WALNUT AFTER FOUR HOURS

Plant Part	% of Total 2,4,5-T-1-^{14}C Applied			ng 2,4,5-T/gm dry weight		
	Control	10 µg ABA	10 µg GA_3	Control	10 µg ABA	10 µg GA_3
Growing point	0.2	0.1	0.2	124.9	105.6	168.7
Upper leaves	19.4	25.4	23.1	199.0	216.7	210.4
Upper stem	4.3	4.2	3.5	980.7	857.7	938.2
Treated area	14.3 b	11.4 a	12.0 a	2315.0 b	1893.0 ab	1800.0 a
Treated petiole	32.1	29.1	29.6	18216.5	18747.6	19593.0
Lower stem	16.3 b	12.8 ab	11.9 a	241.7	191.0	180.5
Roots	0.6 a	1.0 a	0.8 b	7.9 a	13.0 b	8.9 a
Cotyledons	0.6	0.7	0.7	5.4	5.8	5.7
Lower leaves	8.4 b	4.1 a	6.2 ab	93.9	76.8	69.0
Tubing	5.6	5.0	6.1	---	---	---
Nutrient solution	1.2	0.8	0.9	---	---	---

The values are averages of 18 determinations. Values followed by a different letter for a single plant part are significantly different at the 0.05 level. Values for a single plant part without a letter are not significantly different.

plant parts caused by any treatment and consequently no additional significance was noted when the data were expressed in terms of dry weight.

Table VII summarizes the results of a 24 hour experiment. One effect of ABA was threefold enhancement of auxin translocation out of the treated petiole but significantly less auxin was translocated out of the treated area as compared to the control or GA_3 treated seedlings. ABA also enhanced auxin translocation into the growing point, upper leaves, and lower stem 24 hours after treatment. GA_3 stimulated auxin translocation out of the treated petiole and had a greater effect than ABA in stimulating auxin movement into the growing point and upper leaves. GA_3 also enhanced auxin translocation into the lower stem as compared to the control but to a lesser extent than ABA. GA_3 inhibited auxin translocation into the cotyledons. No significant differences existed in the nutrient solution values.

Table VIII summarizes the data from a 48 hour experiment. When the activity was expressed in terms of dry weight, the ABA and GA_3 treated plants had less ^{14}C activity in the upper leaves and upper stem than the controls. The GA_3 treated seedlings had less 2,4,5-T in the treated area and more activity in the treated petiole than either the control or ABA treated plants. Differences due to ABA and GA_3 were not noted in the upper leaves and stems when the data were expressed as a per cent of the total applied.

ABA had several effects on the weight of plant parts as shown in Table IX for the data after 24 hour treatment. ABA treated plants had smaller upper leaves than the control plants. It had been observed earlier in stem injected plants that leaves of ABA treated plants

TABLE VII

EFFECTS OF ABA AND GA_3 TREATMENT ON TRANSLOCATION OF 0.5 µg OF 2,4,5-T-1-^{14}C APPLIED THROUGH A PETIOLE IN WALNUT AFTER 24 HOURS

	% of Total 2,4,5-T-1-^{14}C Applied			ng 2,4,5-T/gm dry weight		
Plant Part	Control	10 µg ABA	10 µg GA_3	Control	10 µg ABA	10 µg GA_3
Growing point	0.8 c	2.5 b	3.8 a	294.3 a	838.8 b	1600.5 c
Upper leaves	11.4 c	13.4 b	18.9 a	362.3 a	673.7 b	990.8 c
Upper stem	6.8	6.7	7.7	2642.0 a	4340.6 b	5753.1 c
Treated area	12.5 b	15.5 a	12.2 b	4761.1 a	7486.5 b	6791.0 a
Treated petiole	34.6 c	27.5 b	20.3 a	61486.4	61124.0	67444.8
Lower stem	10.3 c	16.9 b	13.3 a	332.5 a	588.1 b	578.2 b
Roots	0.5	0.6	0.5	14.7	18.5	18.2
Cotyledons	0.8 b	0.7 b	0.5 a	5.0	4.2	4.4
Lower leaves	1.8	2.8	2.3	82.8	123.0	143.8
Tubing	7.6	7.0	5.8	---	---	---
Nutrient solution	1.1	1.2	1.1	---	---	---

The values are averages of 18 determinations. Values followed by a different letter for a single plant part are significantly different at the 0.05 level. Values for a single plant part without a letter are not significantly different.

TABLE VIII

EFFECT OF ABA AND GA_3 TREATMENT ON TRANSLOCATION OF 0.5 µg OF 2,4,5-T-1-^{14}C APPLIED THROUGH A PETIOLE IN WALNUT AFTER 48 HOURS

	% of Total 2,4,5-T-1-^{14}C Applied			ng 2,4,5-T/gm dry weight		
Plant Part	Control	10 µg ABA	10 µg GA_3	Control	10 µg ABA	10 µg GA_3
Growing Point	1.1	0.9	1.2	248.0	271.0	235.0
Upper leaves	9.7	8.1	7.2	87.6 b	64.1 a	60.6 a
Upper stem	3.8	3.5	3.0	1056.0 b	813.0 a	749.0 a
Treated area	9.6 b	8.8 b	7.0 a	1179.0 b	1161.0 b	910.9 a
Treated petiole	17.8 a	21.1 ab	24.7 b	8141.9 a	8510.4 a	9760.0 b
Lower stem	26.9	25.7	23.8	325.9	290.2	129.8
Roots	2.2	2.2	2.1	17.7	18.0	18.2
Cotyledons	1.7	1.6	1.5	7.5	8.9	6.2
Lower leaves	2.9	2.7	2.1	26.5	27.9	23.7
Treated tubing	4.3	5.1	3.7	---	---	---
Nutrient solution	1.1	1.3	1.4	---	---	---

Each value is the agerage of 18 determinations. Values followed by a different letter for a single plant part are significantly different at the 0.05 level. Values for a single plant part without a letter are not significantly different.

TABLE IX

EFFECT OF ABA AND GA_3 TREATMENT ON THE WEIGHT OF PLANT PARTS IN WALNUT SEEDLINGS AFTER 24 HOURS

Plant Parts	Weight in Grams		
	Control	10 µg ABA	10 µg GA_3
Growing point	0.0229	0.0287	0.0256
Upper leaves	0.3029 b	0.1761 a	0.2418 ab
Lower leaves	0.1860	0.2055	0.2174
Upper stem	0.0222 b	0.0140 a	0.0173 ab
Treated petiole	0.0051	0.0045	0.0040
Treated area	0.0231 b	0.0189 a	0.0216 b
Lower stem	0.2640	0.2568	0.2686
Roots	0.3453	0.3129	0.3128
Cotyledons	1.4312	1.5281	1.5058

The values are averages of 18 determinations. Values followed by a different letter for a single plant part are significantly different at the 0.05 level. Values for a single plant part without a letter are not significantly different.

weighed less than leaves of control plants at the end of 24 hours but this difference was only significant at the 90% level. Upper stem sections and treated areas of ABA treated plants weighed less than the controls. These differences were not significant in the 4 and 48 hour treatment. GA_3 had no significant effect on weight.

These data imply that there is a time effect on auxin translocation and on ABA and GA_3 effects on auxin translocation. Auxin levels in the growing points of control plants increased approximately fourfold between 4 hours and 24 hours after treatment and then increased slowly until 48 hours. Between 4 and 24 hours ABA and GA_3 enhanced auxin translocation by threefold or fivefold respectively over the levels in the control plants. By 48 hours the auxin levels in the ABA and GA_3 treated plants were the same as in the control plants. Translocation of 2,4,5-T to the upper leaves was also time dependent. The amounts of 2,4,5-T in the three treatments were highest 4 hours after treatment and then declined. For the upper stem, the amount of 2,4,5-T increased between 4 hours and 24 hours and then declined between 24 hours and 48 hours for all treatments.

Basipetal translocation of 2,4,5-T also depended upon time and responded to ABA or GA_3 treatment. For the control plants, the amount of 2,4,5-T decreased between 4 hours and 24 hours after treatment and then sharply increased (250%) by the end of 48 hours. The additional 2,4,5-T apparently came from the upper plant parts including the treated area and treated petiole. The level of 2,4,5-T in the cotyledons and roots remained constant between 4 hours and 24 hours but increased fourfold in the next 24 hours. In the lower leaves, the amount of 2,4,5-T decreased sharply in the 4 hour to 24 hour time

period for all treatments but this decrease was not as pronounced for the plants treated with ABA. The level of 2,4,5-T then remained constant for the next 24 hours in the three groups of plants. Amounts of 2,4,5-T in the nutrient solution were constant throughout the course of the experiments.

It appeared that the effects of ABA and GA_3 were time dependent. These hormones enhanced 2,4,5-T translocation to those portions of the plant above the treated area but these effects were transitory. ABA and GA_3 also had transitory effects on translocation to various plant parts below the treated area at different times after treatment. ABA affected the weight of certain plant parts 24 hours after treatment. There were no effects of GA_3 on plant weight at any time period.

ABA had a positive stimulatory enhancement of acropetal auxin translocation in black walnut seedlings in contrast to effects in bean seedlings (5) where ABA decreased acropetal translocation. In preliminary work it had been observed that the cotyledons exerted a positive effect on the growth of black walnut seedlings for at least 6 to 8 weeks after germination. The increased rate of growth did not appear to be due to the cotyledons providing food materials because the amount of materials in the nutmeats of the cotyledons were not measurably decreased. This could be explained by assuming that the cotyledons were producing a growth substance that increased the growth rate of black walnut seedlings. It also seemed possible that this unknown growth substance could have interacted with the ABA in such a manner to cause a stimulation of acropetal auxin translocation. Two experimental approaches were used in attempts to assess this possibility. One of these approaches was to remove the

cotyledons from the black walnut seedlings and repeat the 24-hour experiment to see what effect ABA had on 2,4,5-T translocation in seedlings without cotyledons. The second approach was to try and isolate an active substance from the cotyledons.

The first approach involved removal of the cotyledons 1 week prior to treatment with 2,4,5-T and ABA. Except for removal of the cotyledons, the seedlings were treated as before. The seedlings were separated into two groups. The control group was treated with 0.5 µg of 2,4,5-T-1-^{14}C per plant and the other group was treated with 10 µg ABA and 0.5 µg of the labeled 2,4,5-T. The plants were harvested at 24 hours and the data are summarized in Table X. A very different pattern of auxin movement emerged. ABA inhibited 2,4,5-T translocation into the upper leaves when expressed on a percent of the total auxin injected. When expressed in terms of ng 2,4,5-T per gm dry weight, there are no significant differences. This is because the ABA treated plants had upper leaves which weighed an average of 11% less than the control leaves, and this weight difference negated the difference in auxin levels. The ABA treated plants translocated less auxin out of the treated area. ABA also inhibited the basipetal translocation of 2,4,5-T into the lower stem. This auxin translocation is the opposite of the effects of ABA on auxin translocation in plants with attached cotyledons (see Table VII).

A comparison of the data between Tables VII and X show several striking differences. In control plants, less auxin was translocated to the upper stem and growing points of the plants that had their cotyledons removed while more auxin was translocated to the upper leaves, lower stem, lower leaves, and roots in these plants. In the

TABLE X

EFFECT OF ABA TREATMENT ON TRANSLOCATION OF 0.5 μg OF 2,4,5-T-1-^{14}C APPLIED THROUGH A PETIOLE IN WALNUT SEEDLINGS WITH THE COTYLEDONS REMOVED

Plant Part	% of Total 2,4,5-T-1-^{14}C Applied		ng 2,4,5-T/gm dry wt.	
	Control	10.0 μg ABA/Plant	Control	10.0 μg ABA/Plant
Growing point	0.04	0.04	68.20	52.80
Upper leaves	19.47 b	16.98 a	160.60	153.90
Upper stem	3.97	3.71	805.10 a	881.50 b
Treated area	27.56 a	34.58 b	3077.20 a	3826.10 b
Treated petiole	28.02	27.67	16551.70	17094.90
Lower stem	16.02 b	13.42 a	624.40 b	557.67 a
Roots	1.38	1.60	4.78	5.45
Lower leaves	9.38	6.16	98.50	79.80
Tubing	4.52	5.02	---	---
Nutrient solution	1.10	1.00	---	---

Each value is the average of 36 determinations. Values followed by the different letter for a single plant part are significantly different at the 0.05 level. Values for a single plant part without a letter are not significantly different. The plants were harvested 24 hours after treatment.

plants with their cotyledons removed, ABA inhibited translocation to the upper leaves and lower stem--an effect very different from its effect of enhancing auxin translocation in plants with attached cotyledons.

An attempt was made to partially purify the unknown growth promoting substance from black walnut cotyledons. The crude extract of the black walnut cotyledons was used at various dilutions and injected into bean seedlings along with the labeled auxin 2,4,5-T-1-^{14}C. The bean seedlings were harvested after 24 hours. Table XI summarizes the data with only the control and concentrated crude extract data being listed. Intermediate concentrations of the crude extract gave results intermediate between the two levels shown. The walnut cotyledonary extract enhanced upward movement of auxin into the leaves and epicotyl. It also enhanced translocation of auxin out of the treated area. The compound(s) had no effect on basipetal auxin translocation or on the weight of plant parts. This effect appears to be unique among all compounds known to affect auxin translocation.

Auxin Translocation in Persimmon Seedlings

The effects of ABA on auxin translocation are shown in Figures 6-12. The amount of 2,4,5-T is expressed in terms of ng 2,4,5-T/gm dry weight rather than per plant part because this minimized variation except for the roots. Each figure is for one specific plant part. The data, as either a per cent of the total injected or ng per g dry weight, as well as the statistical results are given in the Appendix. In the case of the roots, it was necessary to express the results as a percentage of the total injected because ABA treatment caused changes

TABLE XI

EFFECT OF WALNUT COTYLEDON EXTRACT ON TRANSLOCATION OF 0.5 µg OF STEM INJECTED 2,4,5-T-1-^{14}C IN BEAN SEEDLINGS AFTER 24 HOURS

Plant Part	% of Total 2,4,5-T-1-^{14}C Injected		ng 2,4,5-T/g dry wt.	
	Control	Concentrate	Control	Concentrate
Growing point	2.77	4.11	845.20	1558.40
Leaves	9.78 a	21.30 b	297.90	599.30
Epicotyl	21.66 a	28.60 b	5850.60 a	7486.70 b
Treated area	36.72 a	27.38 b	11315.00	8664.90
Hypocotyl	19.17	16.26	2183.80	1909.20
Roots	1.41	2.45	117.70	181.40
Nutrient solution	1.51	1.30	6.40	5.57

Each value is the average of 8 determinations. Values followed by a different letter for a single plant part are significantly different at the 0.05 level. Values for a single plant part without a letter are not significantly different.

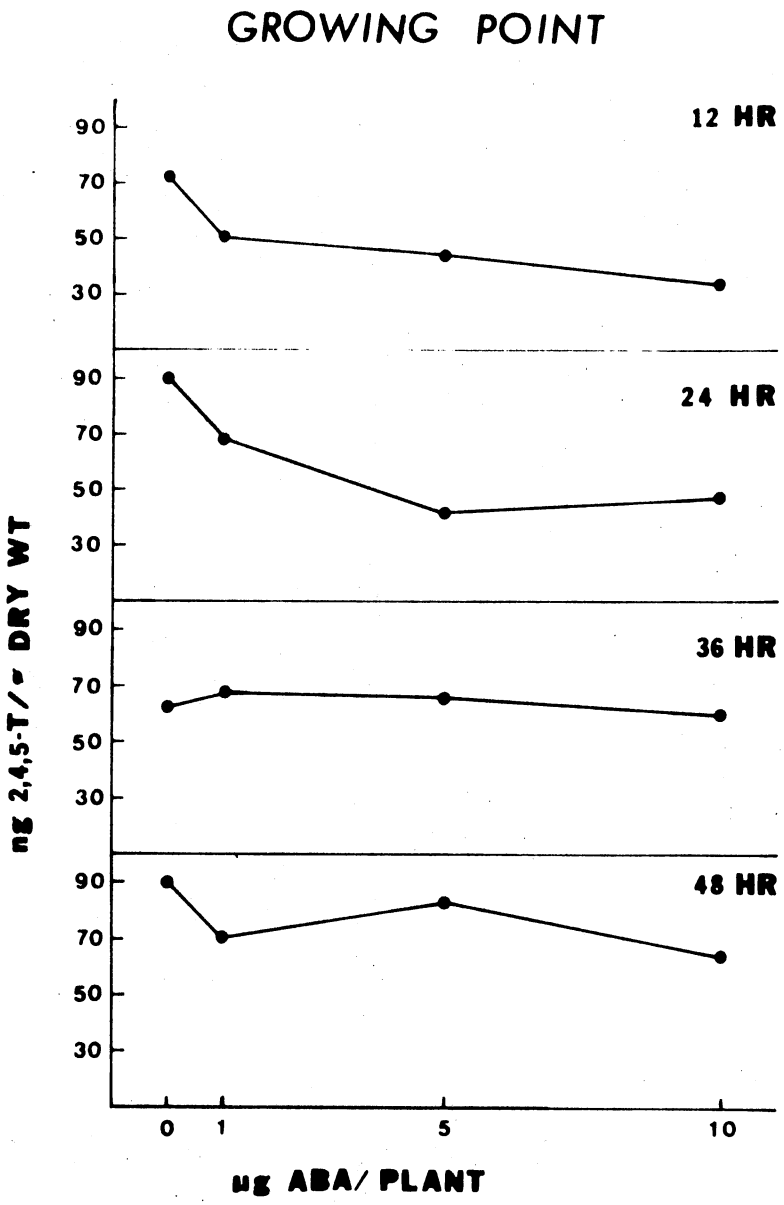

Figure 6. Effects of Time and ABA on Auxin Translocation to the Growing Point. Each Point is the Average of 15 Determinations on Individual Persimmon Seedling.

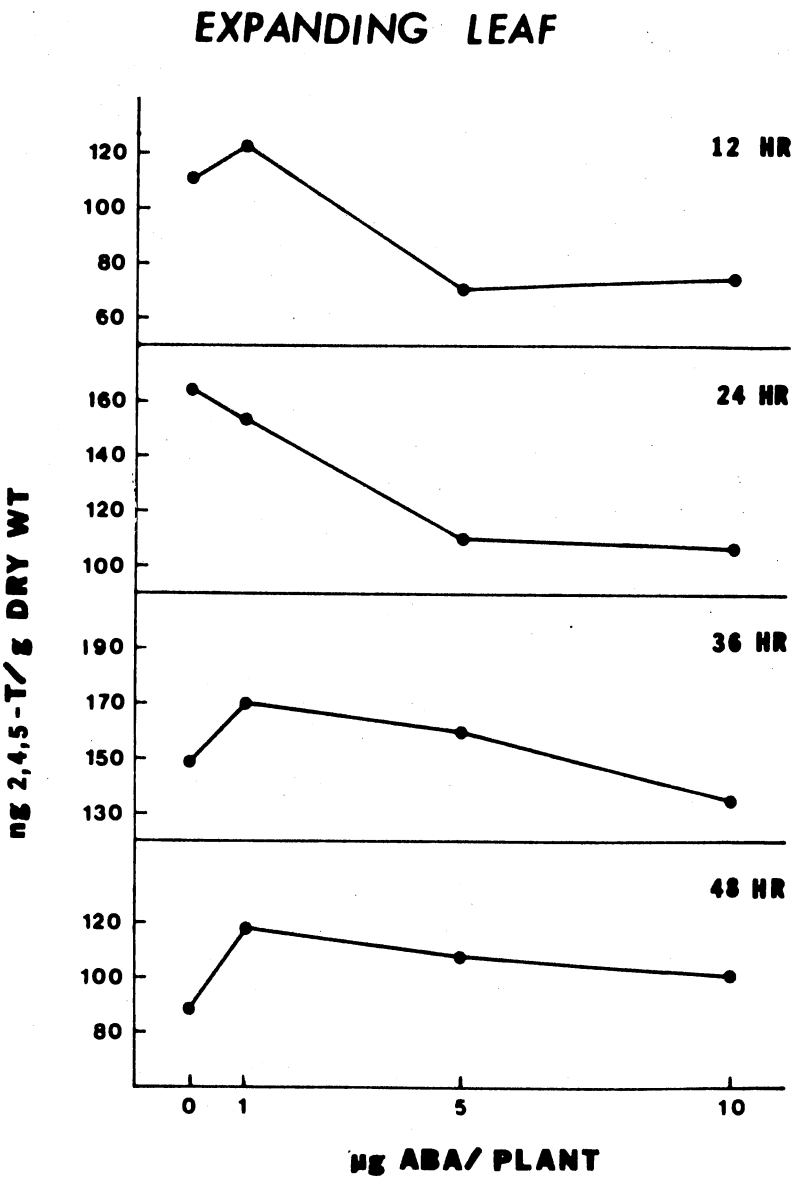

Figure 7. Effects of Time and ABA on Auxin Translocation to the Expanding Leaf. Each Point is the Average of 15 Determinations on Individual Persimmon Seedling.

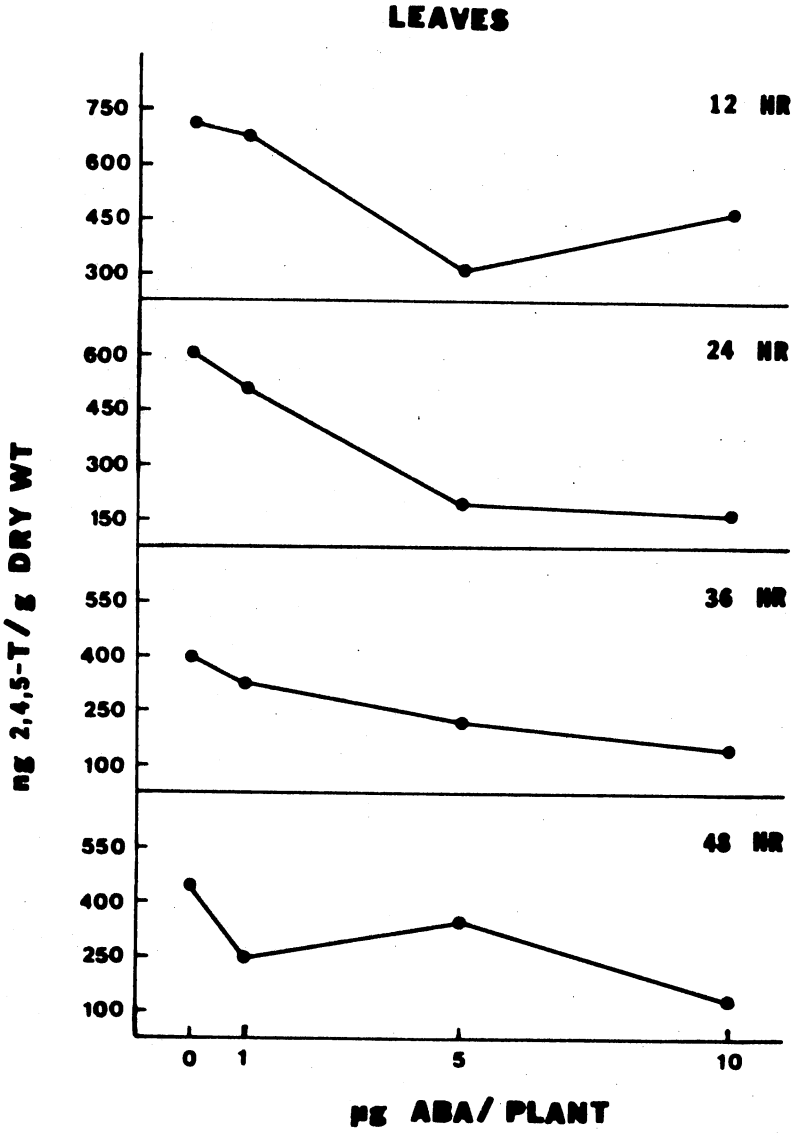

Figure 8. Effects of Time and ABA on Auxin Translocation to the Leaves. Each Point is the Average of 15 Determinations on Individual Persimmon Seedling.

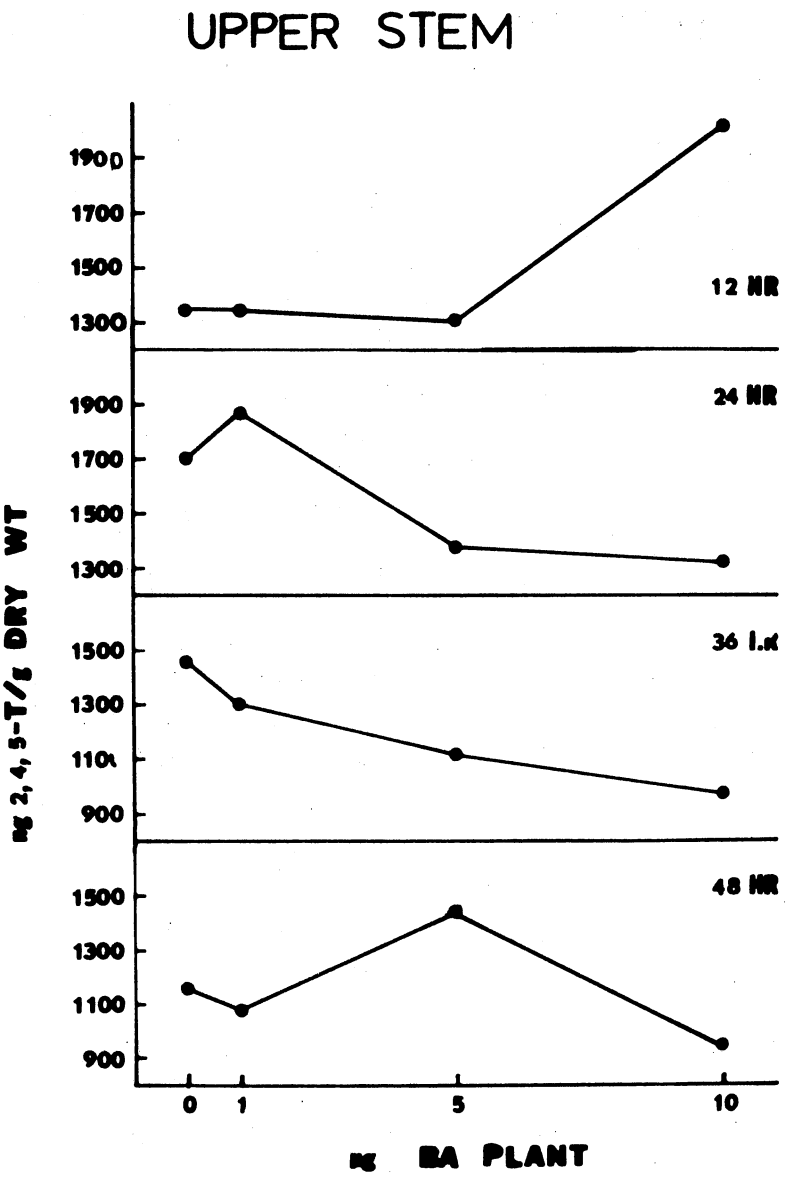

Figure 9. Effects of Time and ABA on Auxin Translocation to the Upper Stem. Each Point is the Average of 15 Determinations on Individual Persimmon Seedling.

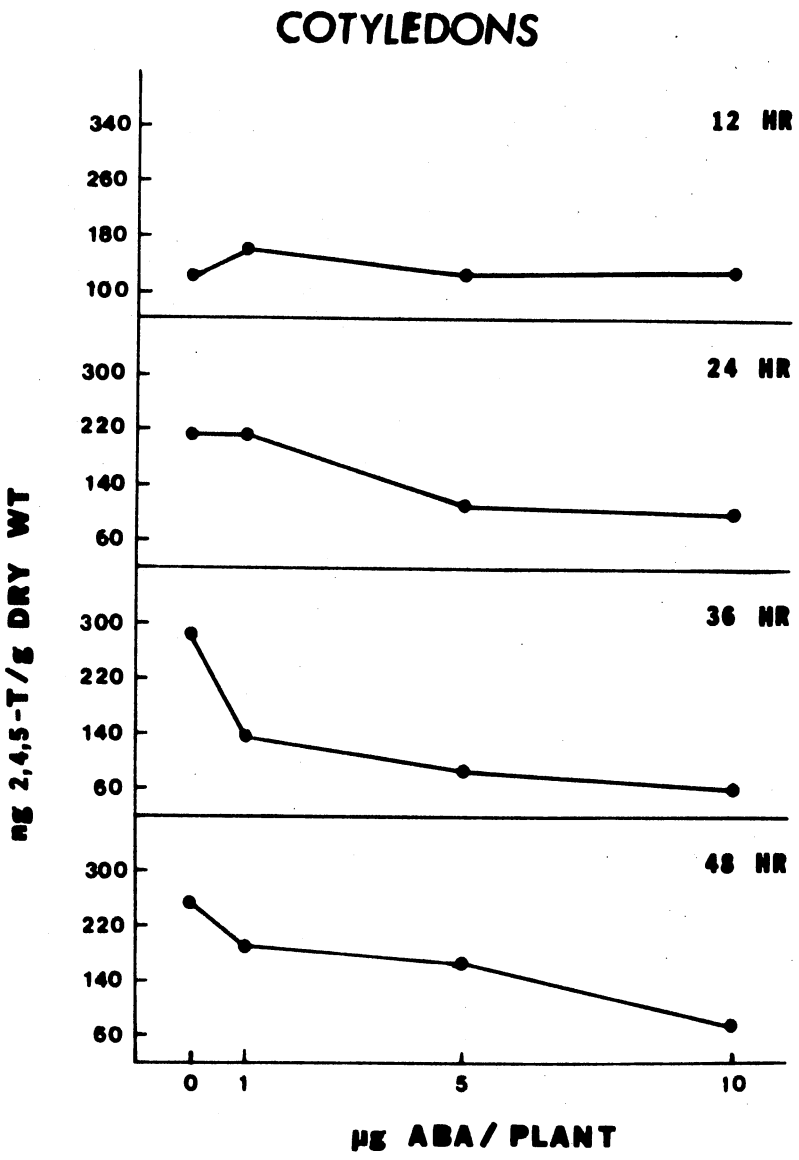

Figure 10. Effects of Time and ABA on Auxin Translocation to the Cotyledons. Each Point is the Average of 15 Determinations on Individual Persimmon Seedling.

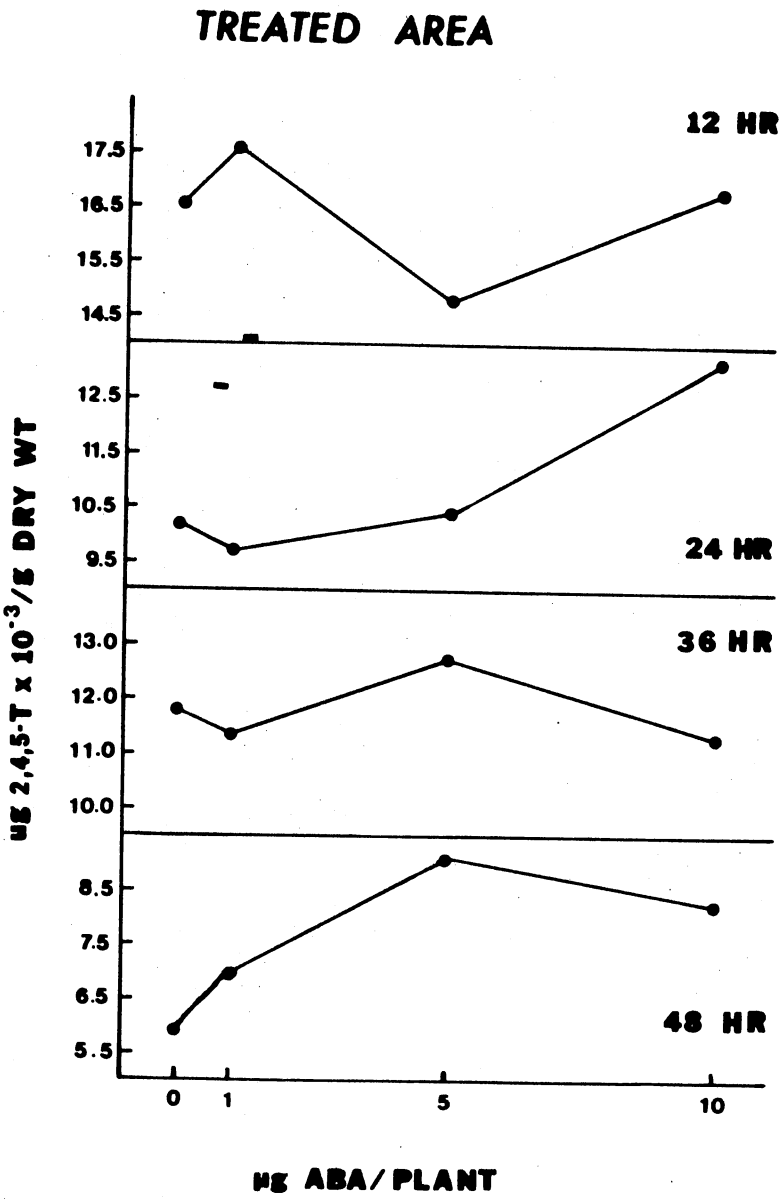

Figure 11. Effects of Time and ABA on Auxin Translocation from the Treated Area. Each Point is the Average of 15 Determinations on Individual Persimmon Seedling.

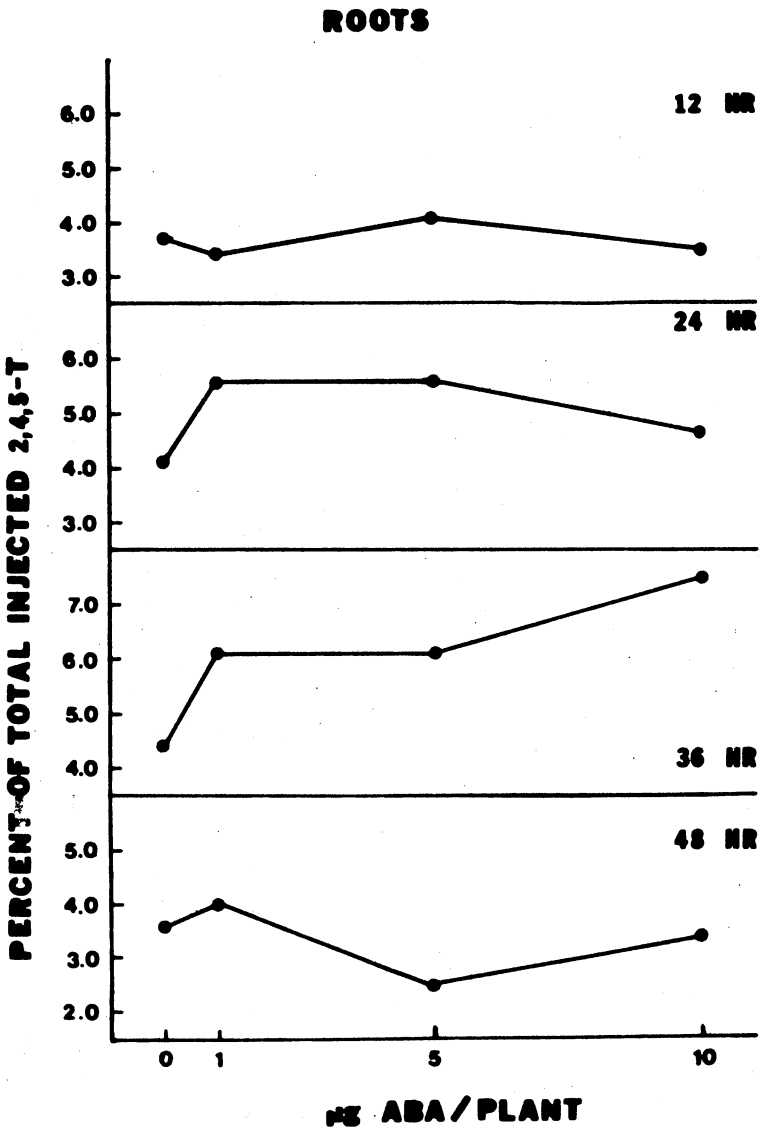

Figure 12. Effect of Time and ABA on Auxin Translocation to the Roots. Each Point is the Average of 15 Determinations on Individual Persimmon Seedling.

in weight which obscured treatment differences.

ABA inhibited auxin translocation into the growing point, expanding leaf and leaves (Figures 6, 7, 8). The inhibition of acropetal translocation into these plant parts was greater at 5 or 10 µg of ABA and at 12 and 24 hours. At time periods longer than 24 hours, ABA treatment appeared to have no effect on auxin translocation into the growing point or expanding leaf.

At 12 hours the 10 µg ABA treated plants contained more of the 2,4,5-T in the upper stem than untreated plants. Since the leaves contained less 2,4,5-T, this may suggest that ABA was inhibiting movement into the leaves from the stem. The higher levels of 2,4,5-T in the upper stem at 12 hours was the only time there were significant differences between the ABA treated plants and the controls (Figure 9).

Although ABA had no significant effect on auxin translocation into the cotyledons at 12 hours after treatment, at 24 and 36 hours after treatment 5 and 10 µg of ABA significantly decreased auxin translocation into the cotyledons (Figure 10). At 48 hours after treatment, only 10 µg of ABA significantly inhibited auxin translocation into the cotyledons as compared to the controls.

The effect of ABA on auxin levels in the treated area (Figure 11) was more complex than in other plant parts. At 12 hours after treatment, there were no significant differences while at 24 hours, the plants treated with 10 µg of ABA had significantly more 2,4,5-T than the other treatments. After 48 hours all plants treated with ABA had significantly more 2,4,5-T in the treated area than the control plants. This suggested that ABA could have been immobilizing the injected auxin by conjugation or chemical inactivation. This hypothesis was tested

by finely grinding tissues from the treated area and applying this to TLC plates. In all cases the ^{14}C label remained at positions on the chromatogram after development corresponding to positions for the intact 2,4,5-T and IAA. Since the auxin was not broken down or conjugated it appears that it simply was not available for translocation.

The effect on 2,4,5-T translocation into the roots (Figure 12) also was complex. No differences between the control seedlings and seedlings treated with ABA were observed after 12 hours. After 24 hours plants treated with 1 or 5 µg of ABA had significantly higher levels of 2,4,5-T in the roots which suggested that these amounts of ABA enhanced basipetal auxin translocation. At 36 hours after treatment, all seedlings treated with ABA had significantly higher amounts of 2,4,5-T than the control seedlings, but after 48 hours the levels of 2,4,5-T in all treatments were not significantly different.

ABA had an effect on the weights of different plant parts after 48 hours. These effects are summarized in Table XII. ABA caused an increase in the weight of the expanding leaf but caused a decrease in the weight of the leaves and roots. It should be noted that ABA caused a decrease in the weight of walnut upper leaves also. ABA had no effect on 2,4,5-T movement into the nutrient solution at any time interval.

The results of an experiment with the effects of ABA on the translocation of the auxin IAA (carboxyl-^{14}C) 36 hours after treatment are summarized in Table XIII. The patterns of auxin translocation were intermediate between the 24 and 36 hour data obtained when 2,4,5-T was used as the auxin (Tables XVII and XVIII). ABA inhibited

TABLE XII

EFFECTS OF ABA TREATMENT ON THE WEIGHT OF PLANT PARTS OF PERSIMMON SEEDLINGS AFTER 48 HOURS

	µg ABA/Plant			
Plant Part	Control	1.0	5.0	10.0
Growing point	0.0011	0.0011	0.0010	0.0013
Expanding leaf	0.0025 a	0.0021 a	0.0038 b	0.0037 b
Leaves	0.0699 b	0.0709 b	0.0573 a	0.0489 a
Cotyledons	0.0412	0.0446	0.0437	0.0398
Upper stem	0.0514	0.0531	0.0476	0.0505
Treated area	0.0375	0.0362	0.0319	0.0322
Roots	0.0242 b	0.0269 b	0.0197 a	0.0199 a

Each value is the average of 15 determinations. Values followed by a different letter for a single plant are significantly different at the 0.05 level. Values for a single plant part without a letter are not significantly different.

TABLE XIII

EFFECT OF ABA TREATMENT ON THE TRANSLOCATION OF 0.5 µg OF STEM INJECTED IAA-1-^{14}C IN PERSIMMON SEEDLINGS AFTER 36 HOURS

Plant Part	% of Total IAA-1-^{14}C Injected			ng IAA/gm dry weight		
	Control	1.0 µg ABA/Plant	5.0 µg ABA/Plant	Control	1.0 µg ABA/Plant	5.0 µg ABA/Plant
Growing point	0.03 b	0.02 a	0.02 a	64.3 b	52.2 a	52.2 a
Expanding leaf	0.08	0.07	0.08	79.6	73.7	84.6
Leaves	1.70 ab	2.00 b	1.41 a	44.9 ab	49.5 b	36.8 a
Cotyledons	1.66 b	0.52 a	0.56 a	170.9 b	58.2 a	60.9 a
Upper stem	4.57 b	4.57 b	2.94 a	263.0 b	239.8 b	168.8 a
Treated area	50.71	59.03	51.40	7640.2	9354.4	7982.9
Roots	10.23 b	8.99 ab	7.47 a	1159.3 b	802.9 ab	684.1 a
Nutrient solution	2.09	1.74	1.67	9.14	7.89	7.49

Each value is the average of 16 determinations. Values followed by a different letter for a single plant part are significantly different at the LSD = 0.05. Values for a single plant part without a letter are not significantly different.

IAA translocation into the growing point, cotyledons, upper stem and roots. The amounts of IAA in the nutrient solution, expanding leaf, and treated area were the same among treatments within a plant part.

The data obtained for the effects of GA_3 on auxin translocation in persimmon seedlings are summarized in Tables XIV and XV. Treatment with 10 µg GA_3 per plant tripled the amount of auxin translocated into the growing point at 24 hours and doubled the amount present at 48 hours. GA_3 at the 1 µg level also enhanced the total 2,4,5-T translocation into the expanding leaf but not on the basis of 2,4,5-T concentration per unit dry weight because GA_3 increased the dry weight of the expanding leaf by 35 per cent during the 24 hour treatment period. GA_3 also inhibited auxin translocation out of the treated area at 24 hours. No other effects of GA_3 on 2,4,5-T translocation were observed in contrast with bean seedlings (5) where GA_3 inhibited basipetal translocation of 2,4,5-T.

TABLE XIV

EFFECT OF GA_3 TREATMENT ON THE TRANSLOCATION OF 0.5 µg OF STEM INJECTED 2,4,5-T-1-^{14}C IN PERSIMMON SEEDLINGS 24 HOURS AFTER TREATMENT

Plant Part	% of Total 2,4,5-T-1-^{14}C Injected				ng 2,4,5-T/gm dry wt.			
	Control	1.0 µg GA_3/Plant	5.0 µg GA_3/Plant	10.0 µg GA_3/Plant	Control	1.0 µg GA_3/Plant	5.0 µg GA_3/Plant	10.0 µg GA_3/Plant
Growing point	0.01 a	0.01 a	0.02 a	0.04 b	53.20 a	49.90 a	69.50 a	163.70 b
Expanding leaf	0.07 a	0.13 b	0.09 ab	0.06 a	139.40	138.30	134.20	119.40
Leaves	2.95	3.59	2.05	3.00	260.00	361.00	408.10	335.50
Cotyledons	0.82	0.96	1.38	0.85	111.50	133.80	199.20	120.40
Upper stem	11.08	12.49	13.84	11.29	1177.70	1598.70	1842.20	1486.10
Treated area	50.76 a	49.30 a	54.54 ab	58.06 b	7982.40 a	8614.40 a	9510.50 ab	10513.90 b
Roots	4.76	6.18	5.32	4.43	1079.00	1595.80	1316.40	1189.00
Nutrient Solution	11.91	7.30	5.40	5.30	---	---	---	---

Each value is the average for 14 determinations. Values followed by a different letter for a single plant part are significantly different at LSD = 0.05 level. Values for a single plant part without a letter are not significantly different.

TABLE XV

EFFECTS OF GA_3 TREATMENT ON THE TRANSLOCATION OF 0.5 µg OF STEM INJECTED 2,4,5-T-1-^{14}C IN PERSIMMON SEEDLINGS 48 HOURS AFTER TREATMENT

Plant Part	% of Total 2,4,5-T-1-^{14}C Injected				ng 2,4,5-T/gm dry wt.			
	Control	1.0 µg GA_3/Plant	5.0 µg GA_3/Plant	10.0 µg GA_3/Plant	Control	1.0 µg GA_3/Plant	5.0 µg GA_3/Plant	10.0 µg GA_3/Plant
Growing point	0.01 a	0.02 a	0.01 a	0.03 b	38.8 a	46.5 a	45.2 a	68.7 b
Expanding leaf	0.12	0.16	0.11	0.12	116.7	150.5	118.0	126.2
Leaves	2.27	2.87	2.97	2.17	143.4	222.5	199.5	145.9
Cotyledons	0.63	0.57	0.45	0.47	76.3	72.3	66.3	59.5
Upper stem	7.36	8.61	8.07	7.73	682.4	843.5	798.7	772.8
Treated area	40.58	42.74	40.16	40.46	7750.4	8140.6	7990.3	7684.3
Roots	7.56	6.88	7.27	9.07	1184.3	1113.3	1186.3	1384.6
Nutrient solution	10.40	10.63	11.68	10.22	---	---	---	---

Each value is the average of 16 determinations. The analysis is based on a randomized block design with each block having 1 replicate. Values followed by the same letter for a single plant part are not significantly different at the 0.05 level. Values not followed by a letter for a single plant part are not significantly different.

CHAPTER V

DISCUSSION

From the experimental evidence, conclusions can be made concerning auxin translocation and the effects of GA_3 and ABA on auxin translocation. One conclusion is that the application of exogenous auxin to several different genera of tree seedlings resulted in both acropetal and basipetal rapid translocation of appreciable quantities of exogenous (and presumably endogenous) auxin except in bur oak. This rapid basipetal auxin translocation and a portion of the acropetal translocation has been characterized as occurring in the phloem (27, 56). This type of auxin translocation has been shown to occur in several herbaceous species. Basler's group (4, 5, 56) have obtained similar results in bean seedlings. Morris' laboratory (65, 66, 67) has also reported similar acropetal and basipetal translocation of foliarly applied auxin in pea seedlings. Goldsmith et al. (27) reported similar movements of exogenous auxin fed into a leaf vein in *Coleus*. Work by Hoad's group (36, 37) and others (47, 57) has shown that the natural auxin IAA is present in the contents of phloem sieve tube members and the level of auxin(s) fluctuates with time. The results reported here and the results cited above suggest that phloem auxin translocation may be a phenomenon occurring in many, if not all, herbaceous and woody plants.

The levels of auxin translocated in bur oak were very low and the bur oak cotyledons appeared to decarboxylate 2,4,5-T since less than 60% of the applied radioactivity was recovered. This was in contrast to other species where 88% to 95% of the radioactivity applied was recovered. This could also be an explanation of why so little auxin was translocated by bur oak. There is also a strong possibility that the low level of translocation is related to the size of the large cotyledons of bur oak and the time it would take for the exogenous auxin to diffuse through the many living cells in the cotyledon to reach the vascular system. Movement through these living cells could also be responsible for the apparent decarboxylation of the 2,4,5-T.

Work by Basler (5) using bean seedlings has shown that GA_3 enhanced auxin translocation into the epicotyl and growing point and inhibited basipetal movement of auxin into the roots and nutrient solution. The results reported here were somewhat similar to his. In the species that translocated appreciable quantities of auxin, GA_3 generally appeared to enhance acropetal auxin translocation although the results were not as pronounced as was reported in bean seedlings. In eastern persimmon seedlings GA_3 did enhance auxin translocation into the growing point although no effect was observed on basipetal auxin translocation. In black walnut GA_3 enhanced auxin translocation into the growing point and upper leaves but this effect was less pronounced than in eastern persimmon.

There could be several reasons why in both eastern persimmon and black walnut GA_3 had far less effect on auxin translocation than Basler (5) reported for bean seedlings. The variety of bean used in Basler's work is a dwarf cultivar and application of exogenous GA_3

will overcome dwarfism suggesting that this cultivar is deficient in gibberellins. The tree seedlings used in these experiments are not dwarfs and apparently are able to synthesize adequate levels of gibberellins. Addition of more GA_3 would be expected to have less effect on growth. It would be consistent that with little effect on growth there should also be a small effect on auxin translocation and this is what was observed. This is the most likely possibility as to why the tree seedlings showed less response to exogenously applied GA_3. There is also the possibility that GA_3 is not an active gibberellin in these two woody species and that application of the proper gibberellin would have more effect on growth and auxin translocation. In his review article, Jones (40) has pointed out that the levels of active gibberellins can be altered by formation of glucosides or that gibberellin may be metabolized to physiologically inactive compounds. It is also possible that the exogenously applied GA_3 had been extensively metabolized or conjugated in either or both of the two species studied.

The growth inhibitor ABA appeared to inhibit acropetal auxin translocation to leaves and growing points in a number of cases. In bur oak, ABA inhibited auxin movement from the injection site. In eastern persimmon, ABA inhibited acropetal translocation and enhanced basipetal movement. The eastern persimmon data as well as the black walnut data also showed that ABA inhibited translocation of auxin to the growing point, but this effect was transitory and was not observed at time periods greater than 24 hours after treatment. This could be due to inactivation of exogenous ABA. This inactivation would not be surprising since the seedlings were maintained under

excellent growing conditions and this inactivation would explain the transitory effects of ABA on auxin translocation. These results are similar to Basler's (5) results on bean seedlings. He found that ABA inhibited acropetal auxin translocation to the young shoots and temporarily enhanced basipetal movement of auxin.

An interesting and unexpected result of this work was the apparent enhancement of acropetal auxin translocation by ABA in black walnut seedlings with attached cotyledons. A crude extract of walnut cotyledons injected into bean seedlings was found to enhance acropetal auxin movement but to have no effect on basipetal auxin translocation. No other compound has been reported to have this activity of increasing acropetal translocation without affecting basipetal transport. It is interesting to speculate that ABA and the cotyledonary factor may have interacted to make more auxin available for translocation to the upper portions of the seedlings. Since the cotyledonary factor enhanced acropetal movement this may have resulted in the observed response to ABA. This is an area that appeared to be worth further study.

The effects of ABA and GA_3 on auxin translocation are consistent with the hypothesis that growth is regulated, at least in part, by the effects of these hormones on auxin translocation to the growing point. GA_3 was shown to increase translocation of auxin into the growing point of black walnut and eastern persimmon while ABA appeared to inhibit auxin movement. This effect of ABA would have the consequence of reducing IAA levels in the growing point and this could in turn lead to slowing or cessation of growth. It should be noted that other factors besides auxin translocation are probably

involved in the control of growth and development, but this does not preclude auxin translocation from having an important role in growth and development.

One mechanism by which ABA and GA_3 could regulate auxin translocation and growth is by regulating the entry of auxin into the phloem or exit of auxin from the phloem. Goldsmith et al. (27) have shown that TIBA pretreatment decreased translocation of auxin by blocking auxin entry into the phloem. Basler (5) has shown that the auxin translocation system is not strictly a source to sink phenomenon since sugar translocation is affected in a different fashion by ABA and GA_3 than auxins. If ABA blocked the entry of auxin into phloem at the sites of auxin synthesis and/or the exit of auxin at meristematic zones and zones of rapid expansion, this would explain many of the observations made here and in other papers. It would certainly explain how ABA decreases auxin levels in the growing point and expanding leaves. This would result in slowing the rate of growth. In contrast with ABA, GA_3 could tend to enhance auxin entry from the site of application and/or enhance auxin exit from the phloem at the meristematic zones or expanding tissues. This would result in an increased rate of growth. Then the balance between ABA and GA_3 could regulate auxin translocation in the phloem and thus determine the levels of auxin in meristematic regions and expanding tissues. The level of auxin would, in turn, determine the rate of growth. This is certainly an attractive hypothesis but the evidence for it is circumstantial and the hypothesis is certainly simplistic. More experimental work would be necessary to prove the hypothesis.

The data also support the idea that ABA may play a role in the

resistance of stressed plants to phenoxy herbicides. Data from many workers (1, 2, 6, 72, 102) have established that stressed plants have greatly decreased rates of herbicide translocation. ABA has been shown to increase in stressed plants by many laboratories (60, 64, 69, 103, 104). The experimental data presented here show that ABA tended to inhibit the translocation of the phenoxy herbicide, 2,4,5-T possibly by inhibiting entry into and/or exit from the phloem. Collectively, these pieces of evidence suggest that stress increases ABA levels which result in decreased phenoxy herbicide translocation. Since translocation of these herbicides is, in general, necessary for them to exert their phytotoxic effects, then decreased translocation of these herbicides caused by ABA would explain, in part, the resistance of stressed plants to the phenoxy herbicides. This is certainly not the only factor involved, however, and more work is necessary to test this hypothesis.

CHAPTER VI

SUMMARY

Stem injection or petiolar application of auxin resulted in both acropetal and basipetal auxin translocation in black walnut and eastern persimmon seedlings.

The primary effect of gibberellic acid on auxin translocation appears to be an enhancement of auxin translocation in the acropetal direction. In eastern persimmon seedlings GA_3 enhanced auxin translocation to the growing point and expanding leaf. In black walnut GA_3 enhanced auxin translocation to the growing point and upper leaves in several instances although this effect appeared to be less pronounced than in eastern persimmon.

ABA appeared to inhibit acropetal translocation to leaves and young shoots in a number of cases. In black walnut ABA and a cotyledonary growth factor interacted to enhance acropetal auxin translocation in black walnut. In black walnut seedlings with the cotyledons removed, ABA inhibited auxin translocation.

The data are consistent with the hypothesis that the regulation of growth and development by ABA and gibberellins could be, in part, due to their effects on auxin translocation.

The data are also consistent with the idea that the resistance of stressed plants to phenoxy herbicides could be due, in part, to ABA inhibiting the translocation of the phenoxy herbicides.

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APPENDIXES

TABLE XVI

THE EFFECT OF ABA ON THE TRANSLOCATION OF 0.5 µg OF 2,4,5-T-1-^{14}C IN PERSIMMON AT 12 HR AFTER TREATMENT

	ng 2,4,5-T/gm dry weight			
Plant Part	Control	1.0 µg ABA/Plant	5.0 µg ABA/Plant	10.0 µg ABA/Plant
Growing point	72.1 b	50.6 ab	44.2 a	33.8 a
Expanding leaf	110.1 b	123.3 b	68.9 a	75.2 a
Leaves	712.2 b	674.8 b	309.3 a	468.5 a
Cotyledons	119.7	166.1	124.0	130.4
Upper stem	1348.1 a	1337.4 a	1305.2 a	2037.1 b
Treated area	16600.2	17634.8	14821.7	16817.6

The values are averages of 15 determinations. Values followed by a different letter for a single plant part are significantly different at the 0.05 level. Values for a single plant part without a letter are not significantly different. Data for the roots and nutrient solutions are shown in Tables XXI and XXII.

TABLE XVII

THE EFFECT OF ABA ON THE TRANSLOCATION OF 0.5 µg OF 2,4,5-T-1-^{14}C IN PERSIMMON 24 HR AFTER TREATMENT

Plant Part	ng 2,4,5-T/gm dry weight			
	Control	1.0 µg ABA/Plant	5.0 µg ABA/Plant	10.0 µg ABA/Plant
Growing point	89.6 b	68.1 ab	42.3 a	47.3 a
Expanding leaf	164.2 b	153.0 b	110.4 a	107.1 a
Leaves	606.1 b	504.9 b	198.2 a	167.7 a
Cotyledons	212.2	211.2	110.8	95.5
Upper stem	1691.8	1859.7	1379.3	1326.8
Treated area	10275.7 a	9698.7 a	10394.7 a	13259.3 b

The values are averages of 15 determinations. Values followed by a different letter for a single plant part are significantly different at the 0.05 level. Values for a single plant part without a letter are not significantly different. Data for the roots and nutrient solutions are shown in Tables XXI and XXII.

TABLE XVIII

THE EFFECT OF ABA ON THE TRANSLOCATION OF 0.5 µg OF 2,4,5-T-1-^{14}C IN PERSIMMON 36 HR AFTER TREATMENT

Plant Part	ng 2,4,5-T/gm dry weight			
	Control	1.0 µg ABA/Plant	5.0 µg ABA/Plant	10.0 µg ABA/Plant
Growing point	62.1	67.4	66.2	60.6
Expanding leaf	147.7	175.1	158.6	133.5
Leaves	404.6 b	322.6 b	222.1 a	148.4 a
Cotyledons	286.5 b	136.1 a	86.2 a	60.4 a
Upper stem	1463.7	1297.3	1118.6	975.5
Treated area	11758.3	11352.0	12751.2	11315.7

The values are averages of 15 determinations. Values followed by a different letter for a single plant part are significantly different at the 0.05 level. Values for a single plant part without a letter are not significantly different. Data for the roots and nutrient solutions are shown in Tables XXI and XXII.

TABLE XIX

THE EFFECT OF ABA ON THE TRANSLOCATION OF 0.5 µg OF 2,4,5-T-1-^{14}C IN PERSIMMON 48 HR AFTER TREATMENT

Plant Parts	ng 2,4,5-T/gm dry weight			
	Control	1.0 µg ABA/Plant	5.0 µg ABA/Plant	10.0 µg ABA/Plant
Growing point	61.2	70.5	62.5	64.2
Expanding leaf	87.5	119.8	110.2	105.7
Leaves	458.9	250.9	326.6	118.9
Cotyledons	265.1 b	188.6 ab	166.2 ab	75.7 a
Upper stem	958.9	964.0	860.5	906.3
Treated area	5820.6 a	6907.1 b	8898.9 c	8217.9 c

The values are averages of 15 determinations. Values followed by a different letter for a single plant part are significantly different at the 0.05 level. Values for a single plant part without a letter are not significantly different. Data for the roots and nutrient solution are shown in Tables XXI and XXII.

TABLE XX

EFFECT OF ABA ON THE TRANSLOCATION OF 2,4,5-T-1-^{14}C INTO PERSIMMON ROOTS AT VARIOUS TIMES

Time of Treatment (Hrs)	% of Total Injected			
	Control	1.0 µg ABA/Plant	5.0 µg ABA/Plant	10.0 µg ABA/Plant
12	3.7	3.4	4.1	3.5
24	4.2 a	5.6 b	5.7 b	4.7 ab
36	4.4 a	6.2 b	6.1 b	7.5 b
48	3.6	4.1	2.4	3.3

The values are the averages of 15 determinations. Values followed by a different letter for a single time are significantly different at the 0.05 level. Values for a specific time without a letter are not significantly different.

TABLE XXI

EFFECT OF ABA ON THE TRANSLOCATION OF 2,4,5-T-1-^{14}C IN PERSIMMON INTO THE NUTRIENT SOLUTION AT VARIOUS TIMES

Time of Treatment (Hrs)	% of Total Injected			
	Control	1.0 µg ABA/Plant	5.0 µg ABA/Plant	10.0 µg ABA/Plant
12	1.7	1.7	1.8	1.8
24	13.2	16.1	13.5	14.1
36	14.4	18.6	12.1	18.1
48	21.8	18.2	16.3	20.8

The values are averages for 16 determinations. Values followed by a different letter for a single time are significantly different at the 0.05 level. Values for a specific time without a letter are not significantly different.

VITA

William Edgar Blanton

Canditate for the Degree of

Doctor of Philosophy

Thesis: THE EFFECT OF ABA AND GA ON THE TRANSLOCATION OF AUXIN IN TREE SEEDLINGS

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