AN ABSTRACT OF THE THESIS OF

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Title: Some Factors Affecting Absorption and Translocation of Phenoxyalkylcarboxylic Acids in Plants

Abstract approved: (Major professor)

Studies were conducted to determine the influence of formulation and molecular configuration of some chlorinated-phenoxyalkylcarboxylic acid herbicides on their absorption, translocation and metabolism in bigleaf maple, Acer macrophyllum, Pursh.

Five to eight year old bigleaf maple seedlings were treated with one percent solutions of carbon 14-labeled herbicides. After three days the treated leaves were washed with alcohol and the plant sectioned into the treated leaves, new growth, stem and roots.

A test of the acids, triethanol amine salts and 2-ethylhexyl esters of 2,4-D and 2,4,5-T revealed an inverse relationship between molecular polarity and absorbability within herbicides. The translocation of the various formulations and chemicals were influenced to a large degree by the amount absorbed. The translocatability of the acids and the amine salts were similar while the ester formulations were lower. In terms of actual amounts of chemical translocated to
the roots, however, 2,4,5-T ester was most effective.

A study of the 2-ethylhexyl ester of 2,4-D, 2,4,5-T, 2,4-DP and 2,4,5-TP revealed some trends in absorbability. In general, the alpha-phenoxypropionic herbicides were absorbed to a greater degree than the phenoxyacetic herbicides. Between herbicides of equal side chain length, the dichlorinated member was more readily absorbed than the trichlorinated member.

The translocatability of these various herbicides from the treated leaves was not significantly different. However, the movement into the roots differed significantly among chemicals. In terms of actual amounts of herbicide translocated to the roots, the following decreasing order was observed: 2,4-DP, 2,4,5-T, 2,4-D and 2,4,5-TP. It is believed that chemical toxicity to the transport mechanism, absorption by the phloem parenchyma cells and leakage to the xylem influenced herbicide movement to the roots.

Studies of the metabolism of 2,4-D, 2,4,5-T, 2,4-DP and 2,4,5-TP in single detached bigleaf maple leaves revealed that decarboxylation was not an important means of detoxification in this species. Paper chromatograms prepared from alcohol extracts of these leaves showed only limited formation of metabolites. The metabolism of 2,4-D and 2,4,5-T in intact seedlings, however, showed various plant parts have a greater ability to alter the form of the applied chemical than the treated leaves. Differences in the rate of chemical alteration between 2,4-D and 2,4,5-T were also observed in the same plant part. It was shown that 2,4,5-T was more stable than 2,4-D in most plant parts. The stability of 2,4,5-T in the roots was considered of
importance in determining the relative effectiveness of these two herbicides on bigleaf maple.

Studies of the absorption, translocation and metabolism of 2,4-DB in bigleaf maple were also conducted. It was shown that detached bigleaf maple leaves rapidly decarboxylated 2,4-DB. Absorption and translocation studies revealed slightly reduced absorption compared to the other herbicides tested, but it was markedly superior in translocatability.

Gas chromatography was used to establish that the primary product of oxidation of 2,4-DB in bigleaf maple was 2,4-D. These tests also indicated the form of the translocated material was 2,4-DB rather than its oxidation product, 2,4-D. Studies using excised root and stem tissue revealed the roots were capable of rapidly converting 2,4-DB to 2,4-D. The stems had only a limited ability to perform this conversion. It is felt the gamma-phenoxybutyric herbicides may have a valuable place in control of some brush species which have resisted control with aerial application of 2,4-D and 2,4,5-T.
SOME FACTORS AFFECTING ABSORPTION AND TRANSLOCATION OF PHENOXYALKYL CARBOXYLIC ACIDS IN PLANTS

by

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SOME FACTORS AFFECTING ABSORPTION AND TRANSLOCATION OF PHENOXYALKYL CARBOXYLIC ACIDS IN PLANTS

INTRODUCTION

The increasing demand on the nation's forests for wood, water and forage makes imperative the intensification of forest management. While the demand for the products and services of the forest increases, the land area to produce them is being decreased by reservoirs, road and powerline rights of way and the removal of large tracts of land primarily for recreational and preservative purposes.

The Timber Resources Review reported about 60 percent of the forests in the Douglas-fir region are less than fully stocked. About half of this is attributed to brush encroachment (73). It is evident that a primary means of increasing the softwood timber supply will be fuller utilization of these lands for tree growth. Brush control will play a major role in accomplishing this end. Of the various means of brush control, such as scarification, burning and the use of chemicals, herbicides offer probably the cheapest and most effective tool for most areas. Chemical brush control affords about the only answer for rehabilitation of areas covered by species which sprout when damaged by logging or other disturbances.

Chemicals for brush control can be applied in several ways, such as basal spraying, injection, mist blowers, high volume broadcast spraying and low volume aerial applications. While each of these methods has utility in certain situations, aerial application of low volumes of chemical offers the best alternative for treatment of
large areas of forest land, particularly where limited access is a problem. The spraying of large areas by helicopter with low volumes of herbicide mixtures may frequently be accomplished for less than ten dollars per acre.

Aerial spray applications have met with variable success. Often, for reasons not readily apparent, operations which should have been successful have failed. There are a great many variables involved in chemical brush control; among them are species composition, timing of application, weather, chemicals, formulations, carriers, additives, rates of application of both herbicide and carrier and plant responses which are peculiar to particular species. Newton stated, "The field of brush control consists of two distinct branches which are seldom pursued together. These are: (1) basic research into the physiology of brush species and the herbicidal reactions; and (2) the study of applied methods which result in effective brush eradication." He further stated, "The point has now been reached at which further progress (in applied methods) is seriously retarded because there is little basic information available in this field." (58, p. 5).

The basic information needed for further advances in applied herbicide research will come from studies of the physiology of brush control. While a great deal of physiological research has been done since the discovery of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in 1941, by Pokorny (62), very little has been done with woody plants. The great majority of research has been directed toward understanding and solving herbicide problems in agriculture. While many basic
principles found in these studies also apply to brush control, characteristics of woody plants, in particular their growth habits and size, make it necessary to determine the degree to which these principles hold true. For example, Freed and Montgomery have shown the absorption of 3-amino-1,2,4-triazole by bean plants is increased more than fourfold when applied in water with 0.013 percent X-77 surfactant (31). It is likely that the relationship of enhanced herbicide uptake when applied with a surfactant also holds true for brush species, but the magnitude of increase probably is different. Many brush species are difficult to control with economical aerial sprays, and small differences in the degree to which a particular principle applies may be sufficient to determine the success or failure of a spray project.

Brush control physiology is a field devoted to basic studies of the mechanisms and factors which influence absorption, translocation, metabolism and mode of action of herbicides in woody plants. From these basic studies will come the knowledge to explain the variability of success in previous brush control projects and reduce the probability of failures in future operations. Of vital importance to the chemical and forest industries will be the knowledge which leads to development of new chemicals, formulations and techniques of application for more efficient and effective brush control.

The purpose of this research is to investigate certain basic factors involved in the response of a particular plant species to a selected series of growth regulator herbicides. The effect of formulation and molecular configuration of the parent herbicide molecule
on the absorption, translocation and metabolism of a series of chlorinated phenoxyalkylcarboxylic herbicides (phenoxy herbicides) in bigleaf maple, *Acer macrophyllum* Pursh., was studied.

The objectives of this research are: (1) a determination of the importance of these factors in the selectivity among chemicals on a single species; (2) a determination of the importance of these factors in the poor degree of control of bigleaf maple which often results from aerial spraying; and (3) the formulation of suggestions for areas of additional applied research. The ultimate goal is the development of more effective chemicals and control methods for brush species which are presently inadequately controlled with aerial application of herbicides.
REVIEW OF LITERATURE

A great deal has been published concerning the absorption and translocation of phenoxy herbicides by plants. Unfortunately, much of the early work depended on plant responses as a quantitative indication of absorption or translocation. While such techniques have their value, the use of radioactive labeled herbicides has allowed the amount of absorption and translocation to be determined with much greater precision.

The use of radioactive labeled herbicides requires that certain precautions in the interpretation of data be observed. Van Overbeek points out that the distribution of radioactivity in the plant shows only the distribution of the labeled element and not necessarily that of the molecule in which it was originally incorporated (74, p. 319). The fact that labeled phenoxy herbicides may be altered in the plant is well established (26; 27; 37; 43). Pallas and Crafts have demonstrated that the treatment of plant sections after exposure to labeled herbicides can markedly influence distribution of radioactivity during any drying process (61).

It is important to determine if the relationship between absorption and translocation has been considered when they are reported as separate entities. As an example, when comparing the amounts of two herbicides translocated to roots from treated foliage, proper consideration must be taken of possible differences in absorption. It is obvious that no more herbicide can be translocated than is absorbed. In addition, differential absorption may markedly
influence translocation due to the toxic effect of the herbicide on transport tissues.

A great many reviews of the absorption, translocation, metabolism and mode of action characteristics of various herbicides have been published during the past ten years. References to many studies which helped develop some of the basic principles of herbicide physiology may be found in reviews by: Crafts (14, p. 253-282), van Overbeek (74, p. 355-372), Mitchel and Linder (52, p. 165-182), Woodford, Holly and McCready (83, p. 311-358), Currier and Dybing (19, p. 195-218), Hull (41, p. 214-231), Crafts (12, p. 14-17, 28-47, 52-70), Dakshindas (20, p. 233-244), and others. Of particular interest in this research, however, are studies which deal with the influence of formulation and molecular configuration of the parent herbicide molecule on absorption, translocation and metabolism of phenoxy herbicides. Further interest is expressed as these factors relate to selectivity between chemicals in bigleaf maple.

Van Overbeek stated "The penetration of herbicide molecules is much dependent upon their molecular structure. When penetration into and through the lipoid phases are involved, a less polar compound has a better chance to achieve penetration than a more polar compound." (74, p. 362). Blackman et al., from their work with variously substituted phenoxyacetic acids, concluded the pattern of uptake of herbicides is determined by the chemical structure of the growth substance and by specific physiological differences at the cell level (7, p. 53-54).
The relative absorption of various formulations of 2,4-D in a variety of plant species has been established. Almost without exception it has been found that the less polar a compound, the greater its absorption by foliage. Crafts showed that esters of 2,4-D are most readily absorbed followed by the ammonium and sodium salts (15). Hauser reported the isopropyl ester of 2,4-D most readily absorbed, followed by an amine salt and the sodium salt in both soybeans and corn (36). Dybing and Currier, working with Zebrina, showed greater absorption of the triethanol amine salt than the sodium salt of 2,4-D (21). Morré and Rogers demonstrated that absorption of the octyl ester of 2,4-D by corn coleoptiles exceeds absorption of the acid (55). Walker, Beck and Dumbroff, working with Quercus nigra and Liquidambar styraciflua seedlings, reported the triethyl amine salt of 2,4,5-T more readily absorbed than the ammonium salt in both species (77). Crafts has reported differences in the effectiveness of a number of ester formulations, an emulsifiable acid and an amine salt formulation. He observed that the bending response of bean plants is greatest with the methyl ester of 2,4-D followed by the hexyl ester and the octadecyl ester. In another test of the same type, Crafts showed the effectiveness of isopropyl, propylene glycol butyl ether and butoxyethanol esters and emulsifiable acid of 2,4-D were of the same degree of magnitude while an isopropyl amine was less than half as effective (16, p. 309-310). It is obvious from these various independent studies that formulation has a marked effect on uptake of phenoxy herbicides.
There have been only a few studies which considered the effect of configuration of the parent herbicide molecule on absorption. A number of studies on absorption were made shortly after the discovery of 2,4-D and 2,4,5-T, but unfortunately the data reported are not precise since plant responses, such as degree of bending or increase in weight, were used as an indication of amount of herbicide absorbed. Such studies assumed that the two herbicides would be equally effective in eliciting the same response. It is possible that such a study might report, on the basis of plant response, that 2,4-D is absorbed to a much greater degree than 2,4,5-T, while in fact, nearly equal amounts were absorbed. In this case, the 2,4,5-T may not have been translocated as well and was less effective in eliciting the measured response—a response which may have nothing to do with ultimate herbicide effectiveness. The use of radioactive herbicides has done much to eliminate such misleading observations.

Weintraub, Reinhart and Scherff reported that the percent absorption of 4-chlorophenoxyacetic acid, 4-fluorophenoxyacetic acid, 2,4-difluorophenoxyacetic acid and 2,4-D was about the same in bean plants while absorption of 2,4,5-T was only 60 percent as great (79). Slife et al., studying the absorption, translocation and metabolism of 2,4-D and 2,4,5-T in wild and cultivated cucumbers, noted that 2,4-D is absorbed to a greater degree than 2,4,5-T (68). Edgerton and Hoffman were able to show a ten to twenty percent increase in absorption by substituting a fluorine for a chlorine in 2,4-D in two varieties of apple (22). Dybing and Currier reported that the triethanol amine salt of 2,4-D was more readily absorbed than the
triethanol amine salt of 2,4,5-T in Zebrina (21). Yamaguchi and Crafts, in work with a number of woody plants, studied the uptake of 2,4-D and 2,4,5-T by the exposed phloem of the stem. They reported the magnitude of uptake and movement of these two herbicides was very similar (84, p. 176-177). These studies point out some definite differences in absorption of related herbicides with minor changes in ring substitution.

The effect of formulation is believed to be restricted to its influence on the absorption-translocation interaction. Phenoxy herbicides absorbed as the acid will be dissociated to a large degree in the leaf depending on their concentration. The amine salt formulations are simple amine salts of phenoxy acids and are probably completely dissociated in aqueous media. It is evident that the acid and amine salts will behave in a similar manner once they are absorbed. The esters on the other hand, present a different problem. The dissociation of the ester in water is not anticipated since it is formed with a covalent bond, rather than the ionic bond of the amine salt. Alteration of the ester to the acid and alcohol moities requires hydrolysis of the molecule.

A few investigators have demonstrated that ester formulations may be hydrolyzed on the leaf surface or in the plant following absorption. Glastonbury, Stevenson and Bell, using infrared spectroscopy for 2,4-DB residue analysis in lucerne, showed nearly complete hydrolysis of an ester of 2,4-DB in 24 hours. They note, however, that a six degree Fahrenheit drop in temperature prolonged the process for four days. The plant parts were not analyzed separately (34).
Crafts used carbon 14 labeled isopropyl alcohol and 2,4-D-1-C\textsubscript{14} in studies of ester hydrolysis in barley. He reported the alcohol group remained in the treated leaf while carboxyl-labeled 2,4-D moved throughout the plant. Crafts felt this indicated hydrolysis of the ester in the treated leaf, and translocation of acid in the phloem (13). Morré and Rogers showed hydrolysis of the octyl ester of 2,4-D by spinach and cucumber enzyme preparations. They also reported release of 2,4-D acid by cucumber root tissue incubated with 2,4-D ester. The authors felt the response lag in bean plants treated with the propylene glycol butyl ether and octyl esters of 2,4-D compared to the acid, is additional evidence of ester hydrolysis (56, p. 442-443). Morré and Rogers studied the uptake of the acid and octyl ester of 2,4-D by corn coleoptiles and found the ester more readily absorbed. They felt part of this response could be due to the internal hydrolysis of the ester to the acid, which maintained a favorable concentration gradient for uptake of the ester (55).

Szabo reported some preliminary work with intact bean and corn plants showing hydrolysis of the propylene glycol butyl ether and butoxyethanol esters of 2,4-D on the leaf surface. Paper chromatographs of extracts from bean and corn plants treated with these esters showed only partial ester hydrolysis in 24 hours (72). However, the temperature at which these tests were run was not noted, and the work by Glastonbury, Stevenson and Ball (34) indicated the rate of hydrolysis is greatly influenced by temperature. Szabo also reported that a bean plant extract incubated with the propylene glycol butyl ether and
butoxyethanol esters of 2,4-D showed partial hydrolysis of the esters (72).

It is fairly clear that plants are able to at least partially hydrolyze the esters of 2,4-D. The question which remains is the extent to which this process proceeds. Szabo's report showed nearly complete hydrolysis of the ester on the leaf surface in three to five hours. Perhaps the more rapid uptake of ester formulations is influenced not only by the ester itself, but also the solubilizing effects of the hydrolyzed alcohol groups on the plant lipid barriers.

The influence of formulation then is probably restricted to its effect on absorption, and the subsequent influence of absorption on translocation. It is obvious that no more herbicide can be translocated than is absorbed, but equally important is the toxic action of the herbicide on the transport mechanism. Crafts has indicated that for effective translocation, the herbicide must move in quantities which do not prevent normal functioning of sieve cells (12, p. 16). Linder, Brown and Mitchell, working with 2,4-D in bean plants, reported the herbicide was translocated in amounts proportional to the applied dose up to a certain level. It was felt that above this level, toxic effects of the herbicide were reducing the effectiveness of the energy-requiring herbicide transport mechanism (48).

The effect of the molecular configuration of the parent herbicide molecule on translocation has not been adequately studied. A number of studies have been reported in which plant responses were used to indicate the quantitative translocation of different
herbicides. The objection to this technique was outlined above. The few studies in which radioactive herbicides were used are more valuable.

Weintraub, Reinhart and Scherff, working with carbon 14 carboxyl-labeled 2,4-D, 2,4,5-T, 4-chlorophenoxyacetic acid, 4-fluorophenoxyacetic acid and 2,4-difluorophenoxyacetic acid in corn, bean, soybean, cotton and potato plants, reported a definite molecular configuration influence on translocation from the treated leaf. Marked differences were noted not only among chemicals in a single plant species, but also among plant species treated with the same chemical (79). Leonard and Yeates reported inferior translocation of 2,4,5-T-1-C14 when compared with 2,4-D-1-C14 in gorse and scotch broom (46). Slife et al., working with 2,4-D-1-C14 and 2,4,5-T-1-C14 in wild and cultivated cucumbers, reported 2,4,5-T appeared to be highly mobile compared to 2,4-D (68). Butts and Fang reported some marked differences in the translocatability of a series of carbon 14 carboxyl-labeled phenoxyacetic acids from the treated leaves of bean plants. They found 59 percent of the 2,4-D in the treated leaf after one day compared to 72 percent for 2,4,6-trichlorophenoxyacetic acid, 79 percent for 2,4,5-T and 80 percent for ortho-chlorophenoxyacetic acid (10).

Van Overbeek stated that translocation is dependent on molecular configuration, and that the introduction of a methoxy group in the alpha carbon atom of the side chain of 2,4-D improves its translocation. Unfortunately, he sites no reference for this work (74, p. 366). However, Mitchell, Marth and Preston reported alpha methyl-substituted
phenylacetic acid showed greater translocation compared to regular phenylacetic acid. This same relationship was found with some carbamate herbicides. They suggested that alpha substitution next to the carboxyl group may improve the translocation of other compounds (53).

It is well established that formulation and molecular configuration of the parent herbicide molecule influence absorption and translocation of phenoxy herbicides in many plant species. What remains to be shown is the extent to which these chemical factors also have an effect on absorption and translocation in woody plants. In addition, a study which incorporates a larger number of phenoxy herbicides such as 2,4-D, 2,4,5-T, dl-2(2,4-dichlorophenoxy)propionic acid (2,4-DP), dl-2(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP) and 4(2,4-dichlorophenoxy)butyric acid (2,4-DB), would give valuable information concerning the effects of nuclear substitution and configuration of the side chain, and their interaction, on absorption and translocation in plants. A study of this kind has not been reported. Such studies will help to show whether or not the principles of herbicide physiology developed with more common test plants are also valid for woody plants. The determination of the extent to which these principles are true may prove valuable in the development of new compounds and methods of chemical brush control.

The metabolism of the phenoxy herbicides, particularly 2,4-D, has been extensively studied by a number of investigators. Their studies have usually been designed to determine either the elusive mode of action of 2,4-D, or detoxification mechanisms on which to base
chemical selectivity between plants. This subject is reviewed by Shaw et al. (66, p. 124-125) and Hilton, Jansen and Hull (38, p. 353-384).

In the recent review by Hilton, Jansen and Hull, the authors noted that the metabolic fate of chlorinated phenoxyalkyl acid herbicides include: (1) physical or chemical conjugation with cellular constituents, (2) degradation of the aliphatic side chain and release of the substituted phenol, and (3) hydroxylation of the substituted benzene ring. The authors felt the relative importance of each process was related to the chemical structure of the herbicide (38, p. 365-368). It is also apparent, however, that the relative importance of these three processes is also influenced by the plant species under investigation.

A number of investigators have shown the formation of various plant-herbicide "complexes" or other herbicide metabolites. Linscott and McCarty, working with ironweed, were able to show a rapid reduction in extractable quantities of 2,4-D-1-C\textsuperscript{14} during a three week period. They showed 14 percent of the radioactivity in unknown compounds in one day; however, this value falls to 1.1 percent in 14 days. The total percent of carbon 14 recovery was dropping at the same time which the authors felt indicated some of the radioactivity was being lost as C\textsubscript{14}O\textsubscript{2} and other portions being incorporated into plant constituents which were not alcohol extractable (49). Luckwill and Lloyd-Jones reported the formation of various water soluble metabolites in leaves of two species of currant and in apple and strawberry leaves. These metabolites have no biological activity in
the oat mesocotyl and coleus abscission test, but yielded 2,4-D on mild hydrolysis with two normal sulphuric acid (50, p. 620; 51, p. 629-630). Morgan and Hall showed the formation of two herbicide-plant constituent complexes in cotton and sorghum which accounted for 52 percent and 36 percent respectively of recovered radioactivity in three days (54). Leafe, in work with chlorine 36 and carbon 14 labeled 2-methyl-4-chlorophenoxyacetic acid (MCPA) and dl-2(4-chloro-2-methylphenoxy)propionic acid (CMPP), reported 75 percent of the applied dose of MCPA appeared as a phenol in the water soluble fraction of Gallium aparine extract, while CMPP was recovered unchanged (45). Butts and Fang (10), Bach and Fellig (4, p. 283-284), (3), Canny and Markus (11, p. 91-93), Holley (39), Jaworski and Butts (42, p. 213), Jaworski, Fang and Freed (43) and Slife et al. (68), all reported the formation of various herbicide-plant constituent complexes or other herbicide metabolites. The extent and type of metabolism was dependent on the chemical and plant studied.

Loss of the side chain of phenoxy herbicides is frequently reported in the literature. However, the extent of cleavage is dependent on the molecular configuration of the herbicide and the plant species under study. Investigators have reported rates of decarboxylation which range from less than one percent of the applied material in one day to more than 50 percent in four days. Morgan and Hall, studying the metabolism of 2,4-D-1-C14 in cotton and sorghum, noted that although the rate of decarboxylation was five to ten times higher in cotton, the total amount released was less
than one percent of the total recovered radioactivity (54). Weintraub, Reinhart and Scherff studied the decarboxylation rates of five variously substituted phenoxyacetic acids in corn plants and found the magnitudes to be small. However, differences in decarboxylation rates between chemicals were found. The authors reported 4-chlorophenoxyacetic acid released 1.2 percent of the applied activity as $^{14}O_2$ in 24 hours compared to one percent for 2,4-D, 0.2 percent for 2,4,5-T, 0.4 percent for 4-fluorophenoxyacetic acid and 0.3 percent for 2,4-difluorophenoxyacetic acid (79). Canny and Markus reported less than six percent of the applied 2,4-D-1-C$_{14}$ appeared as $^{14}O_2$ in 86 hours from broad beans, *Vicia fabia* (11, p. 91-93). Leafe, working with MCPA and CMPP in *Galium aparine*, reported seven percent of the applied MCPA-1-C$_{14}$ activity released as $^{14}O_2$ in ten days with practically no release from CMPP-1-C$_{14}$ (52). Fang *et al.*, using 2,4-D-2-C$_{14}$ reported, 17.5 percent of applied activity released as $^{14}O_2$ in three days by bean plants (27). Edgerton and Hoffman, in studies of chemical selectivity with resistant and susceptible varieties of apple, noted the resistant species decarboxylated 2,4-D-1-C$_{14}$ rapidly. More than 33 percent of absorbed activity was released as $^{14}O_2$ in 24 hours while the susceptible species released only 0.5 percent. It is interesting to note that the decarboxylation rate of 2-chloro-4-fluorophenoxyacetic-1-C$_{14}$ acid was appreciably less in both species (22). Luckwill and Lloyd-Jones reported 57 percent decarboxylation of 2,4-D-1-C$_{14}$ in 92 hours by a resistant variety of apple compared to two percent for a susceptible variety (51, p. 627-628). These authors also showed that red currants, which
are resistant to 2,4-D, released 50 percent of applied activity of 2,4-D-l-C\textsuperscript{14} as Cl\textsubscript{14}O\textsubscript{2} in one week while susceptible black currants released only two percent (50, p. 616-618). It is readily apparent that plants are able to decarboxylate 2,4-D-l-C\textsubscript{14} with varying ability. The importance of this factor in chemical selectivity is uncertain.

Hydroxylation of the ring of phenoxy herbicides has also been proposed as a possible metabolic fate. However, evidence for this is slight, and the importance of this mechanism in herbicide detoxification by plants is doubtful. Wilcox, Moreland and Klingman reported excised oat, barley and corn roots were able to form a hydroxylated metabolic product from non-substituted phenoxy-n-aliphatic acids with side chains ranging from two through six carbons in length. Peanut, soybean and alfalfa plant roots on the other hand, were unable to form this metabolite. Evidence from paper chromatography and ultraviolet spectroscopy showed the hydroxylated product to be 4-hydroxyphenoxyacetic acid. The authors suggested that the molecule is subjected to beta oxidation prior to hydroxylation, since no hydroxylated products were found with more than two carbons in the side chain (81). Faulkner and Woodcock reported the hydroxylation of 2-chlorophenoxyacetic acid and 4-chlorophenoxyacetic acid by Aspergillus niger (28). However, Bach isolated ten metabolic products from bean stems infiltrated with 2,4-D-l-C\textsubscript{14} for three days and although some components contained phenol and alcohol groups, they exhibited no aliphatic unsaturation, and the aromatic nucleus remained intact. No evidence of hydroxylated 2,4-D was found (2).
Ring-hydroxylation of phenoxy herbicides does not appear to be an important means of detoxification in plants.

When considering the metabolism of herbicides one normally thinks of detoxification; however, this is not always the case. A special and important exception is the metabolism of chlorinated phenoxybutyric herbicides. Neither 2,4-DB nor 2,4,5-TB possess biological activity in the butyric form. However, it has been demonstrated that certain plants are able to convert butyric herbicides to biologically active acetic herbicides, i.e., 2,4-D or 2,4,5-T.

The principle of beta oxidation in the fat metabolizing systems of animals has been known for some time. Beta oxidation in plants has only recently been shown. In 1947, Synerholm and Zimmerman demonstrated that plants were able to metabolize certain omega-2,4-dichlorophenoxy-n-aliphatic acids to substances with biological activity equivalent to 2,4-D. The authors reported when the compound C₆Cl₂H₃-(CH₂)ₙ-COOH, where "n" was equal to one, three, five or seven, was applied to tomato leaves for 24 hours, a compound or compounds resulted which showed biological activity equal to 2,4-D. Where "n" was an even number, the resulting compounds were biologically inactive (70, p. 376-377). Synerholm and Zimmerman felt these tests were an indication that the compounds which showed biological activity may have been beta oxidized to 2,4-D. This early work has been confirmed, and the opinions of the authors substantiated.

Fawcett, Ingram and Wain, in 1954, showed that long-chain phenoxy acids with an even number of methylene groups in the side chain were
oxidized to the phenol in flax plants in ten days. Only traces of the phenol were produced from acids with an odd number of methylene groups in the side chain (29, p. 68).

Wain and Wightman studied the oxidation of 4-chloro-, 2,4-dichloro-, and 2,4,5-trichlorophenoxy-n-aliphatic acids with the wheat cylinder, pea curvature and tomato leaf epinasty tests. They found that wheat cylinders were able to beta oxidize all the acids tested with an odd number of methylene groups in the side chain to the active-acetic form in 24 hours. However, ring substitution was shown to have an effect on the oxidation capabilities of other test organisms. The trichlorinated phenoxy-n-aliphatic acids were not degraded in the pea curvature test or tomato leaf epinasty test in 24 hours. If the trichlorinated materials were first incubated with wheat cylinders for 24 hours however, a positive response was noted in these tests (76, p. 529-530 and plates 34-37). This indicates that some degree of selectivity in the beta oxidation process, which is influenced by the molecular configuration of the compound under consideration, exists in plants.

This conclusion is substantiated by work reported by Fawcett et al., in 1959, with a series of various methyl and chlorine substituted omega-phenoxyalkane carboxylic acids. All those acids with an odd number of methylene groups in the side chain showed high levels of biological activity in the wheat cylinder test in 24 hours. With ten members of the series, however, oxidation stopped at the butyric stage in pea and tomato tissue after 24 hours. The authors indicated hindrance to oxidation at the butyric stage
was associated with the presence of an ortho chlorine or methyl group, though the effect was largely removed by introducing an additional chlorine atom at the para but not the meta position (30, p. 99-104). Fryer and Chancellor, in field tests on a number of annual and perennial weeds, reported not all species had equal ability to metabolize 2,4-DB to 2,4-D (32, p. 357-377). Wain reported that neither celery nor clover were affected by applications of 2,4-DB (75). Shaw and Gentner screened 25 phenoxy-n-aliphatic chemicals on ten different plant species. Differences in plant ability to convert these chemicals to the active acetic form were evident both between chemicals on a particular plant species and between species with a single chemical. They noted that if a chemical is to be beta oxidized within a plant it must possess the following characteristics: (1) properties conducive to absorption and translocation, (2) a structure suitable for attachment to sites of oxidation, (3) a side chain structure suitable for oxidation and (4) a ring structure which does not interfere with the beta oxidation of the side chain (67, p. 86-89).

Woodford, Holly and McCready observed that the theory of selective action of the gamma-phenoxybutyric acids is based on the presence or absence of a specific beta oxidizing system in different species. They pointed out, however, that very little is known concerning the location and behavior of enzymes in the living plant, and it is possible that some of the differences between the phytotoxicity of gamma-phenoxybutyrics and acetic homologues could be explained on the basis of differences in absorption and translocation (83, p. 325).
It is apparent that a great deal of research remains to be done with regards to the biochemistry of chlorinated phenoxybutyric herbicides in woody species. Determination of absorption and translocation characteristics and the ability of various plant species and tissues to convert them to the active acetic homologues are vital to their future development for brush control.
METHODS AND PROCEDURES

Moderately suppressed bigleaf maple seedlings, five to eight years old but averaging only 18 inches in height, were collected from the understory of a mixed bigleaf maple Douglas-fir stand on the McDonald Forest in September, 1962. The root ball of each seedling was transferred to a number ten tin can and packed in soil. More than 100 trees were collected in this manner. They were held in a 35-38°F. constant temperature room with an eight hour day (900 foot candles light intensity) until January, 1963. At various intervals, depending on the anticipated need for seedlings, groups of individuals, selected at random, were transferred to the 70-75°F. greenhouse with a 16 hour day (natural light supplemented with fluorescent lamps and incandescent bulbs). The light intensity varied with atmospheric conditions. One month was allowed for new growth development before treatment was made. This procedure was followed through the course of these experiments. The last seedlings were moved to the greenhouse in June, 1963. The majority of plants responded rapidly when moved to the greenhouse, and mortality was held to a minimum. A few extra plants were included in each group which provided replacement plants for those lost through mortality or lack of general vigor.

Absorption and Translocation Studies

The absorption and translocation study is divided into two parts. The first part deals with the effect of formulation of specific chemicals on absorption and translocation, and the second
part is concerned with the effect of the molecular configuration of the parent herbicide molecule on these processes.

The effects of formulation on the absorption and translocation of 2,4-D and 2,4,5-T were tested to determine if the relationship between formulations of 2,4-D were also found between similar formulations of 2,4,5-T. Three formulations each of 2,4-D and 2,4,5-T were included in this experiment. The six treatments included four replications each of carbon 14 carboxyl-labeled acid, triethanol amine salt and 2-ethylhexyl ester of 2,4-D and 2,4,5-T respectively. The chemical was applied at a rate of 2000 micrograms (µgs.) acid equivalent per plant in 200 microliters (µls.) of treatment solution containing one microcurie (µc.) of carbon 14. The carrier solution was water with 0.5 percent Tween 20 as a surfactant or emulsifier added. A detailed procedure for preparation of these treatment solutions is found in the appendix under experiment one. The treatment solutions were applied with a 100 µl. Hamilton syringe in droplets, to two upper leaves nearly fully-expanded of each seedling. Each leaf received 100 µls. of solution. A small aliquot, 25 to 100 µls., of each treatment solution was injected into separate erlenmeyer flasks containing 0.5 NaOH for analysis as a treatment standard.

After 72 hours, the treated leaves of each plant were washed with 100 mls. of 80 percent by volume (v/v) isopropyl alcohol. The plants were sectioned into the treated leaves, the new growth (current stem growth and untreated leaves), the remaining portion of the stem, and the roots. The treatment standards, leaf washings, treated leaves, new growth, stem, and roots were individually extracted by the method
An aliquot of each sample extract was plated in duplicate on one-inch, ringed, stainless steel planchets and counted with a Tracerlab Versamatic II thin window (0.9 mg/cm²), gas flow Geiger-Müller counter to a maximum standard deviation of three percent. Duplicate planchets not agreeing within five percent were discarded and the sample replated. Appropriate corrections were made for background and self-absorption. On the basis of results obtained in the first experiment, remaining absorption and translocation studies employed the 2-ethylhexyl ester formulation.

The 2-ethylhexyl esters of carbon 14 carboxyl-labeled 2,4-DP and 2,4,5-TP were applied at the rate of 2000 µgs. acid equivalent in 200 µls. of water carrier containing 0.5 percent Tween 20 and one µc. of carbon 14 as before. A detailed procedure for the synthesis of dl-2(2,4-D)P-1-C¹⁴OOH and dl-2(2,4,5-T)P-1-C¹⁴OOH and the formulation of their respective 2-ethylhexyl esters is outlined in the appendix under experiment two. The procedure for sample preparation and analysis is as outlined in the preceding experiment.

**Metabolism Studies**

A single, detached leaf technique was used in studying the metabolism of this series of phenoxy herbicides. Four bigleaf maple seedlings were chosen for this study which had large leaves the previous fall when collected. They were brought from the cold room one at a time at about three-week intervals starting in February. The petiole of a single, fully-expanded leaf was cut off near the stem and
immediately immersed in water in a small flask, the total weight of which had been previously determined. If no wilting was evident in 30 minutes, the flask, containing the leaf and water, was weighed and the fresh weight of the leaf determined by difference. If wilting occurred, a second leaf was chosen and the procedure repeated.

The leaf surface was lightly abraded with a piece of tissue to insure maximum uptake of treatment solution, and a ring of silicon grease was applied to the leaf blade around the junction with the petiole. The underside of the leaf was lightly taped in three places, with thin strips of masking tape, to a small wire frame attached to the flask. In an earlier trial some leaves started to curl in a few hours due to effects of the herbicide, and portions of the treatment solution were lost. It did not appear that abrasion of the leaf surface or taping of the underside of the leaf to the wire frame had any appreciable effect except for improved herbicide absorption.

The treatments included in this experiment were four replications each of the triethanol amine salts of carbon 14 carboxyl-labeled 2,4-D, 2,4,5-T, 2,4-DP and 2,4,5-TP. Each leaf received one micromole acid equivalent of herbicide in 200 μls. of water which contained 0.5 percent Tween 20 as a surfactant and one μc. of carbon 14. A detailed description of the preparation of the treatment solutions is found in the appendix under experiment three.

Just prior to being treated, the leaf, with its petiole still in the flask of water, was placed in the bottom part of a three liter desiccator modified to serve as a respiratory chamber. The treatment solution was applied as small droplets with a 100 μl. Hamilton
syringe to the top of the leaf blade. The respiratory chamber had a precision-ground glass fitting between the upper and lower parts which insured a good seal when properly greased. The chamber was swept with 100 mls. per minute of CO₂ free air. The outgoing air from the chamber was scrubbed first in 200 mls. and second in 100 mls. of 0.5 N carbonate free NaOH prepared with CO₂ free water. A slight vacuum, 70 mm. of water, was maintained to prevent loss of radioactive CO₂ from the system. The flow rate and pressure was controlled by using the bottoms of two Bunsen burners as needle valves, one on the incoming air line and another on the vacuum line. A schematic diagram of the respiratory system is shown in figure one.
FIGURE 1.
RESPIRATOR

A. Air line
B. Needle valve
C. CO₂ trap for incoming air
D. CO₂ trap for incoming air
E. Respiratory chamber
F. Manometer
G. CO₂ trap for outgoing air
H. CO₂ trap for outgoing air
I. Drying tube
J. Flowmeter
K. Needle valve
L. Vacuum line
The respiratory studies were run for 72 hours in the dark at 80°F. At the end of the treatment period, the chamber was opened, and the treated leaf washed with 100 mls. of 80 percent v/v isopropyl alcohol. The petiole water and a 200 ml. alcohol and acetone wash of the respiratory chamber were collected separately for counting. The treated leaf was ground in about five mls. of 80 percent v/v isopropyl alcohol with a little clean sand in a small mortar and allowed to stand for 24 hours at 35°F. The homogenate was filtered and washed with 80 percent v/v isopropyl alcohol through Whatman number one filter paper. The first ten mls. of filtrate were saved for counting and chromatography. An additional 25 mls. of alcohol wash proved to be enough to remove nearly all the remaining activity. The residue was digested with one normal NaOH for three hours, filtered and washed with water and the filtrate extracted with benzene by liquid-liquid extraction as outlined in the appendix.

The 0.5 N NaOH CO₂ trapping solution in the outgoing air scrubbing towers was quantitatively transferred into erlenmeyer flasks. The flasks were heated to 70°C. and two mls. of a one normal barium chloride and one normal ammonium chloride solution added to precipitate barium carbonate. The solutions were cooled to room temperature and filtered through previously weighed 2.1 cm. spun-glass filter disks. The disks and precipitate were washed with water and alcohol, dried and re-weighed. Disks containing 20 to 35 mgs. of barium carbonate were prepared to insure maximum accuracy in applying a self-absorption correction factor. The carbonate plates were counted on one-inch, flat-bottomed, stainless steel planchets with a Tracerlab Versamatic II thin window (0.9 mg./cm.²) gas flow Geiger-Muller counter to a
maximum standard deviation of three percent. Suitable correction factors for background and self-absorption were applied to the data.

Aliquots from the ten and 25 ml. treated leaf alcohol extracts, leaf wash, petiole water, chamber wash and leaf residue extract, were plated and counted as before. Paper chromatograms were prepared in duplicate from the initial ten mls. of the treated leaf alcohol extract by spotting 100 μls. of extract on a line across a 1.25 inch-wide Whatman number one filter paper strip 18 inches long. The chromatograms were developed with a descending solvent system of n-butanol, propionic acid and water (12:5.6:8 v/v). The solvent front was marked when it had run about ten inches past the origin. The strips were dried in the hood. The strips were counted with a Tracerlab Versamatic II thin window (0.9 mg./cm.²) gas flow Geiger-Muller counter with a Tracerlab Autostep chromatogram strip scanner and printer. The strip scanner was set with a 1/4 inch window width and 1/4 inch stepwise strip advance.

Standards were prepared by spotting ten μls. of treatment solution on the strips as before. Standards and extracts were chromatographed and scanned in duplicate. Strips were usually counted for 30 minutes per position, and a background count made in connection with each strip. The proper correction was made for background, and the results were plotted as a percent of the total number of counts on the strip by Rf. This study failed to show the expected herbicide complex formation, and another experiment was designed to determine if these complexes might be formed more readily in other parts of the plant.
Four maple seedlings were selected and treated with one ml. of a water solution containing 0.5 percent Tween 20, five micromoles acid equivalent and five μcs. of carbon 14 carboxyl-labeled herbicides per plant. The treatments included two replications each of the tri-ethanol amine salts of 2,4-D and 2,4,5-T. A detailed description of the preparation of the treatment solutions is found in the appendix under experiment four.

The materials were applied with a one ml. Yale tuberculin syringe to a number of leaves. The seedlings were held in the greenhouse under a 16 hour day at 70-75°F. for one week. At the end of a week, the treated leaves were each washed with 50 mls. of 80 percent v/v isopropyl alcohol; the plants were sectioned into the treated leaves, untreated leaves, current stem growth, remaining stem, and the roots. Each section was individually extracted with a small volume of 80 percent v/v ethanol in an Omni-mixer homogenizer. The woody tissue was run through a pencil sharpener prior to extraction. The resulting extracts were chromatographed on paper as before. The strips were dried and cut into 1/2 inch pieces starting from the origin. Each 1/2 inch piece was placed in an individual low K40 glass vial and 15 mls. of a 0.3 percent PPO and 0.01 percent POPOP in toluene solution added.

The vials were counted for one hour each with a Packard Tricarb liquid scintillation counter. The instrument was operated at the balance point with a voltage of 878 volts. The discriminators were set at 10, 50, and 100 volts. Appropriate corrections for background and quenching were made. The quench correction was determined from
a quench correction curve. By adding various amounts of maple stem extract to benzoic acid-1-\textsuperscript{14}C calibration standards, a quench correction curve was constructed by plotting percent efficiency of counting over the ratio of counts per minute in the ten to 50 volt window to the counts per minute in the ten to 100 volt window. The data were plotted as a percent of the total counts on the strip by \( R_f \) as before.

**Gamma-Phenoxybutyric Herbicide Studies**

The poor translocation of the phenoxy herbicides found in the first and second experiments prompted a study with 2,4-DB. As noted in the review of literature, this compound does not appear to have any biological activity per se. Greater translocation was anticipated because this compound lacks the toxic effects which other phenoxy herbicides seem to have on transport tissues. However, greater translocation would be useless if bigleaf maples were unable to convert the inactive butyric to the active acetic form.

Single, detached maple leaves were treated with one micromole acid equivalent of triethanol amine 2,4-DB-1-\textsuperscript{14}C containing one \( \mu \)c. carbon \( 14 \). The procedure previously outlined was followed in this experiment.

Once it was determined that bigleaf maple could decarboxylate 2,4-DB into what was presumed to be 2,4-D, an absorption and translocation study was performed using, instead of the carboxyl-labeled material, carbon \( 14 \) ring-labeled 2,4-DB. The 2-ethylhexyl ester of carbon \( 14 \) ring-labeled 2,4-DB was applied in droplets at a rate of
2000 μgs. acid equivalent in 200 μls. of water with 0.5 percent Tween 20 and one μc. carbon 14 to two upper leaves of each maple seedling with a 100 μl. Hamilton syringe. A detailed description of the preparation of the treatment solution is found in the appendix under experiment six. The rest of the procedure follows that outlined above.

Ring-labeled 2,4-DB was used because movement of the carbon in the carboxyl group was of no interest once it had been cleaved from the molecule, and the metabolism study demonstrated that it is readily cleaved. The translocation study shows that at least the ring portion of 2,4-DB is fairly mobile in bigleaf maple. It is of interest then, to determine if the translocated material is 2,4-DB or the oxidized form, which is presumed to be 2,4-D.

A study was designed to determine the type of oxidation which takes place during the metabolism of 2,4-DB in bigleaf maple, i.e., whether liberation of the carboxyl group occurs through alpha or beta oxidation. In addition, this study would show whether the majority of translocated material is 2,4-D or 2,4-DB.

Four maple seedlings were selected which had been extra plants in previous studies. A 10,000 ppm. acid equivalent treatment solution on non-labeled 2-ethylhexyl ester of 2,4-DB or 2,4,5-TB was liberally applied to all leaves. A detailed description of the preparation of the treatment solutions is found in the appendix under experiment seven. Two seedlings per treatment were used. After 72 hours, the treated leaves were washed by dipping in successive beakers of 80 percent v/v isopropyl alcohol and finally rinsing with water. The plants
were sectioned into treated leaves, stem, and roots. The plant parts were individually subjected to the normal extraction procedure outlined in the appendix. The extracts were submitted to a cleanup procedure prior to analysis with a gas chromatograph. The procedure for sample cleanup and preparation for chromatographic analysis is outlined in the appendix.

The samples were analyzed on an Aerograph Hy-Fi gas chromatograph with hydrogen flame detector. The following columns and operating conditions were used: (1) Two percent m-phenyl ether on 60/80 mesh Gas Chrom Z packed in a six foot glass column operated at 200°C and 20 p.s.i.; (2) three percent Apiezon L on 60/80 mesh Gas Chrom Z packed in a ten foot aluminum column operated at 220°C and 20 p.s.i.; and (3) three percent Carbowax on Gas Chrom W packed in the first five feet of an aluminum column followed by one inch of uncoated Gas Chrom W and four feet of three percent Apiezon L on Gas Chrom W (total column length of nine feet) operated at 200°C and 20 p.s.i. These three columns were interchanged to resolve interfering peaks on a particular sample and to verify the identification of the herbicide. Each sample was run in duplicate in each of two columns. Standard solutions of 2,4-D, 2,4-DB, 2,4,5-T and 2,4,5-TB were used for quantitative determinations of herbicide in each sample. From these studies it became apparent that it would be desirable to determine if plant parts other than leaves were capable of oxidizing the butyric herbicides.

An experiment was designed to determine the rate of oxidation of carbon 14 carboxyl-labeled 2,4-DB in excised root and stem tissue
of bigleaf maple. About five grams of washed root tissue from a single bigleaf maple seedling was placed in each of two 125 ml. erlen-meyer flasks fitted with a center well containing three mls. of 0.5 N carbonate free NaOH solution. The roots were incubated for 24 hours in the dark at 27°C. with 24.7 µgs. of carbon 14 carboxyl-labeled 2,4-DB acid in ten mls. of 2.56 x 10⁻² molar phosphate buffer (pH 6.95). The reaction was stopped by adding three mls. of one normal H₂SO₄ to the incubation medium. The flasks were cooled for 30 minutes on one side to insure air movement in the flask and maximum CO₂ absorption by the NaOH trapping solution. Barium carbonate plates were prepared and counted as before. The incubation mixture was filtered and washed with water. The filtrate was acidified with additional H₂SO₄ to a pH of about one and extracted with three 25 ml. volumes of benzene in a separatory funnel. An aliquot was plated and counted and the activity absorbed by the tissue determined by difference.

Stem tissue was obtained by stripping the outer and inner bark from the xylem. About five grams of stem tissue were incubated in each of two 125 ml. erlenmeyer flasks as before. A control flask containing no tissue was included to check for breakdown of the 2,4-DB in the incubation medium. The results were corrected for background, self-absorption and metabolic rate. The results are expressed as a percentage of absorbed activity liberated as C¹⁴O₂.
RESULTS AND DISCUSSION

The problem of variability in experimental data is present in most experiments. It is usually minimized through careful selection of experimental material, randomization of treatments and large sample sizes. The bigleaf maple seedlings collected for these experiments were chosen for uniformity of size and vigor. The plants used in any single treatment were chosen at random.

The time necessary for preparation of a single sample for analysis was a determining factor in choosing the number of replications to be included in a particular experiment. Examination of the procedures involved in sample preparation will show that a large number of steps and manipulations are involved. The precision desired in these studies demanded the use of the procedures outlined.

Unless otherwise indicated in the previous section, four replications of each treatment were used. The data from all replications are not included in the results in all cases. During the handling of the more than 500 samples included in these experiments, a few were spilled or mislabeled. Unfortunately, the loss of a root sample, for instance, required the deletion of the entire plant from the results. A few additional plants were discarded when treatment solutions were observed running down the petiole and stem from the treated leaf. Atypical results were observed in a few instances with other plants which may have resulted from the loss of treatment solution not observed. These plants were deleted when results differed markedly from others in the same replication.
There are a number of ways of expressing the data found in this series of experiments. In most cases, it has been expressed in relative terms to allow comparisons among treatments to be made. In a few cases the data has been presented in micrograms acid equivalent of herbicide.

**Absorption and Translocation Studies**

**The Influence of Formulation**

Experiment one was designed to determine the relationship between formulations of 2,4-D and 2,4,5-T in terms of their absorbability and translocatability. Each plant was treated with a one percent acid equivalent solution of one of the following: 2,4-D acid, 2,4-D triethanol amine salt, 2,4-D 2-ethylhexyl ester, 2,4,5-T acid, 2,4,5-T triethanol amine salt or 2,4,5-T 2-ethylhexyl ester, containing about one μc. of carbon 14 carboxyl-labeled herbicide. The exact methods and procedures are outlined above.

The data presented in table one are mean values of the percentage of total radioactivity recovered which had been absorbed, and the percentage of total absorbed radioactivity found in different plant parts.
Table 1. The absorption and translocation of carbon 14 after 72 hours in bigleaf maple seedlings treated with acid, triethanol amine salt or 2-ethylhexyl ester formulations of 2,4-D-1-Cl\textsubscript{14} or 2,4,5-T-1-Cl\textsubscript{14}.

<table>
<thead>
<tr>
<th>Number of plants</th>
<th>2,4-D acid</th>
<th>2,4-D amine</th>
<th>2,4-D ester</th>
<th>2,4,5-T acid</th>
<th>2,4,5-T amine</th>
<th>2,4,5-T ester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average percent absorbed</td>
<td>2.91</td>
<td>1.67</td>
<td>20.83</td>
<td>2.35</td>
<td>0.71</td>
<td>16.49</td>
</tr>
</tbody>
</table>

Average percent of the absorbed activity found in the:

- **Treated leaf**: 77.04, 68.81, 95.36, 71.34, 62.03, 95.94
- **New growth**: 10.01, 17.40, 2.64, 21.42, 17.57, 2.15
- **Stem**: 8.06, 9.74, 1.36, 6.52, 7.53, 0.63
- **Roots**: 4.89, 4.04, 0.55, 0.71, 12.93, 1.28

μgs. of herbicide in the roots: 2.8, 1.4, 2.3, 0.3, 2.0, 4.3
The data presented in table one were analyzed by analysis of variance and the new multiple range test for significant differences among treatment means. The results of this statistical analysis are presented in the appendix.

The absorption data reveals the ester formulations most readily absorbed followed by the acid and the amine salt. These results are in agreement with the published results of a number of investigators who have worked with non-woody plants. However, of more direct interest are studies in which woody plants were the test organism.

Walker, Beck and Dumbroff reported the triethanol amine salt of 2,4,5-T was more readily absorbed in *Quercus nigra* and *Liquidambar styraciflua* than the ammonium salt (77). A number of other studies on the absorption and translocation of phenoxy herbicides in woody plants have been reported, but they are of little value in this instance because no effort was made to separate the effects of absorption from translocation. In most cases investigators used plant responses away from the treated area as an indication of the amount of herbicide absorbed and translocated. This technique is perfectly valid for certain objectives, but the accurate determination of rates of absorption are not possible.

Hull, working with the absorption and translocation of 2,4,5-T in velvet mesquite, observed that the acid, triethyl amine salt and sodium salt of 2,4,5-T caused less contact injury to foliage than an ester formulation (40, p. 22-42). If greater contact injury is correlated with greater absorption, it is an indication that the ester was most readily absorbed with little apparent difference
between other formulations. Although the differences in absorption between the acid and amine formulations of 2,4-D and 2,4,5-T in the present study are significantly different, the magnitude is small when compared with differences between acid and amine, and the ester. Hull may not have been able to detect small differences in absorption between the acid and salt formulations because the magnitude of ester contact injury was much larger. These studies with woody plants and the studies cited in the review of literature substantiate the relationship of formulation to absorbability found in this study.

The greater absorbability of the ester is expected if the nature of the leaf and ester molecule are considered. The surface layers of the leaf were characterized by Schieferstein and Loomis as epidermal cell wall material impregnated with pectins overlain by pectin and cutin and covered with wax deposits (65). This material is lipoid in nature and is observed at various degrees of hydration. A given herbicide ester has various degrees of lipoid solubility depending on the alcohol moiety. A good ester formulation possesses sufficient lipoid solubility to penetrate the epidermis, but has an oil-water partition coefficient favorable to movement of the ester into the water phase at a moderate rate.

The expression of the translocation data as a percentage of absorbed radioactivity which is found in a particular plant part gives a good indication of translocatability where differences in absorption are not great. Examination of data in table one reveals some marked differences in translocatability between formulations.
The differences in translocatability between acid and amine formulations of 2,4-D and 2,4,5-T are small and with one exception are not statistically significant within herbicides. Apparently the relative mobility of these two formulations is about the same, which strengthens the opinion that the amine is dissociated on absorption. If this were not the case, greater translocatability of the more water soluble amine would be expected.

The translocatability of the ester formulation is much lower. Greater absorption of this formulation leads to higher herbicide concentration in treated leaves when compared with acid and amine formulations. A simple calculation shows the average amount of 2,4-D as acid equivalent absorbed is 418 µgs. for the ester formulation compared to 58 µgs. for the acid and 33 µgs. for the amine. Plants treated with 2,4,5-T ester absorbed on the average of 330 µgs. acid equivalent compared to 47 µgs. for the acid and 14 µgs. for the amine. How much of the reduced translocatability of the ester is due to this high herbicide concentration is difficult to determine.

Greenham reported the translocatability of 2,4-D was reduced one half when the concentration of applied herbicide was increased 10 fold in skeleton weed (35, p. 633). Linder, Brown and Mitchell, working with various esters of 2,4-D and 2,4,5-T noted that the amount of translocation was proportional to the amount applied but only up to 200 µgs. acid equivalent per plant (48). These results indicate that herbicide concentration in treated leaves has an influence on translocation. The toxic effect of higher concentration of chemical in leaves treated with ester formulations is expected to markedly
reduce the ability of the energy requiring transport system to move the herbicide.

Disregarding concentration effects, little is known concerning the extent to which ester formulations affect translocation of the herbicide. If ester hydrolysis is complete or nearly so, then the translocation of the herbicide would be in the form of the acid as proposed by Crafts (13). However, the lipophilic properties of the ester must be considered. Crafts has pointed out that long chain alkyl esters are readily absorbed due to high lipoid solubility. However, high lipoid solubility makes the ester compatible with the cuticle and lowers the tendency for partition into the water phase. He observed that heavy esters which contain oxygen in the alcohol chains have a more favorable water solubility, but that a proper balance between hydrophilic and lipophilic properties must be maintained (17, p. 336). The hydrolysis of the ester probably does not occur while it is retained in the lipoid phase of the epidermal cell walls. Crafts may be correct that the ester is translocated as the acid, but only that portion of ester absorbed which has partitioned into the water phase is available for transport.

It does not appear likely, however, that these factors are of primary importance in reducing translocatability of ester formulations. The primary influence of the ester on herbicide translocation is probably its influence on absorption, which in turn influences translocation through a concentration effect.

One of the purposes of the formulation study was to determine if one formulation was most effective in translocating both 2,4-D and
2,4,5-T to the roots in terms of actual quantities of chemical. Examination of the data in table one shows this is not the case. However, the ester formulations are most commonly used for aerial application of herbicides in brush control. The 2,4,5-T ester proved to be the most effectively transported chemical tested above, and Rediske has shown that 2,4,5-T is more effective against bigleaf maple than 2,4-D (64). The use of ester formulations in the remaining absorption and translocation studies was a logical choice.

The Influence of Molecular Configuration

The 2-ethylhexyl esters of 2,4-DP and 2,4,5-TP were tested in experiment two to complete the study of the influence of molecular configuration on absorption and translocation. Each plant was treated with a one percent acid equivalent solution containing about one μc. of carbon 14 carboxyl-labeled herbicide. After 72 hours the plants were harvested and analyzed as previously described. The results in table two are expressed as in table one. The absorption and translocation data for the 2-ethylhexyl esters of 2,4-D and 2,4,5-T from table one are included here for convenience of comparison.
Table 2. The absorption and translocation of carbon 14 after 72 hours in bigleaf maple seedlings treated with the 2-ethylhexyl esters of 2,4-D-1-C¹⁴, 2,4,5-T-1-C¹⁴, 2,4-DP-1-C¹⁴ or 2,4,5-TP-1-C¹⁴.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2,4-D ester</th>
<th>2,4,5-T ester</th>
<th>2,4-DP ester</th>
<th>2,4,5-TP ester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of plants</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Average percent absorbed</td>
<td>20.83</td>
<td>16.49</td>
<td>27.35</td>
<td>22.09</td>
</tr>
</tbody>
</table>

Average percent of the absorbed activity found in the:

<table>
<thead>
<tr>
<th>Component</th>
<th>2,4-D ester</th>
<th>2,4,5-T ester</th>
<th>2,4-DP ester</th>
<th>2,4,5-TP ester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated leaf</td>
<td>93.85</td>
<td>95.94</td>
<td>94.87</td>
<td>96.11</td>
</tr>
<tr>
<td>New growth</td>
<td>2.64</td>
<td>2.15</td>
<td>2.83</td>
<td>2.99</td>
</tr>
<tr>
<td>Stem</td>
<td>1.36</td>
<td>0.63</td>
<td>1.14</td>
<td>0.61</td>
</tr>
<tr>
<td>Roots</td>
<td>0.55</td>
<td>1.28</td>
<td>1.17</td>
<td>0.29</td>
</tr>
<tr>
<td>µgs. of herbicide in the roots</td>
<td>2.26</td>
<td>4.23</td>
<td>6.16</td>
<td>1.12</td>
</tr>
</tbody>
</table>
The data presented in table two were analyzed by analysis of variance and the new multiple range test for significant differences between treatment means. The results of this statistical analysis are presented in the appendix.

Examination of the data in table two reveals some differences in the absorbability of these herbicides. The variation found in some treatments prevented drawing statistically significant conclusions. However, certain patterns are observed and remarks pertinent to these can be made.

The mean values presented in table two show that as a group the 2-phenoxypropionic herbicides were more readily absorbed than the phenoxyacetic herbicides. This relationship between structure and absorbability has not been previously reported. So little work has been done with the 2-phenoxypropionic herbicides that no numerical data are available concerning its absorption and translocation characteristics. Leafe reported no significant difference in the absorption and translocation between MCPA and CMPP in Galium aparine (45).

The data in table two also reveals that within molecules with equal side chain length, the dichlorinated member is more readily absorbed than the trichlorinated member. Although this relationship has not been reported for the 2-phenoxypropionic herbicides, it has been observed with phenoxyacetic herbicides.

Weintraub, Reinhart and Scherff showed that 2,4-D was more readily absorbed than 2,4,5-T by a factor of nearly two in bean plants (79). Zebrina pendula leaves absorbed slightly more 2,4-D than
2,4,5-T when applied as triethanol amine salts both with and without surfactants (21). Examination of data presented by Slife et al. revealed that although 2,4-D was more readily absorbed than 2,4,5-T by both wild and cultivated cucumber plants, the magnitude of differences of uptake between these two varieties is much different. The absorption of 2,4,5-T is only 39 percent that of the 2,4-D in wild cucumber but is 71 percent in the cultivated variety after four days (68).

The comparison of the translocatability of this series of phenoxy herbicides is complicated as before by differential absorption. However, careful consideration and study of the data in table two will allow some observations to be made and some conclusions drawn.

The first measure of translocatability is relative mobility of absorbed herbicide from treated leaves. From table two it is seen that regardless of differences in absorption, the dichlorinated herbicides are more mobile than the trichlorinated herbicides. While these differences are small and not statistically significant, they are of interest since they follow the pattern established in the absorption study. It is interesting to observe that 2,4-DP which is most readily absorbed is next to the most mobile from the treated leaves, exceeded only by 2,4-D. 2,4,5-T, on the other hand, is least well absorbed and next to the least mobile. It would appear that some interaction between chemical and plant exists with reference to translocatability.

The relative distribution of the chemicals from the treated leaves to the new growth and stem are essentially the same. However, the movement of these herbicides into the roots deserves careful consideration.
An examination of data in table two reveals that the next to the least mobile herbicide from the treated leaves, 2,4,5-T, is the most mobile with respect to relative movement into the roots. The decreasing order of relative mobility from the treated leaves was 2,4-D, 2,4-DP, 2,4,5-T and 2,4,5-TP. The decreasing order of relative mobility into the roots, however, is 2,4,5-T, 2,4-DP, 2,4-D and 2,4,5-TP. The fact that statistically significant differences should appear concerning the movement of these chemicals from the stem to the roots indicates that molecular configuration has influenced translocatability, particularly the tissue to which the chemical translocates.

Only a few studies have been reported which show the influence of molecular configuration on the translocatability of phenoxy herbicides. It has been reported that the translocatability of 2,4-D from the treated leaves of bean, cotton, corn and potato plants was superior to 2,4,5-T. However, the magnitudes of difference were small and no statistical interpretation was given (79). On the other hand, Slife et al. noted that 2,4,5-T was slightly more mobile than 2,4-D in wild cucumbers and considerably more mobile in cultivated cucumbers. This conclusion is based on intensity of darkening of autoradiographs, and no numerical data are included. The authors reported other experiments showed the majority of 2,4,5-T remained in the treated leaves (68). Thus, the interpretation of this work is uncertain. Butts and Fang reported that 2,4-D was more mobile than 2,4,5-T from treated leaves of bean plants (10). Leafe reported no significant differences in the translocatability of MCPA and CMPP in
**Galium aparine.** This is the only study in which 2-phenoxypropionic and phenoxyacetic herbicides have been compared (45).

The reasons for differences in translocatability from the leaves to the roots has not been well investigated. No investigations have included the 2-phenoxypropionic herbicides and only a few with the phenoxyacetic herbicides have been reported. The majority of the work directly in this area has been done by Crafts and co-workers in California.

Crafts and Yamaguchi studied the uptake and distribution of a number of different herbicides, including 2,4-D and 2,4,5-T, in Zebrina pendula and Tradescantia fluminensis. From their examination of autoradiographs prepared in this study, they concluded the movement of 2,4-D and 2,4,5-T was restricted by accumulation into living cells in leaves and around vascular elements of the stem. To verify this conclusion, they tested the mobility of a number of herbicides in potato tuber cubes with autoradiography. They found both 2,4-D and 2,4,5-T to be strongly bound by parenchyma tissue. 2,4-D was slightly more mobile than 2,4,5-T in this test (18, p. 433, p. 454). Yamaguchi and Crafts, working with 2,4-D, 2,4,5-T and other radioactive herbicides in woody plants, reported 2,4-D and 2,4,5-T were strongly absorbed by live tissue in the stem. Examination of autoradiographs revealed some leakage from phloem to xylem. The authors concluded that both accumulation in live cells around transport tissues and leakage to the xylem were factors which influenced translocation of phenoxy herbicides (84, p. 184, p. 193-196). A histoautoradiographic study of the translocation of 2,4-D from leaves to roots of broadbean...
plants has shown what appears to be increasing accumulation of radioactivity in parenchyma cells of the phloem and leakage to the xylem with increasing distance from the treated leaves (63).

Additional, but less direct, evidence of this phenomenon is also available. Linscott and McCarty showed the most extensive accumulation of radioactive 2,4-D in ironweed plants was in the stems (49). It would appear the higher activity in the stem was caused by accumulation of herbicide in parenchyma tissue of the phloem.

It is interesting to note in the present study that the two most mobile chemicals, 2,4-DP and 2,4,5-T, accumulated more herbicide in the roots than in the stem. Since the amount of herbicide in the new growth is essentially the same in all treatments, it is believed the greater accumulation in the roots is a result of less absorption of 2,4,5-T or 2,4-DP by phloem parenchyma cells.

It has been long accepted that the translocation of phenoxy herbicides follows the pattern of carbohydrate flow. A recent report by Evans, Ebert and Moorby showed that translocated photosynthate was lost from the phloem at a rate of about one percent per cm. traveled (24, p. 221-231). It follows that herbicides might be influenced in similar manner. Little doubt can exist that the loss of herbicide from the translocation stream does occur, although the magnitude of this loss has not been determined.

The differences in translocatability between the members of this series of phenoxy herbicides is probably the result of a combination of factors. The two most important factors are the toxicity of the chemical to the transport mechanism and the extent of accumulation by
parenchyma cells of the phloem. An additional factor of the water solubility of the chemicals may play some part in determining translocatability. Translocatability is the result of the combined influence of these factors.

Metabolism Studies

Following studies of absorption and translocation, the metabolism of this series of phenoxy herbicides in bigleaf maple assumed greater importance. Examination of absorption and translocation data reveals that differences noted between chemicals are probably not sufficient to account for their differential effectiveness on bigleaf maple. A number of investigators have shown that the metabolism, or detoxification, of herbicides plays an important role in determining selectivity in some species. It is logical to study this process in bigleaf maple.

There are two primary means of studying the detoxification of phenoxy herbicides in plants. One method involves determining the rate of loss of the side chain, and the second a determination of metabolite formation.

The rate of decarboxylation, or cleavage of the side chain, of phenoxy herbicides has been successfully studied using a detached leaf technique by a number of investigators. It may be argued that this technique does not give a true indication of the rate of cleavage in intact leaves, but when the metabolic rate of the tissue is taken into consideration, a fairly accurate picture may be obtained. It was not anticipated that major changes in metabolic pathways would
occur. In any case, this technique is valid where comparisons between chemicals in one plant species are the primary objective, and determination of quantitative measures of detoxification secondary.

Metabolism in Detached Leaves

In experiment three, single, detached maple leaves were treated with one micromole acid equivalent of 2,4-D-1-C\textsuperscript{14}, 2,4,5-T-1-C\textsuperscript{14}, 2,4-DP-1-C\textsuperscript{14} and 2,4,5-TP-1-C\textsuperscript{14} formulated as the triethanol amine salt. The treatment solutions contained about one μc. of carbon 14. After 72 hours the leaves were harvested and analyzed as previously described.

Decarboxylation data are usually corrected for the respiration rate or potential of the test organism. Frequently, data is expressed as some value of decarboxylation per weight of CO\textsubscript{2} respired per weight of tissue per unit time. This expression is really a ratio of the actual metabolism to the potential for metabolism, as shown by the fresh weight corrected to a common time base. In this study the metabolic potential was not of interest, and the time was a constant in all experiments. The data has been expressed as a percentage of the absorbed radioactivity which was recovered as C\textsuperscript{14}O\textsubscript{2} in 72 hours adjusted to a common metabolic base of 47.98 mgs. CO\textsubscript{2} respired in 72 hours. Expression in this manner allows accurate comparisons to be made between chemicals metabolized in leaves having slightly different rates of respiration. The results of the decarboxylation study are presented in table three.
Table 3. The release of carbon 14 as C\(^{14}\)O\(_2\) in 72 hours by bigleaf maple seedling leaves treated with 2,4-D-\(\text{-}^1\)C\(^{14}\), 2,4,5-T-\(\text{-}^1\)C\(^{14}\), 2,4-DP-\(\text{-}^1\)C\(^{14}\) or 2,4,5-TP-\(\text{-}^1\)Cl\(^{14}\).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2,4-D</th>
<th>2,4,5-T</th>
<th>2,4-DP</th>
<th>2,4,5-TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of leaves</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Average percent absorbed</td>
<td>92.97</td>
<td>82.27</td>
<td>78.44</td>
<td>61.49</td>
</tr>
<tr>
<td>Average percent of the absorbed activity liberated as C(^{14})O(_2)</td>
<td>0.456</td>
<td>0.582</td>
<td>0.607</td>
<td>0.508</td>
</tr>
</tbody>
</table>

The data presented in table three were analyzed by analysis of variance and the new multiple range test for significant differences between treatment means. The results of this statistical analysis are presented in the appendix.

The data shown in table three were not entirely expected. Decarboxylation rates ranging from less than one to more than ten percent, depending on the chemical tested, had been anticipated. Statistical analyses reveal the small differences noted between treatment means in table three are not significant. While low rates of decarboxylation are often reported, higher rates have also been reported; and in some cases these have been shown to be a major factor in chemical selectivity as pointed out in the review of literature.

Decarboxylation rates ranging from less than one to slightly less than two percent of applied herbicide per day have been reported by a number of investigators for bean plants (79; 4, p. 283; 3; 78). Similar values have been reported for cotton, sorghum, jimson weed, bur cucumber and cocklebur (54; 82, p. 252).
Of direct interest is a recent report by Basler on decarboxylation rates of phenoxyacetic herbicides in detached leaves of woody plants. He reported less than 0.2 percent of the absorbed 2,4,5-T-1-C\(^{14}\) was decarboxylated in 22 hours in blackjack oak compared to less than 0.5 percent for 2,4-D-1-C\(^{14}\) during a six month sampling period. Detached leaves of persimmon, green ash, sweet gum and winged elm had decarboxylation rates ranging from 0.9 percent for persimmon to less than 0.1 percent for winged elm. He found no relationship between decarboxylation ability and 2,4-D susceptibility (5). It is evident that low rates of decarboxylation are not peculiar to bigleaf maple. Decarboxylation does not appear to be an important means of phenoxy herbicide detoxification in bigleaf maple.

The leaves which were used in the decarboxylation study were also used in determining the formation of herbicide metabolites. Extracts from these leaves were chromatographed on paper as previously described, The radioactivity found on each strip was plotted on graph paper. The percentage of total strip activity found at a particular spot was plotted over the R\(_f\) of that spot. A representative example from each treatment is presented in the appendix. The percentages of total activity on the paper chromatograms which corresponds to herbicide, as shown by its R\(_f\) value, are presented in table four.
Table 4. The percentage of total radioactivity which is recoverable as herbicide after 72 hours from alcohol extracts of bigleaf maple seedling leaves treated with 2,4-D-1-C14, 2,4,5-T-1-C14, 2,4-DP-1-C14 or 2,4,5-TP-1-C14.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of leaves</th>
<th>Average percent of the absorbed activity recoverable as herbicide</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D</td>
<td>3</td>
<td>94.98</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>4</td>
<td>93.35</td>
</tr>
<tr>
<td>2,4-DP</td>
<td>4</td>
<td>87.23</td>
</tr>
<tr>
<td>2,4,5-TP</td>
<td>4</td>
<td>84.07</td>
</tr>
</tbody>
</table>

The data presented in table four were analyzed by analysis of variance and the new multiple range test for significant differences among treatment means. The results of this statistical analysis are presented in the appendix.

While the amount of breakdown of herbicide in the treated leaves is small, some interesting relationships are observed. Differences between herbicide recovery of dichlorinated and trichlorinated herbicides with equal side chain lengths are not significantly different at the five percent level. However, the data reveal that the phenoxyacetic herbicides are significantly more stable than the 2-phenoxypropionic herbicides. The differences between any combination of acetic and propionic herbicides are significantly different. It is apparent that the introduction of a methyl group on the α-carbon atom reduced the stability of the herbicide. This is in contrast to the study reported by Leafe. He showed MCPA was less stable than CMPP in Galium aparine which is susceptible to CMPP but not to MCPA (45). It would appear that the configuration of the molecule has an influence on its stability in the plant, but this stability is not constant.
among species.

The graphs of radioactivity on paper chromatograms shown in the appendix reveal one major peak present in all cases which corresponds to unaltered herbicide. These graphs show minimal formation of metabolites with no single predominant breakdown product evident in any treatment.

**Metabolism in Intact Seedlings**

The lack of formation of metabolites in detached leaves prompted an investigation of the metabolism of 2,4-D and 2,4,5-T in intact plants. The objective of experiment four was to determine if other plant parts are better able to metabolize these herbicides than the treated leaves, and to determine if the rate and pathways of metabolism of intact and detached treated leaves are the same.

Intact bigleaf maple seedlings were treated with 2,4-D-1-C\textsubscript{14} and 2,4,5-T-1-C\textsubscript{14} and allowed to metabolize it for one week before harvest. The plants were treated, harvested and analyzed as previously described. The extracts were chromatographed on paper as before, and graphs of the distribution of radioactivity by $R_f$ are presented in the appendix. The percentage of total activity on the paper chromatograms which corresponds to herbicide, as shown by $R_f$ value, is presented in table five.
Table 5. The percentage of the total radioactivity which is recoverable as herbicide after one week from alcohol extracts of plant parts from intact bigleaf maple seedlings treated with 2,4-D-1-C\textsuperscript{14} or 2,4,5-T-1-C\textsuperscript{14}.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2,4-D</th>
<th>2,4,5-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of plants</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Average percent of the recovered activity which is herbicide in the:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated leaves</td>
<td>94.06</td>
<td>93.74</td>
</tr>
<tr>
<td>Untreated leaves</td>
<td>84.41</td>
<td>98.33</td>
</tr>
<tr>
<td>Current stem growth</td>
<td>75.01</td>
<td>60.70</td>
</tr>
<tr>
<td>Old stem</td>
<td>62.23</td>
<td>67.07</td>
</tr>
<tr>
<td>Roots</td>
<td>47.67</td>
<td>85.35</td>
</tr>
</tbody>
</table>

Examination of data in table four and table five show the amount of herbicide recoverable from treated leaves is nearly the same in both studies. The distribution of radioactivity on paper chromatograms is also similar. It would appear that detaching the leaves in experiment three did not alter the pathway of metabolism although the rate of herbicide breakdown may have been increased.

The data in table five reveal that different plant parts alter a particular chemical at different rates. In addition, it is noted that a given plant part alters different chemicals at different rates. These results show differences which may be important in determining the effectiveness of these two chemicals on bigleaf maple.

In most parts of the seedlings, except in the treated leaves where the breakdown is small, 2,4,5-T is more stable than 2,4-D. Although 2,4-D is more stable in the new growth stem, 2,4,5-T is
considerably more stable if the total new growth is considered. It is calculated that 96.29 percent of the total activity in the new growth, i.e., new growth stem and untreated leaves, is 2,4,5-T while only 82.50 percent of the total activity in this same portion is 2,4-D.

Of primary interest is the stability of these two compounds in the roots. The translocation study revealed 2,4,5-T was more mobile than 2,4-D to the roots. This experiment shows nearly twice as much of the translocated 2,4,5-T is unaltered in the roots when compared to 2,4-D after one week. The combined effects of translocation and stability result in appreciably more 2,4,5-T than 2,4-D being available in the roots.

Herbicide alteration has been reported by investigators for a number of plant species. Only a limited amount of work has been reported with woody plants. Morton and Meyer observed that 85 percent of absorbed 2,4,5-T-1-C14 was altered to another form in 25 hours by velvet mesquite (57). Cherry trees, treated with 2,4-D just previous to leaf abscision, yielded half of the applied activity as 2,4-D the following spring (80). Black and red currants have been reported to alter five to ten percent of absorbed 2,4-D and 2,4,5-T to water soluble compounds which are inactive in growth regulation tests. An additional ten to 30 percent is bound in the leaves and is not extractable with water, organic solvents or mild hydrolysis (50, p. 620-623). Cotton plants altered the form of 52 percent of the applied 2,4-D in three days compared to 36 percent in sorghum (54). Galium aparine yielded nearly all of the applied CMPP in an unaltered form.
but practically none of the MCPA in ten days (45). Slife et al. noted that 75 percent of applied 2,4-D was found in two metabolites in 24 hours in wild cucumber while 2,4,5-T was only slightly altered (68). It is evident from these varied reports that stability of herbicides and formation of metabolites is dependent on both the plant and chemical under study.

An examination of the graphs in the appendix of the distribution of radioactivity on paper chromatograms reveals that radioactivity is distributed in three or four primary areas. The $R_f$ of both 2,4-D and 2,4,5-T is 0.87-0.93. The two primary metabolites found in the 2,4-D treated seedlings have $R_f$ values of 0.15-0.20 and 0.42-0.47. The 2,4,5-T treated plants yielded metabolites with $R_f$ values of 0.10-0.15, 0.42-0.47, and 0.62-0.67 and 0.72-0.77. The number of peaks found on chromatograms from 2,4-D treated plants are fairly constant in different plant parts, while the peaks listed above for 2,4,5-T treated plants don't appear on all chromatograms. In all cases where herbicide breakdown has occurred, a primary metabolite with an $R_f$ value of 0.42-0.47 is detected.

The majority of the metabolism work with the phenoxy herbicides has been done with 2,4-D in bean plants. These reports show that some of the metabolites which are formed in bean plants are found in a number of other species as well.

The detection of the primary metabolites found in maple has been reported by other investigators. Jaworski and Butts noted the formation of a metabolite of 2,4-D in bean plants which they labeled as "unknown one!" This metabolite was shown to be a 2,4-D plant-constituent
complex which yielded 2,4-D on hydrolysis with dilute acid or incubation with takadiastase or emulsin. Formation of "unknown one" did not occur to the same extent in all parts of the plant. The highest rate of formation, around 70 percent of the extractable activity, was detected in terminal buds, petioles and the first internode of bean plants. The hypocotyl and roots yielded around 50 percent of the total extractable activity as "unknown one" (42, p. 215). In later work, Jaworski, Fang and Freed reported that "unknown one," which has an Rf of 0.44-0.46 in n-butanol, propionic acid and water (12:5.6:8), was found in higher concentrations in the stem than in the treated leaves of bean plants. The roots were not studied in that investigation. They reported the detection of other metabolites on many of their paper chromatograms which had Rf values of 0.22-0.28, 0.36-0.38, and 0.65-0.68 (43). Bach and Fellig reported the formation of a metabolite having the same Rf as "unknown one" in amounts ranging from 16.5 percent in one day to 45.5 percent in one week in bean stems treated with 2,4-D (3).

Fang and Butts showed that "unknown one," plus certain other metabolites, are found in corn and wheat plants treated with 2,4-D. They noted, however, that the proportion of total activity recovered as "unknown one" was much smaller than reported for bean plants (26). Fang, in later work, reported that "unknown one" was not always the primary metabolite in such 2,4-D susceptible species as peas and tomatoes (25).

"Unknown one" has been characterized as a 2,4-D-protein complex which yields 2,4-D and 12 amino acids on hydrolysis (10). Some
substantiation is offered by other investigators. Bach reported the detection of ten amino acids from the hydrolysis of a 2,4-D metabolite formed in bean stems (2). Brian, working with proteinaceous monolayers from a number of plant species, showed a rough correlation with plant resistance to MCPA and MCPA adsorption to the monolayer (9).

The formation of 2,4-D metabolites which have no biological activity as such, but which yield 2,4-D on hydrolysis, have been found in a number of plants. The 2,4-D bound in roots of tick bean plants was released with acid hydrolysis (11, p. 497). Water soluble metabolites from 2,4-D and 2,4,5-T treated currant plants were inactive in growth regulation tests, but yielded active compounds on acid hydrolysis (50, p. 623). Metabolites previously described by Fang and co-workers were found in cotton and sorghum plants by Morgan and Hall. The formation of the complexes proceeded at different rates in the two species studied (54). Kubutiya reported 2,4-D was rapidly decomposed in bean plants, but prior to decomposition it was bound to plant substances from which it could be released by hydrolysis (44).

These varied reports establish neither the nature nor the importance of the 2,4-D complexes which are formed. These reports seem to indicate the formation of these complexes is species dependent. Thus, the formation of different metabolites of 2,4-D and 2,4,5-T in bigleaf maple seedlings at different rates is not unreasonable.
The poor translocation of the phenoxy herbicides studied thus far prompted a study with 2,4-DB. The lack of biological activity of 2,4-DB indicated it should be fairly mobile in plants in comparison to the other compounds studied. Prior to studying the absorption and translocation characteristics of this compound, it was necessary to determine if bigleaf maple was able to alter 2,4-DB into what was presumed would be 2,4-D. If maples were unable to accomplish this conversion, there would be little point in studying the absorption and translocation of a biologically inactive chemical in this investigation. If decarboxylation occurred, then the use of carboxyl-labeled herbicide would be objectionable because translocation of 2,4-DB would be confused with the translocation of carbon 14 fragments from the side chain.

Although it has been shown in the metabolism study that 2,4-D is rapidly inactivated in the roots of bigleaf maple seedlings, it was felt that information gained with 2,4-DB would be useful for both 2,4-DB and 2,4,5-TB. Unfortunately, 2,4,5-TB was not available as a labeled chemical in this laboratory.

The Metabolism of 2,4-DB in Detached Leaves

In order to determine the rate of decarboxylation of 2,4-DB in bigleaf maple leaves, a single detached leaf technique was used in experiment five as previously described. Determination of the evolution of C\textsubscript{14}O\textsubscript{2} and formation of metabolites was accomplished as before. The results of the decarboxylation study are presented in
The results are expressed as a percentage of the absorbed radioactivity which was recovered as $^{14}\text{O}_2$ in 72 hours as before. The data from table three are included for convenient comparison.

Table 6. The release of carbon 14 as $^{14}\text{O}_2$ in 72 hours by bigleaf maple seedling leaves treated with 2,4-D-1-$^{14}$C, 2,4,5-T-1-$^{14}$C, 2,4-DP-1-$^{14}$C, 2,4,5-TP-1-$^{14}$C or 2,4-DB-1-$^{14}$C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of leaves</th>
<th>Average percent absorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D</td>
<td>4</td>
<td>92.97</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>4</td>
<td>82.27</td>
</tr>
<tr>
<td>2,4-DP</td>
<td>4</td>
<td>78.44</td>
</tr>
<tr>
<td>2,4,5-TP</td>
<td>4</td>
<td>61.49</td>
</tr>
<tr>
<td>2,4-DB</td>
<td>3</td>
<td>92.63</td>
</tr>
</tbody>
</table>

The data presented in table six were analyzed by analysis of variance and the new multiple range test for significant differences between treatment means. The results of this statistical analysis are presented in the appendix.

It is obvious that bigleaf maple seedlings have the ability to decarboxylate 2,4-DB fairly rapidly. The rate of decarboxylation may be higher than indicated by the evolution of $^{14}\text{O}_2$. The distribution of radioactivity on paper chromatograms prepared from alcohol extracts of 2,4-DB treated leaves used in the decarboxylation study is presented in the appendix. The percentage of total radioactivity which corresponds to herbicide, as shown by its $R_f$ value, is presented in table seven. The data from table four is included for convenience of comparison.
Table 7. The percentage of total radioactivity which is recoverable as herbicide after 72 hours from alcohol extracts of bigleaf maple seedling leaves treated with 2,4-D-1-C\textsubscript{14}, 2,4,5-T-1-C\textsubscript{14}, 2,4-DP-1-C\textsubscript{14}, 2,4,5-TP-1-C\textsubscript{14} or 2,4-DB-1-C\textsubscript{14}.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of leaves</th>
<th>Average percent of the absorbed activity recovered as herbicide</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D</td>
<td>3</td>
<td>94.98</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>4</td>
<td>93.35</td>
</tr>
<tr>
<td>2,4-DP</td>
<td>4</td>
<td>87.23</td>
</tr>
<tr>
<td>2,4,5-TP</td>
<td>4</td>
<td>84.07</td>
</tr>
<tr>
<td>2,4-DB</td>
<td>3</td>
<td>73.24</td>
</tr>
</tbody>
</table>

The data presented in table seven were analyzed by analysis of variance and the new multiple range test for significant differences between treatment means. The results of this statistical analysis are presented in the appendix.

The liberation of C\textsubscript{14}O\textsubscript{2} is direct evidence of the decarboxylation of 2,4-DB in these detached leaves. While only 13 percent of the absorbed activity was recovered as C\textsubscript{14}O\textsubscript{2}, the paper chromatograms reveal only 73.24 percent of the activity on the strip was 2,4-DB. A major metabolic product is found with an R\textsubscript{f} of 0.72-0.77. The identity of this spot is not known. This spot is not found in appreciable quantities with the other herbicides tested; the possibility of this peak representing additional cleavage can not be disregarded, although no evidence to support this can be cited. Assuming the majority of the activity not recovered as 2,4-DB is about equal to the production of 2,4-D, nearly 75 percent of the absorbed herbicide is still available for transport as the butyric herbicide. Furthermore, the detached leaves allow for no translocation from the treated area, and it will be shown that a good deal of translocation...
The Absorption and Translocation of 2,4-DB

The 2,4-DB metabolism study showed it would be necessary to determine the absorption and translocation characteristics of 2,4-DB with ring-labeled material. In experiment six, four maple seedlings were treated with one percent acid equivalent solution of the 2-ethyl-hexyl ester of 2,4-DB containing about one μc. of carbon 14 in the ring. After 72 hours the plants were harvested and analyzed as previously described. The results are presented in table eight. Data from table two has been incorporated for convenient comparison. The data is expressed as before.
Table 8. The absorption and translocation of carbon 14 in 72 hours in bigleaf maple seedlings treated with 2-ethylhexyl esters of 2,4-D-1-C¹⁴, 2,4,5-T-1-C¹⁴, 2,4-DP-1-C¹⁴, 2,4,5-TP-1-C¹⁴ or 2,4-DB ring C¹⁴.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of plants</th>
<th>2,4-D</th>
<th>2,4,5-T</th>
<th>2,4-DP</th>
<th>2,4,5-TP</th>
<th>2,4-DB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2,4-D</td>
<td>2,4,5-T</td>
<td>2,4-DP</td>
<td>2,4,5-TP</td>
<td>2,4-DB</td>
<td></td>
</tr>
<tr>
<td>Average percent absorbed</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Treated leaf</td>
<td>20.83</td>
<td>16.49</td>
<td>27.35</td>
<td>22.09</td>
<td>12.29</td>
<td></td>
</tr>
<tr>
<td>New growth</td>
<td>2.64</td>
<td>2.15</td>
<td>2.83</td>
<td>2.99</td>
<td>4.48</td>
<td></td>
</tr>
<tr>
<td>Stem</td>
<td>1.36</td>
<td>0.63</td>
<td>1.14</td>
<td>0.61</td>
<td>11.81</td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>0.55</td>
<td>1.28</td>
<td>1.17</td>
<td>0.29</td>
<td>6.21</td>
<td></td>
</tr>
<tr>
<td>μgs. of herbicide in the roots</td>
<td>2.26</td>
<td>4.23</td>
<td>6.16</td>
<td>1.12</td>
<td>14.68</td>
<td></td>
</tr>
</tbody>
</table>
The data presented in table eight were analyzed by analysis of variance and the new multiple range test for significant differences between treatment means. The results of this statistical analysis are presented in the appendix.

The absorption and translocation study reported in table eight reveals that at least the ring portion of the 2,4-DB molecule is fairly mobile in bigleaf maple when compared to the other herbicides in this series. In terms of actual amounts of herbicide translocated to the roots, the lower absorption is more than offset by the higher translocatability of the chemical. More than twice as much of the labeled portion of the 2,4-DB molecule translocated to the roots compared with 2,4-DP which was the next most mobile.

The mobility of the carbon 14 ring-labeled portion of the 2,4-DB molecule indicated it was not the oxidation product (2,4-D) which was being translocated. The previous translocation study showed 2,4-D was not appreciably mobile in bigleaf maple. There is little reason to believe that treatment with 2,4-DB would improve the translocation of 2,4-D. It was of interest to determine not only the form of the translocated material, but also the identity of the product of oxidation.

The Oxidation Products of the Metabolism of 2,4-DB and 2,4,5-TB and the form of the Translocated Herbicide in Intact Seedlings.

In experiment seven, eight bigleaf maple seedlings were treated with a one percent acid equivalent solution of non-labeled 2-ethylhexyl ester of 2,4-DB or 2,4,5-TB. After three days the plants were
harvested and extracted. The extracts were "cleaned up" and analyzed by gas chromatography as previously described. The results are expressed in µgs. of acid equivalent herbicide in tables nine and ten.

Table 9. The distribution of 2,4-DB and its oxidation product (2,4-D) after 72 hours in bigleaf maple seedlings treated with the 2-ethylhexyl ester of 2,4-DB.

<table>
<thead>
<tr>
<th>Plant number</th>
<th>µg's. of herbicide in the:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated leaf</td>
<td>2,4-DB</td>
<td>1100</td>
<td>1017</td>
<td>1307</td>
<td>545</td>
</tr>
<tr>
<td></td>
<td>2,4-D</td>
<td>110</td>
<td>150</td>
<td>312</td>
<td>140</td>
</tr>
<tr>
<td>percent conversion to 2,4-D</td>
<td>9.09</td>
<td>12.85</td>
<td>19.27</td>
<td>20.43</td>
<td></td>
</tr>
<tr>
<td>Stem</td>
<td>2,4-DB</td>
<td>8</td>
<td>6</td>
<td>23</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>2,4-D</td>
<td>-</td>
<td>6</td>
<td>--</td>
<td>trace</td>
</tr>
<tr>
<td>percent conversion to 2,4-D</td>
<td>0.0</td>
<td>50.0</td>
<td>0.0</td>
<td>trace</td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>2,4-DB</td>
<td>4</td>
<td>23</td>
<td>-</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>2,4-D</td>
<td>47</td>
<td>11</td>
<td>20</td>
<td>58</td>
</tr>
<tr>
<td>percent conversion to 2,4-D</td>
<td>92.16</td>
<td>32.35</td>
<td>100.00</td>
<td>61.70</td>
<td></td>
</tr>
</tbody>
</table>
Table 10. The distribution of 2,4,5-TB and its oxidation product (2,4,5-T) after 72 hours in bigleaf maple seedlings treated with the 2-ethylhexyl ester of 2,4,5-TB.

<table>
<thead>
<tr>
<th>Plant number</th>
<th>Treated leaves</th>
<th>Stem</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2,4,5-TB</td>
<td>2,4,5-T</td>
<td>2,4,5-TB</td>
</tr>
<tr>
<td>1</td>
<td>3577</td>
<td>1598</td>
<td>1162</td>
</tr>
<tr>
<td>2</td>
<td>650</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 10. The distribution of 2,4,5-TB and its oxidation product (2,4,5-T) after 72 hours in bigleaf maple seedlings treated with the 2-ethylhexyl ester of 2,4,5-TB.

<table>
<thead>
<tr>
<th>µgs. of herbicide in the:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated leaves</td>
<td>650</td>
<td>75</td>
<td>75</td>
<td>232</td>
</tr>
<tr>
<td>2,4,5-TB</td>
<td>3577</td>
<td>1598</td>
<td>1162</td>
<td>3305</td>
</tr>
<tr>
<td>percent conversion to 2,4,5-T</td>
<td>15.38</td>
<td>4.48</td>
<td>6.06</td>
<td>6.56</td>
</tr>
<tr>
<td>Stem</td>
<td>92</td>
<td>113</td>
<td>-</td>
<td>192</td>
</tr>
<tr>
<td>2,4,5-TB</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>percent conversion to 2,4,5-T</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>percent conversion to 2,4,5-T</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The ratio of 2,4-D to 2,4-DB found in the treated leaves corresponds fairly closely to the relative amount of C\textsubscript{14}O\textsubscript{2} recovered from carboxyl labeled 2,4-DB in detached maple leaves. It would seem that the primary product of oxidation is 2,4-D. While α-oxidation is not unknown in plants, β-oxidation is more common. On the basis of these results, no attempt was made to determine the nature of other oxidation products. For purposes of this study, the determination that 2,4-D was a primary oxidation product was sufficient.

Observation of the distribution of 2,4-DB and 2,4-D in the treated seedlings, as shown in table nine, reveals that the treated
leaves contain both 2,4-DB and 2,4-D, the stem, except in plant two, contains only 2,4-DB and the roots, except in plant two, contains a greater amount of 2,4-D than 2,4-DB. It is obvious that 2,4-DB is being translocated. The fact that 2,4-D appears in the roots is no indication it also has been translocated. If sufficient 2,4-D were being translocated to correspond to the amounts of herbicide found in the roots, an even larger amount would be found in the stem. The translocation data presented in table eight shows 2,4-D is present in larger amounts in the stem than in the roots. Table eight also shows 2,4-D is not well translocated, while plants treated with ring-labeled 2,4-DB translocated large amount of carbon 14 to the roots. This study indicates the majority of the carbon 14 translocated in the previous study (experiment six) was in the form of the butyric herbicide.

Table ten shows the distribution of 2,4,5-TB and its oxidation product, 2,4,5-T, in bigleaf maple. For an unexplainable reason, the translocation of 2,4,5-TB was inferior to 2,4-DB. However, it is important to notice that the patterns found with 2,4-DB are also evident for 2,4,5-TB. It is observed that 2,4,5-TB is oxidized in the treated leaves to 2,4,5-T. The distribution of 2,4,5-TB and 2,4,5-T in the stem and roots shows the translocated material is 2,4,5-TB.

Since it has been shown that the phenoxybutyric herbicide is the form of the translocated material, the presence of large amounts of phenoxyacetic herbicide in roots indicates they are capable of rapidly beta oxidizing 2,4-DB to 2,4-D.
The Oxidation Capabilities of Stem and Root Tissue

The lack of appreciable acetic herbicide in the stems of plants in the previous section indicated either the material was rapidly transported to the roots, or the roots had a greater oxidation capability than the stem. This prompted experiment eight, a study of the capability of stem and root tissues for the oxidation of 2,4-DB.

Stem and root tissue from two bigleaf maple seedlings were incubated in duplicate with 2.47 ppm carbon 14 carboxyl-labeled 2,4-DB acid in a phosphate buffer at pH 6.9. The liberation of $^14O_2$ was used as a measure of the ability of the tissues to oxidize the butyric herbicide to the acetic. The data from this experiment is presented in table eleven as a percentage of the absorbed radioactivity which was recovered as $^14O_2$ in 24 hours corrected to a common respiratory base of 15.65 mgs. CO$_2$ respired in 24 hours.

Table 11. The release of carbon 14 as $^14O_2$ in 24 hours by bigleaf maple root and stem tissue incubated with 2,4-DB-1-$^14$.

<table>
<thead>
<tr>
<th>Plant number</th>
<th>1</th>
<th>2</th>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>8.607</td>
<td>9.069</td>
<td>4.211</td>
<td>5.080</td>
</tr>
<tr>
<td>Stems</td>
<td>1.383</td>
<td>1.393</td>
<td>1.617</td>
<td>0.779</td>
</tr>
</tbody>
</table>

Plant number two was characterized by relatively few white root tips compared with plant one.
The data in table 11 show that root tissue is considerably better able to decarboxylate 2,4-DB than stem tissue from the same plant. This undoubtedly accounts for the distribution of the butyric and acetic herbicides observed in tables nine and ten.

Canny and Markus have reported that the roots of intact tick bean plants decarboxylated 2,4-D at a considerably higher rate than did the stems of the same plant (11, p. 490-495). These results would appear to substantiate the results found with bigleaf maple both for intact seedlings and for excised plant parts.

The butyric herbicide is pictured as moving readily through the stem and accumulating in the roots where it is converted to the acetic herbicide. The low rate of conversion of 2,4-DB to 2,4-D in the stem may be an important factor in the translocation of 2,4-DB. If large amounts of 2,4-DB were converted in the stem to 2,4-D, reduced translocation would be expected to occur.

The interpretation of the results reported in these experiments with respect to chemical selectivity with aerial application of herbicides to bigleaf maple is not without difficulty. The primary problem is lack of information concerning the relative effectiveness of these various herbicides in aerial applications. Studies of chemical toxicity to bigleaf maple have been performed, but with objectives other than aerial control in mind.

Chemical selectivity is based on a number of factors. Spray retention, absorption, translocation, detoxification and herbicide toxicity are commonly included. As indicated in the review of literature, a number of early investigations, as well as a few later ones,
attempted to demonstrate that the basis of chemical selectivity between species depended on single factors. A number of early investigations showed lack of phenoxy herbicide uptake and movement in resistant monocots when compared to susceptible dicots. Some investigators felt this was a primary mechanism of selectivity. However, Butts and Fang reported that although differential uptake and distribution was observed between various monocots and dicots, when equal amounts of herbicide were present in the plants, dicots showed greater response than monocots. They concluded that absorption and translocation were factors in determining selectivity, but detoxification also played a major role (10). Luckwill and Lloyd-Jones reported the detoxification of phenoxy herbicides to be the primary basis of selectivity in a few species, but this pattern was not observed in most species tested (50, p. 613-625; 51, p. 626-636). These reports and others indicate that the basis of chemical selectivity is not a single factor in most cases.

Chemical selectivity in bigleaf maple, as indicated by the experiments reported in this study, is a combination of factors. These factors include absorption, translocation, detoxification and the inherent toxicity of the chemical to the test organism.

The results of this study indicate that absorption is not of primary importance in determining the selectivity of this series of compounds to bigleaf maple. In fact, many differences in absorbability between various chemicals were negated by differential translo- catability. In table two, for instance, it is observed that 2,4,5-TP was absorbed to a greater extent than 2,4,5-T, but the greater
translocatability of 2,4,5-T resulted in more 2,4,5-T in the roots than 2,4,5-TP.

It was pointed out previously that the translocation of phenoxy herbicides is influenced by leakage of chemicals from the phloem to the xylem, and by accumulation in parenchyma cells of phloem. It was also noted that these processes probably do not occur to the same extent for two different herbicides. It follows that the greater the distance two chemicals have to be translocated, the greater the difference between amounts reaching the roots will be.

A histoautoradiographic study of the translocation of 2,4-D-1-\textsuperscript{14}C from leaves to roots of bean plants has shown what appears to be increasing accumulation of carbon 14 in the parenchyma cells of the phloem and leakage to the xylem with increasing distance from the leaves (63). Eliasson, working with a number of phenoxy herbicides in aspen seedlings, reported an inverse relationship between plant size and amount of herbicide translocated to roots following foliage treatment (23, p. 213). Newton observed a definite inverse correlation between shrub size and herbicide effectiveness with aerial application to vine maple (58, p. 63). These reports substantiate the contention that differences in the translocatability of various herbicides will be greater with larger trees.

Unfortunately, data on metabolism in intact seedlings for the entire series of herbicides tested in this study is not available. Data obtained for the metabolism of 2,4-D and 2,4,5-T indicates detoxification is an important mechanism in determining chemical selectivity in bigleaf maple.
Differences in chemical stability in the roots is particularly important when considered with the translocation data. A combination of translocation and detoxification have resulted in three times more 2,4,5-T than 2,4-D being present in the roots. This difference is considered to be of extreme importance in determining the relative effectiveness of 2,4-D and 2,4,5-T when applied as aerial sprays to bigleaf maple.

The question of herbicide toxicity to bigleaf maple is unclear. Rediske has compared the effects of different herbicides and formulations on bigleaf maple and other brush species. In these tests the plants were sprayed to the runoff point with four pounds of herbicide acid equivalent per 100 gallons of carrier. While this is a high volume application and does not approximate aerial application, certain observations may be made. The present study shows that herbicide translocation is not extensive in bigleaf maple. Assuming this is also the case in the study reported by Rediske, a good deal of the effect noted must have been contact injury which should be a fair indicator of inherent chemical toxicity.

Rediske reported the following degrees of kill of bigleaf maple after one year when sprayed with the triethyl amine salts of the following herbicides: 2,4-D, 55 percent; 2,4-DP, 100 percent; and 2,4,5-T, 100 percent (64). On this basis, 2,4-D is probably less toxic to bigleaf maple than either 2,4-DP or 2,4,5-T. The 100 percent kill by 2,4-DP and 2,4,5-T prevent a comparison of their relative toxicities. Data on the toxicity of 2,4,5-TP in foliage treatments of this type are not available. Newton reported, however, that aerial
application of 2,4,5-TP as a pre bud burst spray was relatively ineffective, while 2,4,5-T was more satisfactory (58, p. 63). However, Bernsten reported 2,4,5-TP to be markedly superior to 2,4,5-T when applied as a basal spray to bigleaf maple trees (6). Newton reported 2,4,5-TP to be most effective against bigleaf maple trees when applied in frilling treatment, followed by 2,4,5-T and 2,4-D (58, p. 32). From these reports, it would appear that 2,4,5-TP is fairly toxic to bigleaf maple. It is possible that the poor degree of translocation noted for this compound is a direct result of its toxicity to the phloem.

Chemical selectivity in bigleaf maple is pictured as a multifactored process. It is similar in nature to a linked chemical reaction in living organisms. The first step in the process of determining selectivity, in terms of effectiveness of control with aerial application of herbicides, is absorption. Absorption is the first limiting step. No more herbicide can be translocated than is absorbed. Translocation is the next step in the process; in terms of effective control, translocation to the roots is considered to be the primary factor. Once the herbicides are translocated to the roots, the rate of detoxification becomes important. When the factors of absorption, translocation and detoxification have had their respective effects, certain quantities of the different herbicides will be available to destroy the roots. The final factor in this scheme of control is the inherent toxicity of the various herbicides to root tissues. As an example, if twice as much 2,4,5-T is present in the roots compared to 2,4-D after a given period, but 2,4-D is twice as
effective as 2,4,5-T in destroying root tissue, their apparent effectiveness will be about the same.

This scheme of selectivity may be reduced to a simple equation:

$$\text{Eff.} = \text{Int.} \times \text{Abs.} \times \text{Trans.} \times \text{Stab.} \times \text{Tox.}$$

where

Eff. = ultimate effectiveness as an aerially applied herbicide on a particular species.

Int. = amount of herbicide intercepted by the foliage.

Abs. = percent absorption/100

Trans. = percent of the absorbed material which translocates to the roots/100.

Stab. = percent of the material in the roots in an active form after a given period of time/100.

Tox. = toxicity to root tissue as a percent of the toxicity of another compound to which the comparison will be made/100.

As a hypothetical case, consider the use of this equation in determining the probable control of plant species "X" with 2,4-D and 2,4,5-T applied in equal concentrations as aerial sprays. The leaves of both plants intercept 100 mgs. of acid equivalent herbicide. The absorption of 2,4-D is 35 percent compared to 28 percent for 2,4,5-T. Of the material absorbed, six percent of the 2,4-D is translocated to the roots compared to eight percent for 2,4,5-T. After a given period of time, 58 percent of the 2,4-D transported to the roots remains unaltered compared to 38 percent for 2,4,5-T. The 2,4-D is only 38 percent as effective as 2,4,5-T in destroying root tissue.
The equation for 2,4-D is as follows:

\[
100 \text{ mgs.} \times 0.35 \times 0.06 \times 0.58 \times 0.38 = 0.46 \text{ mgs. equivalent 2,4,5-T.}
\]

The equation for 2,4,5-T is as follows:

\[
100 \text{ mgs.} \times 0.28 \times 0.08 \times 1.0 = 0.85 \text{ mgs. equivalent 2,4,5-T.}
\]

In terms of 2,4,5-T, 2,4-D is little more than one half as effective as 2,4,5-T in controlling plant species "X".

The phenoxybutyric herbicides must be considered separately. The toxicity of these compounds depends on the ability of the plant to convert them to the phenoxyacetic homolog. It has been demonstrated in this study that bigleaf maple is capable of making this conversion.

The data reported show the majority of the translocated 2,4-DB has been converted to 2,4-D in three days. Most, if not all, of the translocated butyric herbicide will probably be converted to the active acetic homolog in time. The picture for 2,4,5-TB is not clear. The roots show apparently complete conversion of the butyric to the acetic, but the amounts translocated were small. However, Rediske reported 2,4,5-TB to be more toxic to bigleaf maple than 2,4-DB when applied as a foliar spray (64).

The interpretation of these results with respect to bigleaf maple control is uncertain, because information concerning the relative effectiveness of these chemicals as aerial sprays is not available. Although 2,4-D, 2,4,5-T and 2,4,5-TP have been employed as herbicides in aerial spray projects, the results have been largely disappointing in terms of bigleaf maple control. The usual picture is varying degrees of crown kill followed by vigorous sprouting and eventual site reoccupation.
This study shows some of the reasons for poor control of bigleaf maple. The absorbability of these compounds is fairly low in this test but still acceptable. Raising the absorption will further reduce translocation due to contact injury. With less than two percent of the absorbed material being translocated to the roots in these treatments, it is clear that translocation constitutes the primary limiting step in effective control of bigleaf maple.

There are a number of steps which might be taken to help alleviate this problem. Among them are the development of new formulations and chemicals which will have better translocation characteristics. A step in this direction has been the development of 4-amino-3,5,6-trichloropicolinic acid (Tordon), an experimental herbicide of the Dow Chemical Company, which appears to be readily translocated in a number of plants, although it has a high degree of toxicity to many species. However, Tordon and some other chemicals, which might prove effective in bigleaf maple control, suffer from low selectivity for desirable plant species, such as conifers, or long persistence in the environment.

The gamma-phenoxybutyric herbicide studies have revealed that they may have a real place in brush control. 2,4-DB has been shown to be absorbed and readily translocated in bigleaf maple, and is readily converted to an active form in the roots. Newton has reported 2,4,5-TB to be relatively non-toxic to Douglas-fir when applied as a foliar spray at rates comparable to those used in aerial application (59).
Considerable work remains to be done before the butyric herbicides can be recommended for broadscale use in the field. Their absorption, translocation and metabolism characteristics should be determined in a number of woody plant species which have resisted aerial control with 2,4-D and 2,4,5-T. Extensive field testing will be necessary to determine proper rates and times of application.

The development of new herbicides and formulations will be aided by studies which determine the mechanisms involved in absorption and translocation of herbicides. Studies which elucidate the influence of physiological, chemical and environmental factors on these processes would be invaluable. Determination of the modes of action and breakdown products of these different herbicides are needed. The logical development of new compounds suitable for brush control is hampered by lack of basic information in these areas.
SUMMARY AND CONCLUSIONS

Increasing the productivity of forest lands in the Western United States demands more complete utilization of the growth potential of the site by desirable species. To attain this end, the control of undesirable brush and weed trees species by the aerial application of low volumes of growth regulator type herbicides is advocated. Aerial application of herbicides has met with variable success in the past, and certain species appear to be quite resistant to control, although it has been established they are susceptible to these chemicals.

The effective control of woody plants with aerial sprays depends on adequate absorption and movement of herbicide to the roots in a form which has a high degree of biological activity. Bigleaf maple has been used to determine the influence of formulation and molecular configuration on the absorption, translocation and metabolism characteristics of a series of phenoxy herbicides in woody plants. These characteristics were used to determine the factors which have prevented adequate control of bigleaf maple.

This study has shown formulation has a marked influence on the absorption of herbicides in woody plants. Within a series of formulations of a single herbicide, an inverse relationship exists between the polarity of the compound and its absorption by bigleaf maple foliage.

The influence of formulation on translocation was not clearly defined. Large differences in absorption between formulations
influenced translocation. The acid and amine formulations were about equally absorbed and showed the same degree of translocatability. It is believed the acid and amine formulations are translocated as the acid. Ester formulations have been shown to be at least partially hydrolyzed in the leaves. It is recognized that the ester formulation probably has some direct influence on translocation. However, the primary influence of the ester formulation on translocation of herbicides is believed to be exerted through its influence on absorption, which in turn reduces the translocatability of the herbicide through a concentration effect.

The influence of molecular configuration on absorption is not clear. Variation within treatments prevented drawing statistically significant conclusions. The data indicate, however, that both degree of chlorination of the ring and configuration of the side chain influence the absorption of this series of phenoxy herbicides. The addition of a methyl group on the alpha carbon of the side chain enhanced the absorption of the molecule, while lengthening the normal two-carbon chain to four reduced it.

The translocation characteristics of these compounds showed that extent of ring-substitution and configuration of side chain are also important in their translocation. The order of mobility of this series was not the same from the treated leaves as it was entering the roots. This indicates that movement through the stem is a critical factor in determining their effectiveness. The non-toxic 2,4-DB proved to be the most readily translocated herbicide tested.
Metabolism studies with detached maple leaves indicated that
decarboxylation is not an important detoxification mechanism in
bigleaf maple. The formation of metabolites in the treated leaves
was at a minimum. Experiments with 2,4-D and 2,4,5-T in intact
seedlings, however, showed that other plant parts metabolize these
chemicals at a faster rate. 2,4-D was shown to be less stable than
2,4,5-T, particularly in the roots.

The more mobile 2,4-DB was chosen for further testing. It was
demonstrated that bigleaf maple leaves and roots were able to alter
2,4-DB to the biologically active 2,4-D at moderate rates. The stem
was less able to do so. The lack of conversion of the butyric to the
acetic in the stem is believed to be important in determining the
translocation characteristics of this chemical.

The results of these experiments indicate that chemical selectiv-
ity in bigleaf maple is a multifactored process involving absorption,
translocation, chemical stability in the roots and the inherent
toxicity of the herbicide. It is believed that a simple equation
involving these factors may be used to determine the relative effective-
ness of a number of different chemicals in controlling a particular
plant species.

The performance of 2,4-DB in this series of tests indicates it
may have real promise in brush control. Basic information of the
mechanisms of absorption, translocation, metabolism and modes of action
of herbicides is vital to the development of new herbicides and
formulations. Adequate field testing and determination of absorption,
translocation and metabolism characteristics of new compounds will aid in the development of better chemicals and techniques of brush control.


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APPENDIX
Preparation of Treatment Solutions

Experiment one.

2,4-D acid.
1. Evaporate to dryness in a one ml. volumetric tube, one ml. of a 9503 ppm. 2,4-D acid in acetone solution.

2. Add and evaporate to dryness in aliquots, 2.80 mls. of a 177.5 ppm., 8.85 μcs. per mg., 2,4-D-1-C14 acid in acetone solution.

3. Add enough 80% v/v isopropyl alcohol to dissolve the acid.

4. Add 25 μls. of a 20,000 ppm. Tween 20 solution.

5. Make to volume with distilled water.

2,4-D triethanol amine.
1. Repeat steps one and two above.

2. Add 343 μls. of a 14,900 ppm. triethanol amine solution and heat gently for 30 minutes in warm water.

3. Add 25 μls. of a 20,000 ppm. Tween 20 solution.

4. Make to volume with distilled water.

2,4-D 2-ethylhexyl ester.
1. Repeat step one above.

2. Add 50 μls. 2-ethylhexyl alcohol and heat in a water bath for two hours with the stopper loosely in place.

3. After cooling to room temperature, add 25 μls. of a 20,000 ppm. Tween 20 solution.

4. Make to volume with distilled water.

2,4,5-T acid.
1. Evaporate to dryness in a one ml. volumetric tube, one ml. of a 9682 ppm. 2,4,5-T acid in acetone solution.

2. Add and evaporate to dryness in aliquots, 1.4 mls. of a 227.2 ppm, 15.5 μcs. per mg, 2,4,5-T-1-C14 acid in acetone solution.

3. Follow steps outlined for 2,4-D acid.
2,4,5-T triethanol amine.
1. Repeat steps one and two above.
2. Add 450 μls. of a 14,900 ppm. triethanol amine solution.
3. Follow steps outlined for 2,4-D triethanol amine.

2,4,5-T 2-ethylhexyl ester.
1. Repeat step one above.
2. Follow steps outlined for 2,4-D 2-ethylhexyl ester.

Experiment two.

Synthesis of 2,4-DP-1-C$^{14}$ and 2,4,5-TP-1-C$^{14}$ acids.
1. Sodium 2,4-dichlorophenate and sodium 2,4,5-trichlorophenate were purified and recrystallized four times from redistilled benzene and dried in vacuo over CaSO$_4$ for 48 hours.

2. 16.7 mgs. of six μcs. per mg. 2-bromopropionic-1-C$^{14}$ acid were refluxed in 100 mls. of freshly purified anhydrous methanol under a CaSO$_4$ water trap for 24 hours to form the methyl ester.

3. The methyl 2-bromopropionate in methanol was divided into two portions and refluxed with three grams of sodium 2,4-dichlorophenate or three grams of sodium 2,4,5-trichlorophenate for 24 hours.

4. The products were diluted with water, adjusted to pH five and extracted with three 75 ml. aliquots of carbon tetrachloride to remove the unreacted phenol.

5. The water phase was acidified with HCl to about pH one and liquid-liquid extracted continuously for 12 hours.

6. The products were chromatographed on paper in the following solvent systems in duplicate:
   a. n-butanol, propionic acid and water 12:5.6:8.
   b. n-butanol saturated with water.
   c. 65 percent lutidine.
   d. isooctane, 95 percent ethanol, acetone and 90 percent acetic acid 4:4:1:0.05.

All systems showed only a single radioactive peak. The products showed the expected degrees of biological activity on maple seedlings during the experiments in which they were used.

This Williamson synthesis yielded 6.77 mgs. of 3.9 μcs. per mg. 2,4-DP-1-C$^{14}$ and 3.62 mgs. of 3.4 μcs. per mg. 2,4,5-TP-1-C$^{14}$. 
2,4-DP 2-ethylhexyl ester.
1. Evaporate to dryness in a one ml. volumetric tube, one ml. of an 8647 ppm. 2,4-DP acid in acetone solution.

2. Add and evaporate to dryness in aliquots, 20.0 mls. of a 67.6 ppm., 3.9 µcs. per mg., 2,4-DP-1-C\textsubscript{14} acid in benzene solution.

3. Follow steps outlined for 2,4-D 2-ethylhexyl ester in experiment one.

2,4,5-TP 2-ethylhexyl ester.
1. Evaporate to dryness in a one ml. volumetric tube, 1.7 mls. of a 5140 ppm. 2,4,5-TP acid in acetone solution.

2. Add and evaporate to dryness in aliquots, 35.0 mls. of a 36.25 ppm., 3.40 Fics. per mg., 2,4,5-TP-1-C\textsubscript{14} acid in benzene solution.

3. Follow the steps outlined for 2,4-D 2-ethylhexyl ester in experiment one.

Experiment three.

2,4-D triethanol amine.
1. Evaporate to dryness in a one ml. volumetric tube, 100 µls. of a 5370 ppm. 2,4-D acid in acetone solution.

2. Add and evaporate to dryness in aliquots, 3.2 mls. of a 177.6 ppm., 8.85 µcs. per mg., 2,4-D-1-C\textsubscript{14} acid in acetone solution.

3. Add 550 µls. of a 1490 ppm. triethanol amine solution and heat gently in warm water for 30 minutes.

4. Add 25 µls. of a 20,000 ppm. Tween 20 solution.

5. Make to volume with distilled water.

2,4,5-T triethanol amine.
1. Evaporate to dryness in a one ml. volumetric tube, 100 µls. of a 9682 ppm. 2,4,5-T acid in acetone solution.

2. Add and evaporate to dryness in aliquots, 1.4 mls. of a 227.2 ppm., 15.5 µcs. per mg., 2,4,5-T-1-C\textsubscript{14} acid in acetone solution.

3. Follow the steps outlined for 2,4-D triethanol amine in experiment three.
2,4-DP triethanol amine.
1. Add and evaporate to dryness in aliquots in a one ml. volumetric tube, 17.4 mls. of a 67.6 ppm., 3.9 μcs. per mg., 2,4-DP-1-Cl\textsubscript{14} acid in benzene solution.

2. Follow the steps outlined for 2,4-D triethanol amine in experiment three.

2,4,5-TP triethanol amine.
1. Evaporate to dryness in a one ml. volumetric tube, 100 μls. of a 5140 ppm. 2,4,5-TP acid in acetone solution.

2. Add and evaporate to dryness in aliquots 23.0 mls. of a 36.25 ppm., 3.40 μcs. per mg., 2,4,5-TP-1-Cl\textsubscript{14} acid in benzene solution.

3. Follow the steps outlined for 2,4-D triethanol amine in experiment three.

Experiment four.

2,4-D triethanol amine.
1. Evaporate to dryness in a two ml. volumetric tube, 200 μls. of a 5370 ppm. 2,4-D acid in acetone solution.

2. Add and evaporate to dryness in aliquots, 6.4 mls. of a 177.6 ppm., 8.85 μcs. per mg., 2,4-D-1-Cl\textsubscript{14} acid in acetone solution.

3. Add 1.1 mls. of a 1490 ppm. triethanol amine solution and heat gently in warm water for 30 minutes.

4. Add 50 μls. of a 20,000 ppm. Tween 20 solution.

5. Make to volume with distilled water.

2,4,5-T triethanol amine.
1. Evaporate to dryness in a two ml. volumetric tube, 200 μls. of a 9682 ppm. 2,4,5-T acid in acetone solution.

2. Add and evaporate to dryness in aliquots, 2.8 mls. of a 227.2 ppm., 15.5 μcs. per mg., 2,4,5-T-1-Cl\textsubscript{14} acid in acetone solution.

3. Follow steps outlined for 2,4-D triethanol amine in experiment four.
Experiment five.

2,4-DB triethanol amine.
1. Evaporate to dryness in a one ml. volumetric tube, 700 μls. of a 1792 ppm. 2.96 μcs. per mg. 2,4-DB-1-C14 acid in acetone solution.
2. Follow steps outlined for 2,4-D triethanol amine in experiment two.

Experiment six.

2,4-DB 2-ethylhexyl ester.
1. Evaporate to dryness in a one ml. volumetric tube, one ml. of an 8740 ppm. 2,4-DB acid in acetone solution.
2. Add and evaporate to dryness in aliquots, 3.6 mls. of a 350.0 ppm four μcs. per mg. 2,4-DB ring C'-4 acid in acetone solution.
3. Follow the steps outlined for 2,4-D 2-ethylhexyl ester in experiment one.

Experiment seven.

2,4-DB 2-ethylhexyl ester.
1. Evaporate to dryness in a ten ml. volumetric flask, 5.07 mls. of a 19,748 ppm. 2,4-DB acid in acetone solution.
2. Add 600 μls. of 2-ethylhexyl alcohol.
3. Heat for two hours in a water bath with stopper loosely in place.
4. After cooling to room temperature, add 400 μls. of a 20,000 ppm. Tween 20 solution.
5. Make to volume with distilled water.

2,4,5-TB 2-ethylhexyl ester.
1. Evaporate to dryness in a ten ml. volumetric flask five mls. of a 20,000 ppm. 2,4,5-TB acid in acetone solution.
2. Follow the steps outlined for 2,4-DB 2-ethylhexyl ester in experiment 7.

Experiment eight.

2,4-DB acid.
1. Evaporate to dryness in a 100 ml. volumetric flask 3.13 mls. of a 79.0 ppm. 2.96 μcs. per mg. 2,4-DB-1-C14 acid in acetone solution.
2. Make to volume with a $2.56 \times 10^{-2}$ molar phosphate buffer solution with a pH of 6.9.

Extraction Procedures

Extraction Procedure used in Experiments One, Two, Six and Seven.

1. Woody material is cut into pieces measuring a maximum of 1 x .5 inches and dried at 80°C. for 24 hours.

2. After drying, the woody material is ground in a Wiley mill through a 20-mesh sieve.

   The ground woody material and all other plant parts are extracted as follows:

3. Macerate in 100 mls. of 80 percent v/v isopropyl alcohol in a Waring Blendor for two minutes.

4. The homogenate is heated for two hours on the steam bath and filtered and washed with 100 mls. of 80 percent v/v isopropyl alcohol followed by 25 mls. of acetone.

5. Add sufficient ten percent KOH to raise the pH to around 13, and evaporate off the alcohol, washing the sides down periodically with water.

6. Add water to make about 200 mls., and acidify with conc. HCl to about pH one.

7. Add two mls. of ten percent phosphotungstic acid and liquid-liquid extract continuously for at least 12 hours with benzene.

8. Evaporate benzene extract down and transfer quantitatively to volumetric flask and make to volume with benzene.

Sample Clean-up and Preparation for Gas Chromatography Procedures.

1. Transfer benzene extract from step six above to a six-inch Woelm basic alumina column.

2. Wash the column with 100 mls. of distilled chloroform, 100 mls. of distilled ether and 100 mls. of distilled chloroform.

3. Draw air through the column to evaporate solvent for 30 minutes; elute with 100 mls. of one percent NaHCO$_3$ solution and wash with 50 mls. of distilled water.
4. Acidify with conc. HCl to about pH one and liquid-liquid extract continuously for at least 12 hours with benzene.

5. Evaporate benzene extract down and transfer quantitatively to a 25 ml. volumetric flask and evaporate to dryness.

6. Add five mls. of 15 percent BF₃ in methanol solution, and heat for two minutes on the steam bath.

7. Cool and add 15 mls. of two percent Na₂SO₄ and swirl.

8. Add purified CS₂ quantitatively and shake on a mechanical shaker for at least 30 minutes to extract the methyl ester from the water phase.

9. Transfer the CS₂ layer to a small test tube and cover the organic layer with water. Inject quantitatively into the gas chromatograph.

Statistical Analysis

The data in experiments one, two, three, five and six were subjected to analysis of variance by one-way classification. In cases where significant differences in treatment effects at the five percent level were found, the data were analyzed by the new multiple range test for differences between individual treatment means. In some cases it was necessary to perform a logarithmic transformation prior to analysis. The results of the statistical analyses are listed by the table number. The form in which the data were analyzed and the calculated F value are given. Where significant F values were found, the data were analyzed by the new multiple range test. The mean values of each treatment expressed in the form in which they were analyzed by the multiple range test are shown. Those values which are underscored by a common line are not significantly different from one another at the five percent level. In cases where lines overlap, the question of significance is
uncertain. As an example, some hypothetical values are listed below, and their statistical interpretation indicated.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment means</td>
<td>23.5</td>
<td>31.7</td>
<td>35.8</td>
<td>36.0</td>
</tr>
</tbody>
</table>

At the five percent level, treatment A is significantly different from B, C, and D. Treatment B is significantly different from A and D, but the question of significance between B and C is uncertain. Treatments C and D are not significantly different.

Data from table one:

Average percent absorption plus ten: Logarithmic transformation.

One-way analysis of variance: F-91.055 with five and 11 degrees of freedom. Results of the new multiple range test are listed below.

<table>
<thead>
<tr>
<th>2,4,5-T amine</th>
<th>2,4-D amine</th>
<th>2,4,5-T acid</th>
<th>2,4-D acid</th>
<th>2,4,5-T ester</th>
<th>2,4-D ester</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0295</td>
<td>1.0670</td>
<td>1.0912</td>
<td>1.1109</td>
<td>1.4218</td>
<td>1.4859</td>
</tr>
</tbody>
</table>

Average percent of absorbed activity in the treated leaves:

Logarithmic transformation.

One-way analysis of variance: F-12.948 with five and 11 degrees of freedom. Results of the new multiple range test are listed below.

<table>
<thead>
<tr>
<th>2,4,5-T amine</th>
<th>2,4-D amine</th>
<th>2,4,5-T acid</th>
<th>2,4-D acid</th>
<th>2,4-D ester</th>
<th>2,4,5-T ester</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7905</td>
<td>1.8352</td>
<td>1.8523</td>
<td>1.8860</td>
<td>1.9723</td>
<td>1.9820</td>
</tr>
</tbody>
</table>
Average percent of absorbed activity in the new growth: Logarithmic transformation.

One-way analysis of variance: $F = 8.306$ with five and 11 degrees of freedom. Results of the new multiple range test are listed below.

\[
\begin{array}{cccccc}
2,4,5-T & 2,4-D & .2,4-D & 2,4-D & 2,4,5-T & 2,4,5-T \\
ester & ester & acid & amine & amine & acid \\
0.3255 & 0.5232 & 0.9408 & 1.1911 & 1.2407 & 1.3191 \\
\end{array}
\]

Average percent of absorbed activity in the stem plus ten:

Logarithmic transformation.

One-way analysis of variance: $F = 4.257$ with five and 11 degrees of freedom. Results of the new multiple range test are listed below.

\[
\begin{array}{cccccc}
2,4,5-T & 2,4-D & 2,4,5-T & 2,4,5-T & 2,4-D & 2,4-D \\
ester & ester & acid & amine & acid & amine \\
1.0264 & 1.0542 & 1.2178 & 1.2240 & 1.2561 & 1.2806 \\
\end{array}
\]

Average percent of absorbed activity in the roots plus ten:

Logarithmic transformation.

One-way analysis of variance: $F = 19.301$ with five and 11 degrees of freedom. Results of the new multiple range test are presented below.

\[
\begin{array}{cccccc}
2,4-D & 2,4,5-T & 2,4,5-T & 2,4-D & 2,4-D & 2,4,5-T \\
ester & ester & acid & amine & acid & amine \\
1.0234 & 1.0524 & 1.0761 & 1.1455 & 1.1729 & 1.3599 \\
\end{array}
\]

Average number of micrograms of herbicide as acid equivalent in the roots: No transformation.

One-way analysis of variance: $F = 7.093$ with five and 11 degrees of freedom. Results of the new multiple range test are listed below.
Data from table two.

Average percent absorption: Logarithmic transformation.

One-way analysis of variance: F-1.533 with three and nine degrees of freedom. Not significant.

Average percent of absorbed activity in the treated leaves: No transformation.

One-way analysis of variance: F-0.819 with three and nine degrees of freedom. Not significant.

Average percent of absorbed activity in the new growth: No transformation.

One-way analysis of variance: F-0.2159 with three and nine degrees of freedom. Not significant.

Average percent of absorbed activity in the roots plus ten: Logarithmic transformation.

One-way analysis of variance: F-5.688 with three and nine degrees of freedom. Results of the new multiple range test are presented below.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>2,4,5-TP</th>
<th>2,4-D</th>
<th>2,4-DP</th>
<th>2,4,5-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td>0.3</td>
<td>1.4</td>
<td>2.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Amines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ester</td>
<td>2.3</td>
<td>2.8</td>
<td>4.3</td>
<td></td>
</tr>
</tbody>
</table>

Average number of micrograms of herbicide as acid equivalent in the roots plus ten: Logarithmic transformation.
One-way analysis of variance: $F = 8.901$ with three and nine degrees of freedom. Results of the new multiple range test are presented below.

<table>
<thead>
<tr>
<th></th>
<th>2,4,5-TP</th>
<th>2,4-D</th>
<th>2,4,5-T</th>
<th>2,4-DP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>1.0465</td>
<td>1.0885</td>
<td>1.1530</td>
<td>1.2015</td>
</tr>
</tbody>
</table>

Data from table three.

Average percent of absorbed activity recovered as $\text{C}^{14}O_2$ plus ten: Logarithmic transformation.

One-way analysis of variance: $F = 0.708$ with three and 12 degrees of freedom. Not significant.

Data from table four.

Average percent of activity in alcohol extracts recoverable as herbicide: No transformation.

One-way analysis of variance: $F = 17.126$ with three and 11 degrees of freedom. Results of the new multiple range test are presented below.

<table>
<thead>
<tr>
<th></th>
<th>2,4,5-TP</th>
<th>2,4-DP</th>
<th>2,4,5-T</th>
<th>2,4-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>84.07</td>
<td>87.23</td>
<td>93.35</td>
<td>94.98</td>
</tr>
</tbody>
</table>

Data from table six.

Average percent of absorbed activity recovered as $\text{C}^{14}O_2$ plus ten: Logarithmic transformation.

One-way analysis of variance: $F = 61.869$ with four and 14 degrees of freedom. Results of the new multiple range test are presented below.
Data from table seven.

Average percent of activity in alcohol extracts recoverable as herbicide: No transformation.

One-way analysis of variance: F-15.016 with four and 13 degrees of freedom. Results of the new multiple range test are presented below.

<table>
<thead>
<tr>
<th></th>
<th>2,4-D</th>
<th>2,4,5-TP</th>
<th>2,4,5-T</th>
<th>2,4-DP</th>
<th>2,4-DB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data</td>
<td>1.0193</td>
<td>1.0215</td>
<td>1.0245</td>
<td>1.0256</td>
<td>1.3545</td>
</tr>
</tbody>
</table>

Data from table eight.

Average percent absorption: Logarithmic transformation.

One-way analysis of variance: F-3.464 with four and 11 degrees of freedom. Results of the new multiple range test are listed below.

<table>
<thead>
<tr>
<th></th>
<th>2,4-D</th>
<th>2,4,5-T</th>
<th>2,4-DP</th>
<th>2,4,5-T</th>
<th>2,4-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data</td>
<td>73.24</td>
<td>84.07</td>
<td>87.23</td>
<td>93.35</td>
<td>94.98</td>
</tr>
</tbody>
</table>

Average percent of absorbed activity in the treated leaves: No transformation.

One-way analysis of variance: F-32.638 with four and 11 degrees of freedom. Results of the new multiple range test are presented below.

<table>
<thead>
<tr>
<th></th>
<th>2,4,5-TP</th>
<th>2,4,5-T</th>
<th>2,4-DP</th>
<th>2,4-D</th>
<th>2,4-DB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data</td>
<td>96.11</td>
<td>95.94</td>
<td>94.87</td>
<td>93.85</td>
<td>77.48</td>
</tr>
</tbody>
</table>
Average percent of absorbed activity in the new growth: No transformation.

One-way analysis of variance: $F=1.404$ with four and 11 degrees of freedom. Not significant.

Average percent of absorbed activity in the stem plus ten:
Logarithmic transformation.

One-way analysis of variance: $F=70.181$ with four and 11 degrees of freedom. Results of the new multiple range test are presented below.

<table>
<thead>
<tr>
<th></th>
<th>2,4,5-TP</th>
<th>2,4,5-T</th>
<th>2,4-DP</th>
<th>2,4-D</th>
<th>2,4-DB</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4,5-TP</td>
<td>1.0256</td>
<td>1.0264</td>
<td>1.0468</td>
<td>1.0542</td>
<td>1.3373</td>
</tr>
</tbody>
</table>

Average percent of absorbed activity in the roots plus ten:
Logarithmic transformation.

One-way analysis of variance: $F=39.348$ with four and 11 degrees of freedom. Results of the new multiple range test are presented below.

<table>
<thead>
<tr>
<th></th>
<th>2,4,5-TP</th>
<th>2,4-D</th>
<th>2,4-DP</th>
<th>2,4,5-T</th>
<th>2,4-DB</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4,5-TP</td>
<td>1.0124</td>
<td>1.0234</td>
<td>1.0469</td>
<td>1.0524</td>
<td>1.2090</td>
</tr>
</tbody>
</table>

Average number of micrograms of herbicide as acid equivalent in the roots plus ten: Logarithmic transformation.

One-way analysis of variance: $F=31.071$ with four and 11 degrees of freedom. Results of the new multiple range test are presented below.

<table>
<thead>
<tr>
<th></th>
<th>2,4,5-TP</th>
<th>2,4-D</th>
<th>2,4,5-T</th>
<th>2,4-DP</th>
<th>2,4-DB</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4,5-TP</td>
<td>1.0465</td>
<td>1.0885</td>
<td>1.1530</td>
<td>1.2015</td>
<td>1.3897</td>
</tr>
</tbody>
</table>
Graphs of the Distribution of Radioactivity on Paper Chromatograms

The figures which follow show the distribution of radioactivity on representative paper chromatograms prepared in experiments three and four. The paper chromatograms were prepared by placing 100 µls. of an alcohol extract of treated bigleaf maple leaves on Whatman number one filter paper strips. The strips were developed in n-butanol, propionic acid and water (12:5.6:3). Treatment standards were also chromatographed in a similar manner, and they have been included to aid in identification of the herbicide peak. The distribution of radioactivity on each chromatogram is shown by the percentage of the total radioactivity found on the strip which was located at a particular point. This percentage has been plotted over the Rf of the point.
Figure 2

The distribution of radioactivity on a paper chromatogram of 2,4-D-1-C¹⁴ treatment standard. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the $R_f$ of that point.
Figure 3

The distribution of radioactivity on a paper chromatogram of an alcohol extract of a single, detached bigleaf maple leaf treated with 2,4-D-1-C\textsuperscript{14}. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the R\textsubscript{f} of that point.
Figure 4

The distribution of radioactivity on a paper chromatogram of 2,4,5-T-1-Cl⁴ treatment standard. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the $R_f$ of that point.
Figure 5

The distribution of radioactivity on a paper chromatogram of an alcohol extract of a single, detached bigleaf maple leaf treated with 2,4,5-T-1-C14. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the $R_f$ of that point.
Figure 6

The distribution of radioactivity on a paper chromatogram of 2,4-DP-1-C¹⁴ treatment standard. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the $R_f$ of that point.
The distribution of radioactivity on a paper chromatogram of an alcohol extract of a single, detached bigleaf maple leaf treated with 2,4-DP-1-C<sub>14</sub>. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the R<sub>f</sub> of that point.

Figure 7
Figure 8

The distribution of radioactivity on a paper chromatogram of 2,4,5-TP-1-C\textsuperscript{14} treatment standard. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the $R_f$ of that point.
Figure 9

The distribution of radioactivity on a paper chromatogram of an alcohol extract of a single, detached bigleaf maple leaf treated with 2,4,5-TP-1-C\textsuperscript{14}. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the R\textsubscript{f} of that point.
Figure 10

The distribution of radioactivity on a paper chromatogram of 2,4-DB-1-C\textsubscript{14} treatment standard. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the R\textsubscript{f} of that point.
Figure 11

The distribution of radioactivity on a paper chromatogram of an alcohol extract of a single, detached bigleaf maple leaf treated with 2,4-DB-1-C\textsuperscript{14}. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the R\textsubscript{f} of that point.
Figure 12

The distribution of radioactivity on a paper chromatogram of 2,4-D-1-C¹⁴ treatment standard. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the $R_f$ of that point.
The distribution of radioactivity on a paper chromatogram of an alcohol extract of the treated leaves of an intact bigleaf maple seedling treated with 2,4-D-l-C\textsubscript{14}. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the $R_f$ of that point.
Figure 14

The distribution of radioactivity on a paper chromatogram of an alcohol extract of the untreated leaves of an intact bigleaf maple seedling treated with 2,4-D-1-C\textsuperscript{14}. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the $R_f$ of that point.
Figure 15

The distribution of radioactivity on a paper chromatogram of an alcohol extract of the new growth stem of an intact bigleaf maple seedling treated with 2,4-D-[1-\textsuperscript{14}C]. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the \( R_f \) of that point.
Figure 16

The distribution of radioactivity on a paper chromatogram of an alcohol extract of the old stem of an intact bigleaf maple seedling treated with 2,4-D-1-C\textsuperscript{14}. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the $R_f$ of that point.
Figure 17

The distribution of radioactivity on a paper chromatogram of an alcohol extract of the roots of an intact bigleaf maple seedling treated with 2,4-D-1-C\textsubscript{14}. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the R\textsubscript{f} of that point.
Figure 18

The distribution of radioactivity on a paper chromatogram of 2,4,5-T-1-C14 treatment standard. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the Rf of that point.
Figure 19

The distribution of radioactivity on a paper chromatogram of an alcohol extract of the treated leaves of an intact bigleaf maple seedling treated with 2,4,5-T-1-C\textsuperscript{14}. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the $R_f$ of that point.
Figure 20

The distribution of radioactivity on a paper chromatogram of an alcohol extract of the untreated leaves of an intact bigleaf maple seedling treated with 2,4,5-T-1-C^14. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the R_f of that point.
The distribution of radioactivity on a paper chromatogram of an alcohol extract of the new growth stem of an intact bigleaf maple seedling treated with 2,4,5-T-l-C14. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the Rf of that point.
Figure 22

The distribution of radioactivity on a paper chromatogram of an alcohol extract of the old stem of an intact bigleaf maple seedling treated with 2,4,5-T-l-Cl\(^{14}\). The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the \(R_f\) of that point.
Figure 23

The distribution of radioactivity on a paper chromatogram of an alcohol extract of the roots of an intact bigleaf maple seedling treated with 2,4,5-T-1-C\textsuperscript{14}. The percent of the total radioactivity on the paper chromatogram found at a particular point has been plotted over the R\textsubscript{f} of that point.