PHYSIOLOGICAL ASPECTS OF MOVEMENT AND TOXICITY OF 2,4,5-TRICHLOROPHENOXYACETIC ACID IN BLACKJACK OAK

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TABLE OF CONTENTS

LIST OF APPENDIX TABLES

Table | Page

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The grazing value of a large portion of the rangeland in the Southern Great Plains has been greatly reduced by the invasion of undesirable woody plants. In Oklahoma alone an estimated 10 million acres are in this vegetation type, largely dominated by oak species. Although some of this land is unsuited for the growth of grass, much of it is capable of producing desirable forage grass species.

The use of 2,4,5-T has become an accepted practive for controlling blackjack oak. Aerial application of this chemical has been used widely since its recommendation in the early 1950's, and it continues to give effective economical plant control. However, the results of any given 2,4,5-T treatment may vary greatly. Due to this variability in the results obtained from spray treatments, more information is needed on the action of 2,4,5-T in oak in the hope that this can help increase the dependability of the chemical treatment. The present study was initiated to determine the extent and rate of absorption, translocation, and breakdown of 2,4,5-T in blackjack oak.

CHAPTER II

REVIEW OF LITERATURE

The occurrence of undesirable brush and trees is of considerable economic importance on nearly every type of land, including rangelands, pastures, highways, recreation areas, irrigation canals, and grounds surrounding homes and industrial plants (1).

All kinds of mechanical, biological, and chemical methods have been used to control brush and each has its proper place in the program (64, 65). The method which is preferable at any one site depends on a number of factors including the kind and size of brush, the extent of infestation, the degree of control desired, and the availability of equipment (1, 2).

Today the chemical control methods are of prime concern and are in general use because they offer the greatest promise as to effectiveness and low cost (11, 38, 63). The use of chemicals for control of undesirable plants has expanded most rapidly since about 1946, shortly after the introduction of growth regulating compounds (38, 60, 73).

Literature dealing with research on physiological aspects of the chemical control of woody species is limited (5, 50). The size of trees and shrubs makes it impractical to study them in the greenhouse. The bulkiness of the samples hinders the use of radioisotopes which have greatly facilitated research on the absorption, translocation, and mechanisms of toxic action of herbicides in herbaceous plants (27).

To be systemically effective in a program of brush control, the foliar applied chemicals must first be absorbed by the leaf and then be translocated through the phloem into an active root system in a high enough concentration to bring about the death of the plant (15, 16, 62).

Herbicidal Tracer Techniques

To trace the path and extent of movement of the applied chemical within a plant, different methods have been employed.

Autoradiography, which has probably been the most important technique for obtaining data on translocation of herbicides in woody species (5), is a relatively rapid and simple method. Here, advantage is taken of the fact that ionizing radiations act upon photographic emulsion in the same manner in which light acts. The image so produced is known by such terms as autoradiogram, autoradiograph, autogram, and radioautogram (13). To apply this technique to studies concerning herbicidal movement within a woody plant, radioactive herbicides are usually applied to the leaf. Later at desired intervals, autograms are made of leaves, bark samples, or small branches which are located short distances from the treated spot. The great advantage of the autoradiographic technique is that it may enable the precise distribution of labeled material within a tissue, or even within a single cell, to be established with accuracy (28). Using histoautoradiography, the exposure of a piece of film to a thin tissue section containing a radioisotope, Radwan et al. (54) could show the particular cells involved in translocation of two herbicides in broad bean.

In 1955, Carvel (12) was able to show by use of the autoradiography of small seedlings the pattern of movement of S^{35}-labeled ammate in hickory and privet. From this study he concluded that the chemical moves up into the leaves more rapidly than it moves down into the root system. His results also indicated that downward movement of ammate was through the phloem and upward movement through the xylem. The author relates the variation in effectiveness of ammate applied at different times to the rate of translocation of water in the xylem.

Leonard and Crafts (39) applied urea* and 2,4-D* to the leaves of live oak, toyon, and coyote brush. One week later, they sampled and autographed the bark. From study of the autograms, the authors concluded that 2,4-D* was absorbed slightly better than urea* by all three species, and that urea* moved more freely in live oak than did 2,4-D* indicating some chemical binding of the latter chemical either in the sieve tubes or other cells. Some lateral movement of both herbicides was also noted.

In addition, Leonard and Crafts (39) studied absorption and translocation of 2,4-D* by seven species of brush with the same method mentioned above. They showed that 2,4-D* penetrated into the leaf through the cuticle in the absence of stomata. The autoradiograms indicated that different species require different treatment and that the rate and direction of translocation of 2,4-D in plants may vary considerably with the season of the year. The authors emphasized the deleterious effect of contact injury on the uptake and transport of 2,4-D and the positive correlation between food movement and the transport of this chemical in plants.

Walker et al. (67) traced the movement of 2,4,5-T* in the seedlings of four woody plants. The autoradiograms of bark samples indicated a different pattern of translocation in different species for the same phytocide. They also suggested a higher rate and a greater amount of 2,4,5-T in downward movement than in upward movement.

Autoradiography, although an excellent technique in herbicidal studies, is in most cases of little value as a means of measuring the amount of labeled material in a tissue (27, 28, 74). This method also suffers from the fact

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*The asterisk indicates a carbon-14 labeled compound.

that separation of bark samples from some woody species and their subsequent preparation are difficult (5, 74).

When data is needed on a quantitative basis, the counting method is usually employed. In this technique, the plant treated with radioactive herbicide is cut into parts and the radioactivity of each part is measured separately. One of the earliest attempts to employ this method for herbicidal studies in woody species is that of Byron and Fuller (10). They studied the movement of the radioactive herbicide 2,4-dichloro-5-iodophenoxyacetic acid (2,4-DI^{131}) from treated tops to untreated lower stem, hypocotyl, and root tissue in 8-week-old mesquite seedlings. Results obtained from Geiger-count analyses showed that four days after treatment less than 3% of the applied radioactive material had moved downward. Addition of surfactants did not result in greater total translocation of the herbicide from the treated area but substantially increased the degree of acute local toxicity, probably as a result of increased absorption. The authors relate the erratic performance of 2,4-D in mature velvet mesquite trees to the extremely low mobility of 2,4-DI^{131} as measured in their study.

In 1954, Weintraub et al. (70) studied metabolism and persistance of 2,4-D* in dormant plant tissue. When applied to buds of two-year-old cherry trees, 20% of the chemical did not penetrate the bud at the end of one week regardless of dosage form. They reported further that a small amount of carbon-14 in 2,4-D was transferred to other products.

To obtain quantitative results on translocation of ammate in privet seedlings, Carvel (12) used the counting method in addition to autoradiography. The counting of ashed leaves and roots confirmed the results obtained from the radiograms reported above.

To study the absorption of various formulations of 2,4,5-T by water oak and sweet gum, Walker et al. (66) used a simple counting method. After various intervals, the leaves treated with 2,4,5-T* were removed and washed with 80% ethanol to remove the unabsorbed chemical. The amount of C^{14} in the leaf extract was taken as a measure of absorption. The results indicated that the rate of absorption of some chemicals was much greater than that of other chemicals. The order of superiority of formulations was not the same for the two test plants. While absorption did not increase after one day for water oak, concentrations within sweet gum leaves increased with time.

Morton (49) employed the same technique and showed that the type of carrier is an important factor in the efficiency of an herbicidal treatment. Greater quantities of 2,4,5-T were absorbed by mesquite leaves with the oil-water carrier than with the ethanol-water carrier.

The absorption and translocation of 2,4,5-T by blackjack oak were studied by Dalrymple and Basler (19). Here, 2,4,5-T* was applied to the leaf surface, and at various intervals the treated leaves were removed from the trees. The unabsorbed 2,4,5-T-1-C^{14} was determined by washing these leaves with 80% alcohol and determining the C^{14} in each wash sample. Values for absorption were obtained by subtracting the C^{14} in the leaf wash from that of the total applied. Subtracting the leaf wash C^{14} and the leaf homogenate C^{14} from the total applied gave an estimated measure for translocation. Their results showed a high rate of absorption and translocation in early spring and a sharp decline in both processes during May and June. There was a gradual increase in absorption and translocation during July, August, and September. The translocation rates were not correlated with water stress as measured by the soil moisture level.

Bioassay techniques have also been used to determine the herbicidal translocation in woody plants. These methods have an obvious advantage over those in which a radioactive herbicide is used. Any organic compound when introduced into a plant is subject to possible degradation, and it follows that the presence of C^{14} in plant tissue does not necessarily indicate the intact labeled molecule that was originally applied.

Using slit pea tests, Hay (30) studied the translocation of 2,4,5-T in woody shoots of marabu. When the chemical was applied on to the bark, very little downward movement was detected; however, upward movement was shown to occur. The extent of basal movement of 2,4,5-T was much greater when applied to freshly cut stumps, and the downward movement of the compound occurred primarily in the xylem with subsequent lateral movement to the bark.

In another study, Hay (31) followed the path of foliar applied 2,4-D in marabu by the same method. The tests showed the occurrence of both the upward and downward movement of this chemical in the plant. A rapid translocation, degradation, or both was also indicated. A large portion of the herbicide moved in the mature bark.

Behrens and Morton (9) developed and employed a bioassay technique in which the degree of root inhibition of mesquite seedlings is used as a measure of the presence of 2,4-D and 2,4,5-T in a given tissue. The results showed that 2,4,5-T translocated more readily than 2,4-D in mesquite seedlings. This difference in the movement of 2,4-D and 2,4,5-T could partially explain the better performance of the latter on the mesquite trees.

A bioassay method has been described by Leonard et al. (40). In this technique the suppression of leaf-widths of cotton seedlings following the application of a tissue extract to the cotyledons is considered as indicating the presence of phenoxy herbicides. With this refined method, the authors showed that a concentration as low as 0.3 parts per billion of 2,4-D in the plant material could be detected.

Translocation of Systemic Herbicides in Relation to Transport of Sugars

There are a number of nutritional factors which influence the translocation of phenoxy herbicides in Plants. Rohrbaugh and Rice (58) showed that the level of phosphorus in tomato plants has a marked effect on the translocation of 2,4-D. 2,4-D was not readily translocated by tomato plants which were deficient in phosphorus. In another study, Rice and Rohrbaugh (56) investigated the effect of potassium on the translocation of 2,4-D by tomato plants. Potassium deficient plants translocated considerably less 2,4-D than plants supplied with adequate levels of potassium. Since potassium plays an important role in CO_2 assimilation and carbohydrate accumulation, the authors stipulate that the decrease in rate of translocation of 2,4-D through the tomato plants could be due to a lowering of the carbohydrate supply.

Boron is another element which has proved to have a great influence on the translocation of plant growth regulators. Mitchell et al. (48) found that boron markedly accelerated the translocation of 2,4-DNH_4 in bean plsnts. Boron also increased the translocation of 3-indoleacetic, 2,4,5-trichlorophenoxyacetic, and alpha naphthaleneacetic acids from leaves to the stems of bean plants. The authors believe that this effect of boron is due to its effect in increasing the rate of translocation of sugar.

A number of workers have followed food reserve trends in perennial weeds and have established a positive correlation between the translocation of the growth substances and the transport of the carbohydrates in the so-called assimilate stream of the phloem (41, 47, 69).

Today it is believed that the translocation of chemicals downward primarily parallels the movement of food materials from the leaf to the other parts of the plant (18, 26, 55), a theory first advanced by Weaver and DeRose (68). Therefore, the general metabolic activity of the plant probably plays an important role in distribution of herbicides within the treated plants and consequently in the final results.

Rohrbaugh and Rice (57) demonstrated that there was no translocation of 2,4-D in bean plants if the readily available carbohydrate supply had been diminished by continued darkness. However, when sugar was applied to the leaves, the translocation of the chemical in the decarbohydrated plants did occur.

Using C^{14}-labeled 2,4-D, Jaworski et al. (36) found that the herbicide was absorbed by the treated leaves in the etiolated plants but was not translocated from them. Translocation, however, occurred when sugar solutions were applied to the treated leaves.

Employing a bioassay technique, Hay and Thimann (32) showed that 2,4-D transport did not take place in the darkened bean plants but could be induced by applying sucrrose solution to the leaves of these plants. The sucrose solution did not increase the movement of 2,4-D in the light. Barrier and Loomis (4) reported that the foliar absorption of 2,4-D and P^{32} was not affected by depletion of carbohydrates. Transport from such leaves, however, was reduced or stopped.

Crafts and Yamaguchi (17) demonstrated that C^{14}-labeled 2,4-D, amino triazole, and maleic hydrazide moved readily out of green leaves of tradescantia. The chemicals did not move out of chlorotic leaves of this plant. Comparative tests of these compounds showed that the translocation of 2,4-D was slowed because of its absorption by living cells. Active root growth was essential to the translocation of 2,4-D* from leaves to roots.

Crafts (14) stated,

It seems well established that movement of 2,4-D in plants occurs with food material, and the autographs indicate that it travels with the assimilated stream by some sort of mass flow. No other mechanism can account for the rapidity of movement so often found and the fact that dosage applied has no effect on linear rate of movement.

On the other hand, Hay and Thimann (32) stated, "It is deduced that transport of 2,4-D is not due to simple osmotic forces in the mass flow hypothesis, but involves a metabolic component." In other words, according to them, carbohydrates may also be required as a source of energy.

In contrast to the majority of workers, Penfound and Minyard (53) reported that the greatest bending and destruction, in both diffuse light and in darkness, occurred in starch free plants. Epinasty and necrosis were much greater under shaded conditions than under full sunlight. Therefore they have questioned the idea that these herbicides travel with soluble carbohydrates. Their results are not generally supported by others. The instances in which shaded plants were killed or injured more rapidly indicate that the physiological condition of the plants was such that systemic herbicide was absorbed and translocated; and because of low carbohydrates, it was extremely toxic (20).

Crafts and Yamaguchi (17), in their survey of this debatable problem, hypothesized the movement of systemic herbicides in a common stream of assimilates, moving from the source in green leaves to various sinks in the plants that are maintained by metabolism, growth, and storage.

The practical application of this relationship is to spray the plants when greatest downward movement of assimilates and consequently of herbicides is expected in order to obtain a high percentage of kill. This means that for securing the best results in a program of weed control, phytocides should be applied when the plant is in the stage of active growth with photosynthesis and the movement of food materials at high rates (14). Currier and Dybing (18) explained that when herbicides are loaded or accumulated in plant cells, particularly in parenchyma cells of the veins, the concentration gradient extending to the leaf surface will be effectively increased. The production of photosynthate and stimulation of the movement of the herbicide may steepen the absorption gradient.

For these reasons the majority of investigators have emphasized the importance of the time factor in chemical weed control. It is now generally accepted that the best time for foliar application of chemicals is from the time of budding till early blossoming (15, 26). Although during late blossoming and seed maturation, translocation of herbicides may be rapid, the mature root tissues are not as susceptible to phytocides as young, meristematic, actively growing tissues (11, 15). Also, destruction of the latter tissues is believed to be more detrimental to the plant than destruction of the former ones. In a study of mesquite control, Valentine and Norris (62) reported that tests of spraying dates showed a consistent pattern of kill from year to year. Kill was relatively poor early in the season, then rose rapidly to its peak about the first of June; thereafter, it declined at a slower rate than that of the rise in early season.

The thickening of the cuticle progressively with time should also be mentioned. Young leaves have a thinner cuticle and are, therefore, more penetrable than old ones (18).

Effects of Soil Moisture on the Absorption and Translocation of Systemic Herbicides

There are a number of environmental factors which influence the performance of an herbicide on a plant. Fisher et al. (26) correlated the effectiveness of 2,4,5-T on mesquite plants with soil moisture conditions. They reported that under drought conditions the accumulation of 2,4,5-T in the crown area was not sufficient to prevent sprouting. Under conditions of adequate moisture, however, excellent control of mesquite was obtained. The authors believed this differential behavior to be due to a more efficient translocation of toxic amounts of 2,4,5-T with adequate moisture as compared to the amounts translocated under drought conditions.

Pallas (51) showed that the soil moisture level causes marked effects on translocation of phenoxy herbicides in plant tissue. The results of his experiment indicate that with approaching permanent wilting point, translocation of 2,4-D* in red kidney bean plants decreased to approximately one half that found near field capacity. He found that a direct relationship existed between soil moisture percent and the amount of 2,4-D translocated to the epicotyl. Soil moisture did not have any effects on the absorption of the herbicide. Hauser (29) reported, however, that 2,4-D was absorbed more slowly by soybean and corn plants grown continuously with decreased soil mositure as compared with those plants supplied with adequate water.

Basler et al. (8) showed that translocation of the foliar applied 2,4-D* in bean plants may be significantly affected by moisture stress as measured by the turgidity of leaf tissue. When turgidity of leaf cells decreased to below 80 percent, only trace amounts of 2,4-D were translocated. There was a gradual decrease in 2,4-D translocation with a decrease in soil moisture level.

In summing up the effect of moisture on the herbicidal behavior, Miller and Starr (46) stated that herbicidal effectiveness is dependent upon absorption and translocation of the chemical and that the mechanism of absorption and translocation are to some degree regulated by moisture regime.

Metabolism of Herbicides

Resistance and susceptibility of different plants to phenoxy herbicides have in some instances been attributed to the rate of metabolism of the compounds by the plants (59). The use of C^{14}-labeled herbicides has confirmed the fact that the rate and nature of the metabolic processes undergone by a compound may vary greatly from plant to plant.

In the process of degradation of the phenoxy herbicides, it seems that the side chain is first metabolized leaving the ring unattacked (3). Splitting off of the side chain will in general lead to its oxidation (33). This probably accounts for the results of several workers who have demonstrated that when 2,4-D with C^{14} in the side chain is given to plants the C^{14} comes off in CO_2 (23, 35, 43) or is converted to numerous compounds other than 2,4-D (33, 71).

Holley et al. (35) and Holley (34) used carboxyl-C^{14}-labeled 2,4-D and reported that CO_2* was produced by bean plants treated with 2,4-D*. In addition to 2,4-D* they found two radioactive, water-soluble, ether-insoluble organic acids in the plant homogenate. Jaworski and Butts (37) used both carboxyl and methylene-C^{14}-labeled 2,4-D and found that after treatment with 2,4-D two major radioactive compounds in addition to unchanged 2,4-D* were present in an 80% alcohol extract of the stems of bean plants. One of the major compounds was found to be a complex containing 2,4-D. Over-all, half of the isolated radioactive material was not 2,4-D*.

Weintraub et al. (72) reported experiments in which CO_2* was produced by bean plants treated with 2,4-D containing C^{14} in either the carboxyl or the methylene position. No CO_2* evolved from the position-1 carbon of the ring. In another paper, Weintraub et al. (71) reported that C^{14} in either the carboxyl or the methylene position was incorporated into other substances within a few days after application of 2,4-D*. The radioactive carbon was found in a variety of plant constituents including acids, sugars, dextrin, starch, proteins, and cell wall substances.

To determine the effect of metabolism of 2,4-D and 2,4,5-T on the degree of susceptibility of various plants to them, Luckwill and Lloyd-Jones (43, 44) traced the degradation of these compounds in detached leaves of different species. The resistant species of currant, apple, and strawberry decarboxylated 2,4-D and 2,4,5-T rapidly, whereas in the susceptible species the rate of CO_2* evolution was much slower. The extensive conversion of 2,4-D to a breakdown product in resistant species of red maple, yellow poplar and sweet gum to this compound is reported by Pallas (52). Similarly, Morton (50) found that as much as 80% of the 2,4,5-T absorbed by mesquite leaves is altered within 24 hours.

While Basler (6) showed that the rates of decarboxylation of phenoxyacetic acid herbicides were not extensive in blackjack oak, Basler et al. (7) showed that the breakdown of the same compounds was extensive in this species. The rates of breakdown were found to be quite variable during the growing season. The authors believe that this variability could in some cases be responsible for variability in control measures of blackjack oak utilizing these herbicides.

Slife et al. (59) showed that the resistance of wild cucumber to 2,4-D and its susceptibility to 2,4,5-T could be due to the differences in the extent of degradation of these two compounds. The authors reported that considerably more 2,4-D* was converted within 24 hours into two major metabolites which were not very mobile in the plant. The absorbed 2,4,5-T formed only traces of these metabolites even after eight days.

CHAPTER III

MATERIALS AND METHODS

The test plants were blackjack oaks (Quercus marilandica Muenchh.) growing either as mature trees in their natural habitat or as seedlings in the green house. The studies reported here consist of 1) seasonal variation in absorption and translocation of 2,4,5-T, 2) effectiveness of 2,4,5-T as influenced by time of spraying, 3) absorption and translocation of 2,4,5-T as affected by dosage, soil moisture, and plant sugar content, and 4) metabolism of 2,4,5-T.

The experimental plan was a randomized complete-block design where one leaf per plant was treated and a randomized complete-block design with equal subsample numbers where two leaves per plant were treated. Analyses of variance of the data were made according to methods outlined by Steel and Torrie(61) with significant differences calculated by use of Duncan's new multiple range. All the data were processed at 5% level of confidence.

Seasonal Variation in Absorption and Translocation of 2,4,5-T

This study was initiated in May, 1963 and continued for two growing seasons, terminating in September, 1964. Six blackjack oaks, each about ten feet tall, were selected in a pasture located just south of Lake Carl Blackwell approximately 10 miles west of Stillwater. The absorption and translocation of 2,4,5-T-1-C^{14} were studied by tracing the movement of C^{14} in the leaves and stems of these trees. Each treatment consisted of application of radioactive 2,4,5-T on the surfaces of two leaves located near the tip of a branch for each of the six oaks.

The radioactive 2,4,5-T used during the summer of 1963 was a potassium salt of the herbicide labeled with C^{14} in the carboxyl position with a specific activity of 4.16 mc/mmole. Each leaf application was of 50 μg of 2,4,5-T (acid basis), equivalent to 0.82 μc, in water solution with 0.5% sterox, a non-ionic surfactant with the formulation: polyoxyethylene thioether. On the second treatment date two herbicidal preparations were used, the one just described plus a second with a WEEDONE 2,4,5-T base replacing the sterox. This was an exploratory attempt for deciding which preparation would result in the higher absorption and translocation of 2,4,5-T. For the 1964 growing season a butoxy ethyl ester of 2,4,5-T-1-C^{14} with a specific activity of 14.1 mc/mmole was used. Applying this compound as an emulsion, each leaf received approximately 25 μg of 2,4,5-T, equivalent to 1.39 μc. The emulsion was prepared by mixing 1 mg 2,4,5-T butoxy ethyl ester (acid basis), 1.5 μl WEEDONE 2,4,5-T base, and 200 μl water.

At the time of treatment 10 μl of 2,4,5-T* were placed on the upper surface of each leaf to the left of the midrib. The herbicide was confined within a 5 mm diameter ring of stopcock grease on the leaf surface. The two treated leaves on each branch were usually the fourth and fifth from the apex. Treatments were made at about 2-week intervals from May 11 until August 11 for 1963, and 4-week intervals from May 15 until September 24 for 1964. Since there has been some indication that 2,4,5-T is somewhat less effective in control of the regrowth of blackjack oak than the original tree itself, on the second treatment date in the summer of 1964 six regrowth tips were given the same treatments as the six original trees. All treatments were made around 9 AM.

Twenty-four hours after treatment the branch tips bearing the two treated leaves, the untreated leaves above these two, and as many as three to four leaves located basipetally from the treated leaves were severed and taken to the laboratory for analyses. They were then subjected to two radioassay procedures, counting and autoradiography as described below.

1. Counting.

The treated areas were punched out with a 9 mm cork borer, and the unabosrbed 2,4,5-T* was washed from the surface into a test tube with 25 ml of 80% ethanol. Absorption values were obtained by subtracting the leaf wash C^{14} from the total C^{14} originally applied onto the leaf surface. The radioactivity measurements were made by a liquid scintillation counter. The C^{14} content of each disk was determined by the following methods. 1) The disks were submerged in 10 ml of absolute alcohol for three months afterwhich an aliquot of the alcohol extract was counted in a gas-flow Geiger counting assembly. 2) The disks were homogenized in 10 ml of 80% ethanol in a hand operated glass grinder. A 0.2 ml aliquot of the homogenate was analyzed for C^{14} by a liquid scintillation unit.

The first method was used in 1963 and the second in 1964. Values for translocation out of the treated area were obtained by subtracting the disk homogenate C^{14} from the total absorbed C^{14}.

In 1964, the remainders of the treated leaves were also analyzed for their C^{14} content partly by liquid scintillation and partly by wet combustion of the leaf tissue and trapping of the evolving C^*O_2 in an ionization chamber and its subsequent measurement with a Nuclear-Chicago Dynacon Electrometer. Values for translocation out of the leaves were estimated by subtracting the disk homogenate C^{14} and the leaf homogenate C^{14} from the total C^{14} absorbed.

2. Autoradiography.

Two bark samples were withdrawn from each branch tip, one from below and one from above the treated leaves. After lyophilizing the bark samples for two to three days, they were dampened and left overnight in a press to flatten. The samples were then mounted on a heavy paper, covered with Saran-Wrap, and placed in contact with Royal Blue X-ray medical film for a two-month exposure period. The exposed films were developed and processed by standard procedures. In 1964, autograms were also made of two leaves, one immediately below and one immediately above the treated leaves. The autoradiographic procedure was similar to that of the bark samples.

Effectiveness of 2,4,5-T as Influenced by Time of Spraying

The purpose of this study was to find out whether or not there is any relationship between the level of root food reserves, translocation of 2,4,5-T, and effectiveness of the herbicide in the chemical control of blackjack oak. The experimental trees were located in the same area as those of the previous experiment. This study lasted one growing season, and treatments were made at monthly intervals from May 11, 1963 until September 20, 1963. Each treatment consisted of spraying three blackjack oak trees with 2,4,5-T butoxy ethyl ester at three pounds per acre. The chemical was emulsified in water and applied at a total spray volume of 100 gallons per acre. The percentage kill was determined a year after the last treatment. Triplicate samples from roots in the crown bud area were taken at the time of treatment for carbohydrate analysis and three days after spraying for 2,4,5-T bioassay on cotton seedlings.

In the carbohydrate analysis the root samples were first cut to small pieces and then lyophilized for three days. The dried tissue was ground and passed through a 60-mesh sieve. A 100 mg sample of this powder was extracted and its free sugars were determined colorimetrically according to the methods of Dubois et al. (22).

For the 2,4,5-T bioassay, the root samples were powdered as for the carbohydrate analysis. 200 gm of each sample were first extracted with ethanol and then subjected to frequent purifications as described by Leonard et al. (40). The purified extract was taken up in 5 ml of 95% ethanol containing 0.5% sterox and used as stock solution.

Twelve-day-old cotton seedlings were treated in triplicate with 10, 20, 40, and 80 µl aliquots of stock solution, with one half of the solution being placed on each cotton cotyledon. The average widths of the first and second leaves were determined 20 days after treatment and compared with those of standard plants treated with 2,4,5-T solutions of known concentrations. The suppression of seedling leaf-width was considered to be an indication of the presence of 2,4,5-T. The bioassay for 2,4,5-T was considered negative if no strapped-shaped or cupped leaves developed on the cotton seedlings.

Absorption and Translocation of 2,4,5-T as Affected by Dosage, Soil Moisture, and Plant Sugar Content

This study was undertaken to measure the effect of soil moisture, herbicidal dosage, and presence of sugar on the movement of 2,4,5-T in blackjack oak. The experimental plants were three-month-old blackjack oak seedlings. The seedlings were germinated in vermiculite and then transferred to sandy loam soil for further growth in a greenhouse.

Thirty uniform seedlings comprised of three groups of ten were used for the dosage study. The third leaf from the growing point of each seedling was treated with 10 µl of an emulsion of a 2,4,5-T* butoxy ethyl ester having a specific activity of 14.1 mc/mmole. Each plant in group 1, group 2, and group

3 received 10 μg, 25 μg, and 50 μg respectively. Twenty-four hours later the treated leaves were severed and radioassayed. The absorption and translocation were determined by the counting method using liquid scintillation described in the first experiment.

In studying the effect of soil moisture on absorption and translocation of 2,4,5-T, 24 uniform seedlings were selected and divided into three groups of eight each. To develop a soil moisture differential, the first group received irrigation once every three days, the second once every other day, and the third once every day. After three moisture regime cycles, all the seedlings were transferred one day before treatment to a growth chamber providing a light period of 14 hours and a dark period of 10 hours. The temperature and relative humidity during the light period were 90°F and 45% and during the dark period 65°F and 80% respectively.

The time of treatment was so selected that no additional irrigation would be necessary after the application of the herbicide without changing the irrigating intervals. The 2,4,5-T* was applied on two leaves—the second and third from the growing tip of each seedling. Each leaf received approximately 20 μg of 2,4,5-T* with a specific activity of 14.1 mc/mmole. Soil samples were taken five hours after treatment with a 5/8" x 2" sampling tube and dried at 105°C. The moisture content was determined on a dry weight basis. Twenty-four hours after the treatments the treated leaves were radioassayed by the same method as that of the dosage study.

For studying the effect of application of sugar on the translocation of 2,4,5-T by blackjack oak, 27 seedlings were transferred to complete darkness prior to the herbicidal application. This was done to deplete the seedlings of their native sugars.

The seedlings were divided into three groups. About one half hour before the application of 2,4,5-T*, the tips of the leaves of the first group were removed and the cut ends dipped in a 10% solution of sucrose. The leaves of the second and third groups were left intact. Eighteen hours after the beginning of the dark treatment, each seedling was treated with 10 μl from the same stock solution as was used in the above moisture study. During the treatment low intensity illumination was obtained by the use of a low intensity red light covered with a sheet of blue cellophane.

Following the 2,4,5-T treatment, the first and second groups were left in the dark for 24 more hours and the third group was transferred to a growth chamber with a light intensity of 2,500 foot candles. The temperature of the growth chamber was adjusted to that of the dark room with a constant temperature of 86°F.

The radioassay of the treated leaves which followed after 24 hours from the time of herbicidal application was the same as that for the two previous experiments. In addition, from each seedling, a 16 mm section of stem located immediately below the treated leaves was removed with a microtome, homogenized, and its activity determined with a liquid scintillation counter.

Metabolism of 2,4,5-T in Blackjack Oak

In this experiment the variation in the extent of breakdown of 2,4,5-T with time and the rate of 2,4,5-T breakdown were studied. Four mature blackjack oaks growing in the pasture described in the first experiment were selected. In studying the variation in the extent of 2,4,5-T breakdown, leaf samples were collected from the four trees at two to four week intervals from early spring until near the end of the growing season in 1963. At each sampling date, one sun-leaf located near the end of a branch was removed from each tree and immediately placed in a beaker of tap water. The leaves collected at any given date were assumed to be of the same age since blackjack oak in this locality characteristically initiates only one set of leaves during the growing season.

In the laboratory each leaf was treated through its transpiration stream with 2,4,5-T* having a specific activity of 4.18 mc/mmole. Half a milliter of a 2×10^{-4} M herbicidal solution was placed in a small vial with a constricted mouth to decrease evaporation. The petiole and lower part of each leaf were trimmed to fit the vial which contained the herbicide. After placing the leaves in the vials, they were kept illuminated with 200 foot candles of incandescent light, and air was blown over them to increase transpiration and thereby increase the rate of herbicide uptake. The leaves were allowed to take up the solution until one of the leaves had absorbed nearly all of the herbicide. The petioles were then placed in small beakers of tap water and left until 24 hours had elapsed from the beginning of treatment.

Then each leaf was removed from the tap water and its petiole severed and discarded. The blade portion of the leaf was lyophilized for two days and then either stored at $-20^{\circ}C$ or immediately homogenized for analysis.

Homogenization of the leaf was accomplished by grinding it for three minutes in 25 ml of cold distilled water in a high speed Vitris homogenizer. The homogenate was acidified with 5 ml of ice cold perchloric acid to make a final concentration of 0.2 N and then centrifuged at 12,000 x g for 30 minutes to remove the coarse material. The supernatant solution was extracted two times in a separatory funnel with two volumes of reagent ethyl ether. This resulted in an acidic aqueous fraction and an ether soluble fraction.

The ether fraction was backwashed with a few milliters of distilled water and then evaporated to dryness. The residue was dissolved in 1 ml of 95% ethanol and stored at $0^{\circ}C$ until being analyzed by chromatography.

The water fraction was adjusted to pH 7 with KOH, centrifuged at 12,000 x g to remove $KClO_4$, and evaporated to near dryness. The brownish residue was washed with 35 ml of 95% ethanol, centrifuged, evaporated to dryness, and taken up in 1 ml of 95% ethanol.

A 0.2 ml aliquot of each sample was spotted on Whatman No. 1 filter paper for chromatography. In order to identify 2,4,5-T, the extracts were co-chromatographed with a standard solution of 2,4,5-T. The paper was developed by ascending chromatography in an isopropanol-water-15.3 N ammonium hydroxide solvent (8:1:1 v:v:v) for 16 hours. After having been dried, the chromatograms were scanned by the use of a Picker gas-flow scanner and recorder set at a constant speed of 12 inches per hour. The instrument provided a direct 1:1 ratio between the chromatograms and the recorder chart. R_f values were determined from the peaks produced by the recorder, and the relative amounts of radioactivity were calculated by measuring the areas under the peaks. The activity under each peak was then converted to a percent of the total activity found in the water soluble and ether soluble components of the leaf homogenate.

In an experiment in which the rate of the breakdown of 2,4,5-T was under study, three leaves were removed from each of the four trees on June 11, 1964. The leaves were subjected to the same treatment and analyses mentioned above except that one set of the leaves was left in tap water until the end of 24 hours, the second set until the end of 48 hours, and the third set until the end of 72 hours from the beginning of treatment.

CHAPTER IV

RESULTS AND DISCUSSION

Seasonal Variation in Absorption and Translocation of 2,4,5-T

The 1963 data for the absorption of 2,4,5-T-1-C^{14} and its subsequent translocation out of the treated area in blackjack oak are shown in Fig. 1. The data for translocation are much higher than the actual values since the method employed for extraction of radioactivity out of the treated areas was an ineffective one. As previously explained, the extraction technique for the 1963 study consisted of submerging the leaf disks in absolute alcohol for three months. This method proved to be completely inadequate and was subsequently abandoned. The translocation data presented in Fig. 1 has, therefore, little bearing on the true picture of translocation and is reported here only to show the total research performed. Appendix Table II gives the numerical values and the statistical analysis for this part of the study.

The absorption data show considerable variation during the growing season. At the 5% level of probability, absorption values for May and the early part of June were significantly higher than those for the rest of the treatment dates (Appendix Table I). Statistical analysis failed to detect any significant differences in the absorption data for the latter part of the season due to great differences in absorption values from tree to tree and leaf to leaf. Such random variation in absorption has also been encountered by other workers (33, 45). Holley et al. (33) observed considerable variation in the absorption of 2,4-D by individual bean plants. They report that the amount of 2,4-D ab-

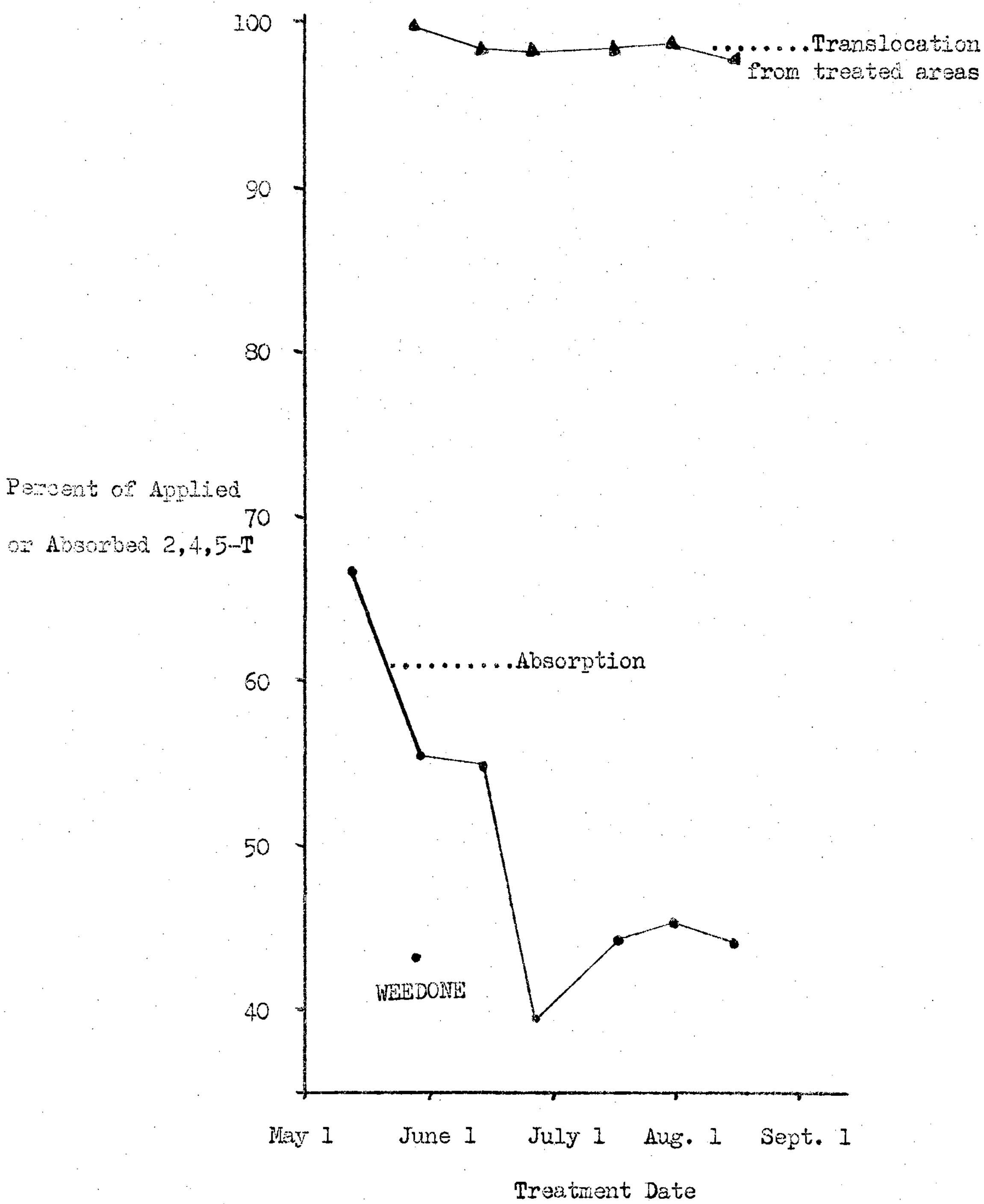

Figure 1. Absorption and Translocation of 2,4,5-T* by Blackjack Oak During the 1963 Growing Season. Absorption and Translocation Values are Presented as a Percent of the Total 2,4,5-T* Applied and Absorbed Respectively.

sorbed varied from 25 to 60% even though the application of the chemical was the same and the major environmental factors were closely controlled.

The WEEDONE preparation decreased the absorption by more than 10% as shown in Fig. 1. The use of the WEEDONE base in the formulation was discontinued after the exploratory trial on the second treatment date in May.

The 1964 data for absorption and translocation of 2,4,5-T-1-C^{14} are presented in Fig. 2. Here variations among the absorption values for most of the growing season are less pronounced than those of 1963. The only significant difference detected was between the last treatment and the rest of the treatments (Appendix Table III).

Both the 1963 and 1964 series of data indicate a fairly high rate of absorption for the early part of the growing season and a low rate for the end of the season. This trend is usually expected and might be explained by an increase in the thickness of the cuticle layer.

Data showing the trend of translocation out of treated leaf and treated area are also given in Fig. 2. Translocation was fairly low at the beginning of the study in May and then increased till late in June. After this, translocation showed a declining trend which was followed by an increasing trend lasting until near the end of the growing season. The analysis of data for the treated area (Appendix Table IV) shows that the treatment dates brought about significant differences in the translocation values. For the leaf data, however, no significant differences were detected (Appendix Table V). This is believed to be due to a leak in the combustion apparatus which was used in the counting method for the leaves treated in the early part of the season as described in the materials and methods section. This failure caused a change in the method and much variation in the data obtained (coefficient of variation 35.6).

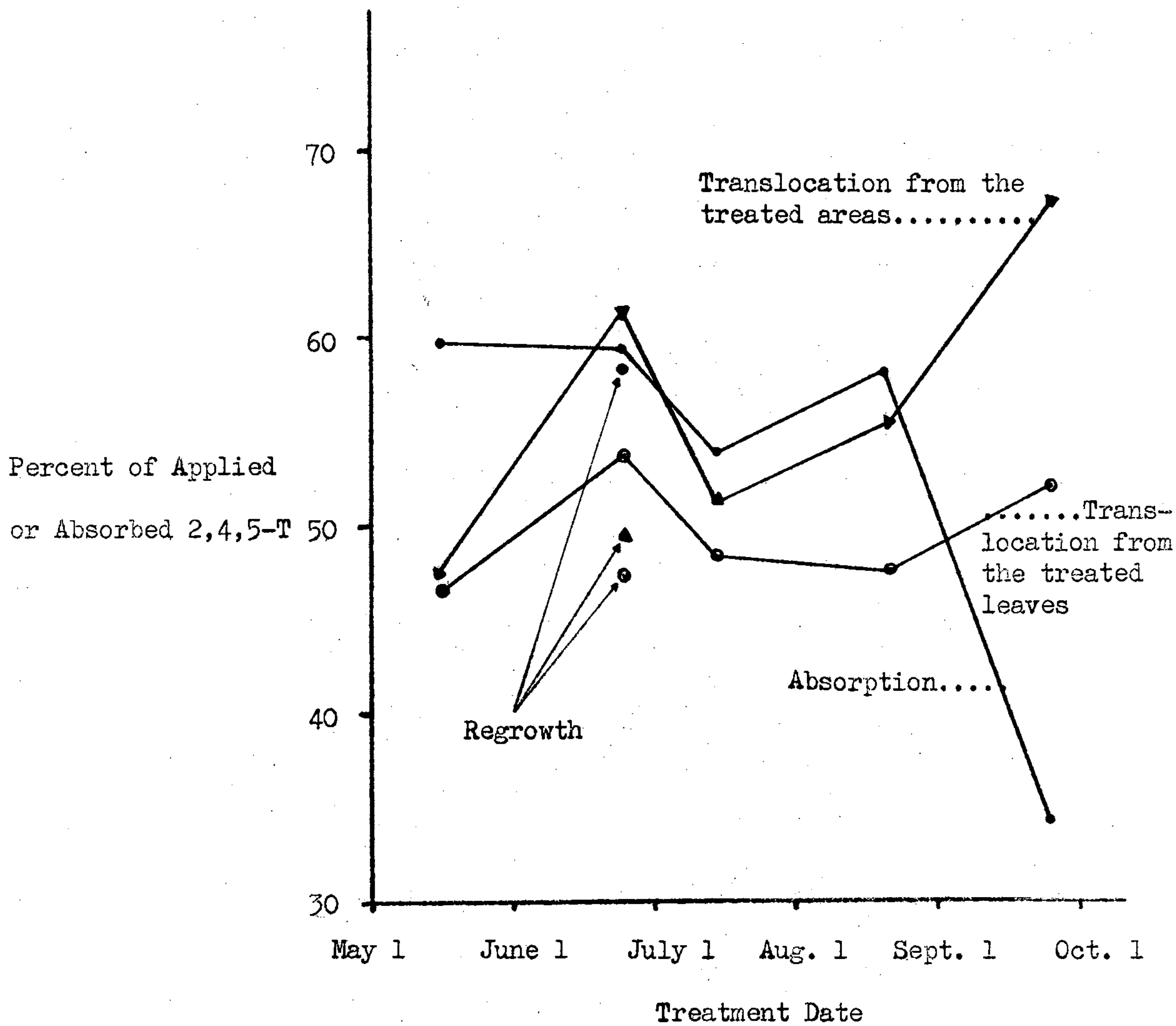

Figure 2. Absorption and Translocation of 2,4,5-T* by Blackjack Oak During the 1964 Growing Season. Absorption and Translocation Values are Presented as a Percent of the Total 2,4,5-T* Applied and Absorbed Respectively.

The data suggest that over-all about half of the absorbed herbicide was trapped by the tissues of the treated area throughout the growing season. The rest of the leaf tissue, however, did not exert much binding effect and transported about 90% of the herbicide exported from the treated area. In other words, the translocation values and trend for the entire leaf were close to and parallel to those for the treated area. To avoid confusion, it should be reemphasized that translocation out of the treated area and leaf is presented as a percent of the total herbicide absorbed into the leaf.

The data for absorption and translocation of 2,4,5-T by blackjack oak regrowth are shown in Fig. 2. The lower values for translocation in regrowth than in original trees might be one reason for the higher resistance of regrowth to 2,4,5-T than the original trees to the herbicide (25).

Fig. 3 and Fig. 4 show the autoradiograms of the 1963 and 1964 bark samples respectively. The 1963 autoradiograms indicate a low rate of translocation for the beginning as well as the end of the growing season. Translocation is highest for the months of June and July. Elwell (24) has reported that blackjack oak could be controlled more effectively by 2,4,5-T if the chemical is applied from May 15 to July 15. Treatments made either early in the spring or mid-summer were not effective. The translocation pattern shown by 1963 bark samples roughly corresponds with Elwell's results. Working with several woody species, Leonard and Crafts (39) showed that the rate of translocation of an herbicide may be a determining factor in the effectiveness of a chemical control program. The variability in effectiveness of 2,4,5-T might well be due to its translocation trend in blackjack oak.

The radiograms of the 1964 bark samples show that June treatment gave a higher and more uniform rate of translocation than other treatments. The August treatment gave the next highest translocation rate. The intensities of

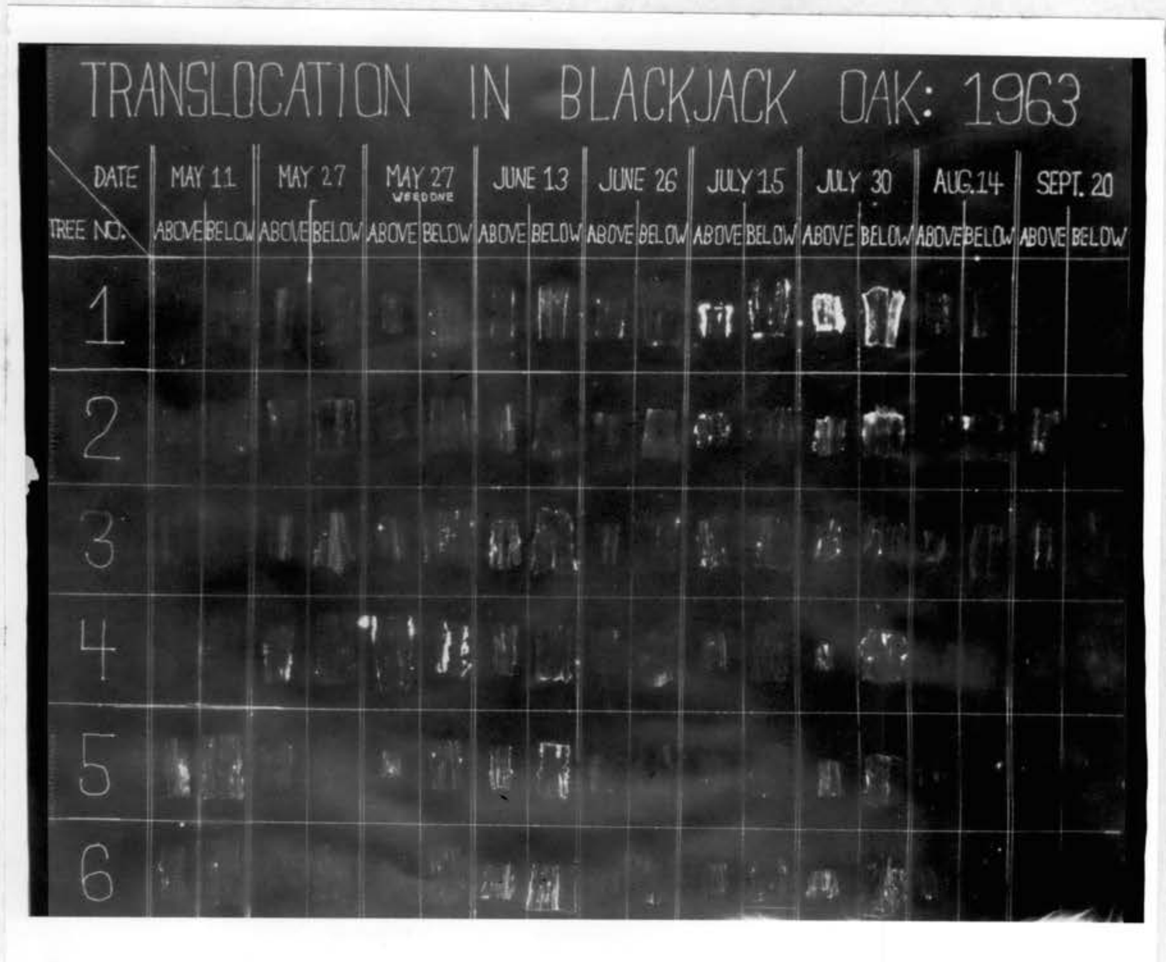

Figure 3. Radioautograph of the Bark Samples from Blackjack Oak Trees Treated with 2,4,5-T* Throughout the 1963 Growing Season. Two Bark Samples were Removed from each Branch, One from Below and One From Above the Treated Leaves. Dosage was 50 μg, Treatment Period 24 Hours.

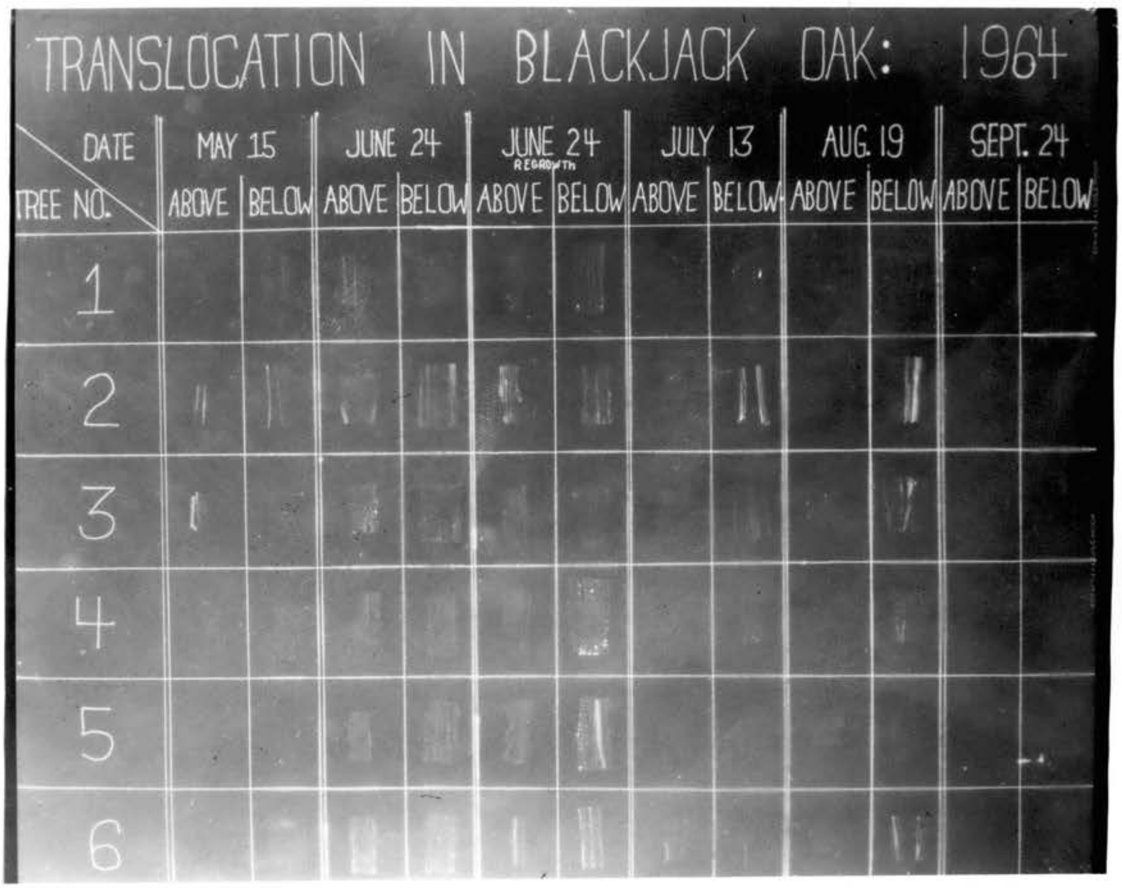

Figure 4. Radioautograph of the Bark Samples from Blackjack Oak Trees Treated with 2,4,5-T* Throughout the 1964 Growing Season. Two Bark Samples were Removed from Each Branch, One from Below and One from Above the Treated Leaves. Dosage was 25 μg, Treatment Period 24 Hours.

the autographs for June bark samples from regrowth and original trees seem to be the same. This would indicate a similar translocation rate for both groups of test plants. The counting method, however, detected lower translocation values for regrowth plants than for original trees, as mentioned above. That the original trees translocated more 2,4,5-T* could also be observed from the leaf autoradiograms (Fig. 5).

All the autographs indicate a bidirectional transport of 2,4,5-T in blackjack oak, that is, the 2,4,5-T movement in blackjack oak, when it occurs, is both upward and downward.

Effectiveness of 2,4,5-T as Influenced by Time of Spraying

Data showing the amounts of free sugars and 2,4,5-T in the root samples and the percentage kill of blackjack oak are given in Fig. 6. As is shown, the free sugars in the roots were most plentiful during mid-summer and the least amounts occurred in June. This cycle indicates that the food reserves are utilized early in the season and replenished later in the summer.

The data for 2,4,5-T translocation represent the quantities of this chemical extracted from pulverized root samples as determined by the bioassay method on cotton seedlings. The data suggest that the transport of 2,4,5-T into the root tissues was greatest in May and least in August. This trend seems somewhat different from those reported in the previous experiment. The discrepency could be partly attributed to differences in the methods employed. In the present experiment, the extent of 2,4,5-T translocation into the root tissue was measured by determining the degree of suppression of leaf-widths of cotton seedlings. Although the bioassay used in this experiment is a sensitive one, it is the author's belief that this criterion for quantitative measurement is not very dependable. The extract of August root samples caused definite formative ef-

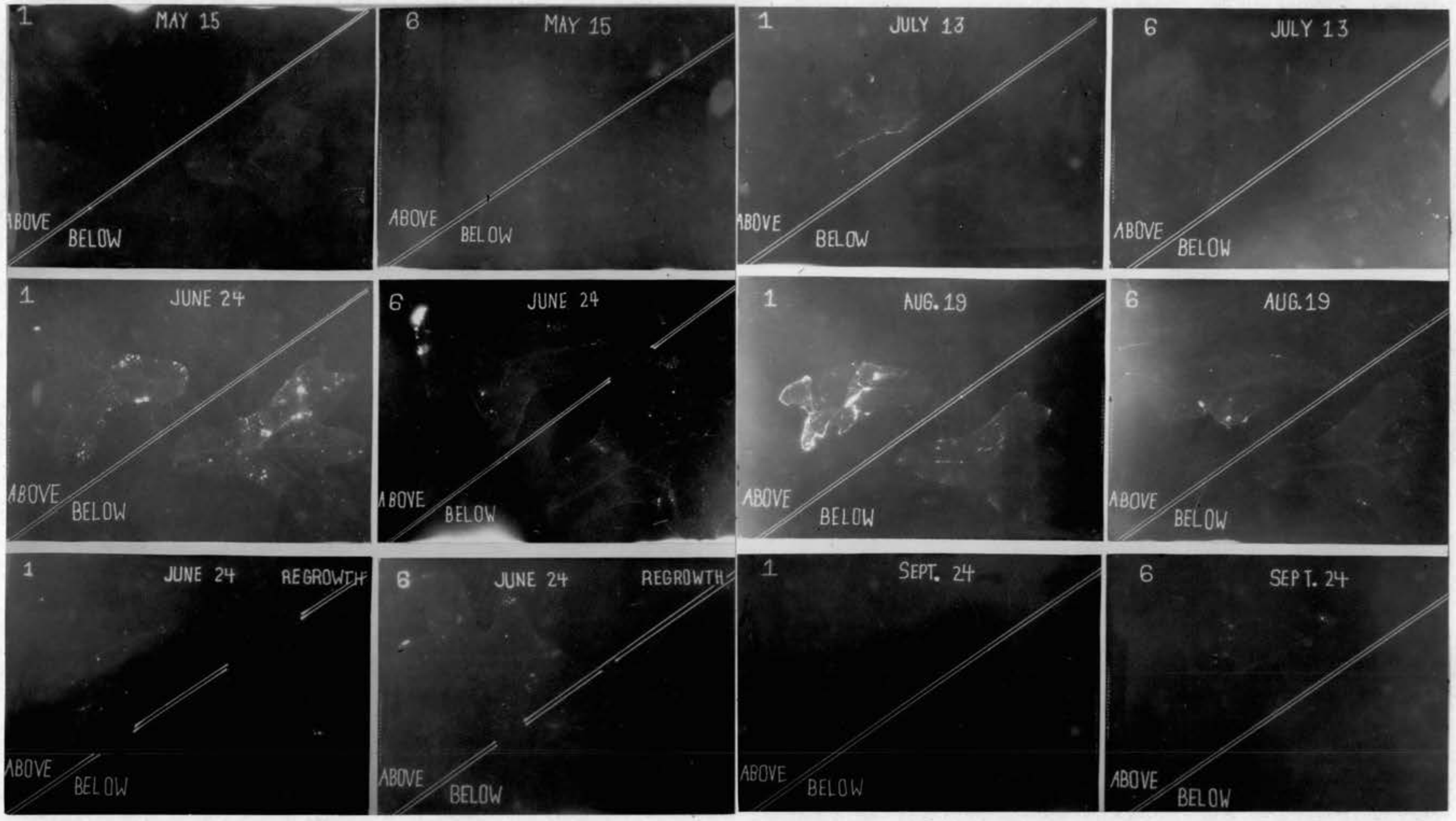

Figure 5. Radioautograph of the Leaves from Blackjack Oak Trees Treated with 2,4,5-T* Throughout the 1964 Growing Season. Two Leaves were Removed from Each Branch, One Immediately Below and One Immediately Above the Treated Leaves.

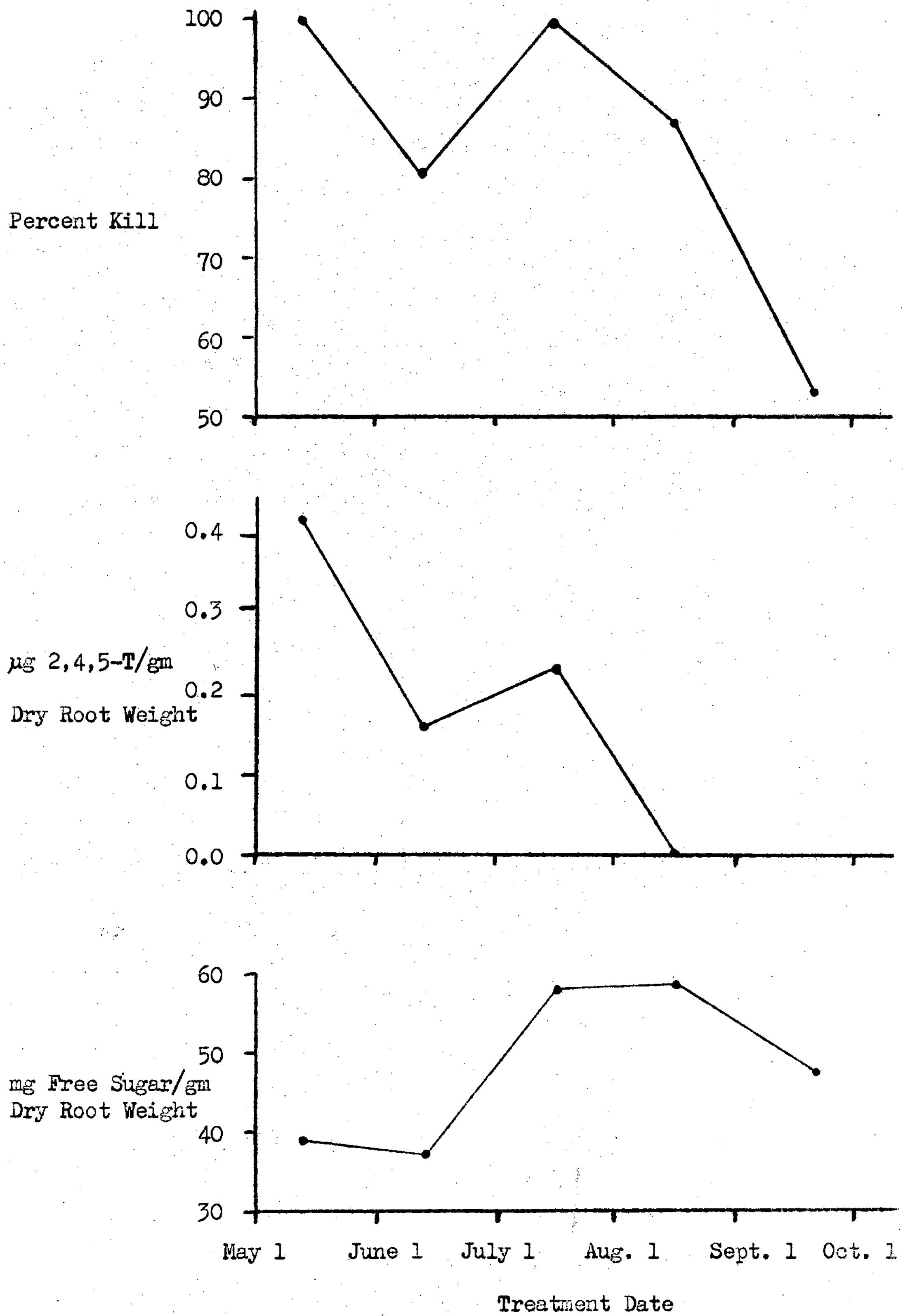

Figure 6. Relationship Among Root Food Reserves, Translocation of 2,4,5-T, and Percent Kill in Blackjack Oak.

fects on the leaves of cotton seedlings, but leaf widths were not suppressed or decreased to below those of the check plants. Therefore, a zero value is reported for 2,4,5-T translocation in August despite the fact that visible signs proved the presence of 2,4,5-T in the extract. Leonard et al. (40), who developed this bioassay, have themselves apparently encountered the same problem. They reported that "the response varied with cotton seedlings, so the quantity of 2,4-D necessary to produce a given response was variable."

The trend in percentage kill follows that of translocation which in turn is somewhat similar to that of root food reserves. The trends, at least for the most part, suggest that downward translocation of 2,4,5-T in blackjack oak takes place coincidentally with the movement of assimilates and that the herbicidal application is most effective when large amounts of assimilates are moving downward.

Absorption and Translocation of 2,4,5-T as Affected by Dosage, Soil Moisture, and Plant Sugar Content

1. Dosage Study.

The absorption and translocation data for this study are graphically presented in Fig. 7. The percentage absorption linearly decreased with an increase of 2,4,5-T from 10 μg to 50 μg. As the data show, the decrease in absorption was more than 20% of the applied 2,4,5-T*. Statistical analysis of the absorption values showed that the percent of absorbed 2,4,5-T at a rate of 10 μg per leaf was significantly higher than that of 25 μg per leaf which in turn was significantly higher than that of 50 μg per leaf (Appendix Table VI).

Analyses of variance of the translocation data did not show any significant differences between the rates of 10 μg per leaf and 25 μg per leaf. At

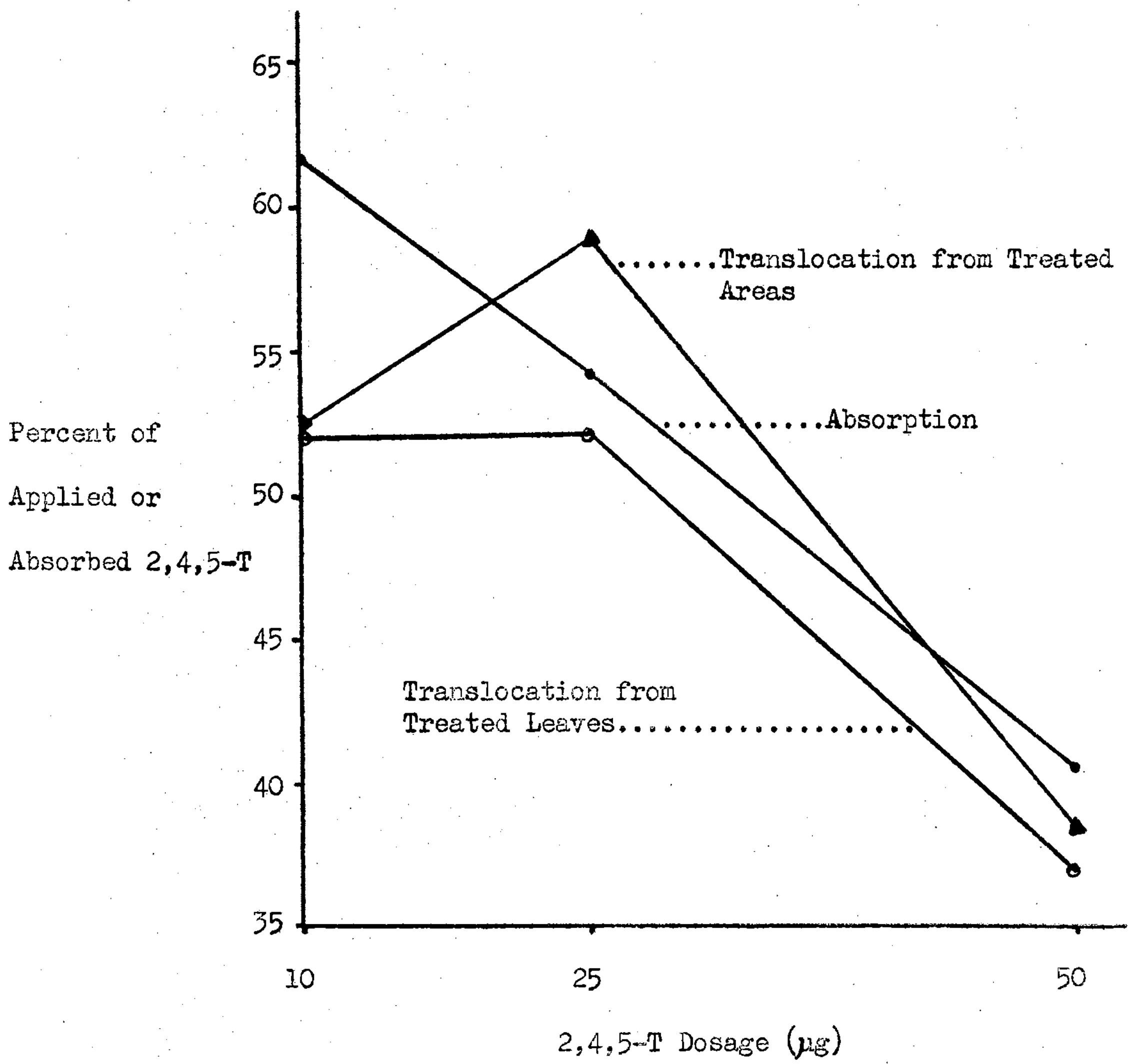

Figure 7. Effect of Herbicidal Dosage on Absorption and Translocation of 2,4,5-T* by Blackjack Oak Seedlings. Absorption and Translocation Values are Presented as a Percent of the Total 2,4,5-T* Applied and Absorbed Respectively.

the rate of 50 μg per leaf, however, translocation values for leaf and treated area were significantly lower than those for 10 and 25 μg per leaf (Appendix Tables VII and VIII).

The marked decrease in absorption and translocation of 2,4,5-T caused by an increase in dosage from 25 to 50 μg is most probably caused by the contact effect of 2,4,5-T on the young leaves.

Applying different doses of 2,4-D to bean seedlings, Hay and Thimann (32) noted that there was a decrease in the amount of 2,4-D transported into the epicotyl and hypocotyl when the dosage was increased beyond a limit. The authors believe this to be due to the effect of 2,4-D in damaging the transport mechanism. They showed evidence that when 25 μg or more were applied, the amount of 2,4-D destroyed was essentially constant, and they argued that the system or systems which lead to destruction, or other modes of disappearance of 2,4-D, were saturated at this concentration.

In the present study the 25 μg dosage not only showed a higher translocation in percent of 2,4,5-T* applied but also in the absolute amounts of 2,4,5-T transported out of the treated leaves (4.2 μg 2,4,5-T transported for 25 μg applied versus 3.0 μg transported for 50 μg applied). Therefore, in all the subsequent experiments, a dosage of 20-25 μg per leaf was used.

2. Soil Moisture Study.

Fig. 8 depicts the absorption and translocation data for this experiment. The soil moisture content for each seedling is given in Appendix Table IX. Figures 2.8, 7.5, and 16 recorded for the abscissa in Fig. 8 refer to the average soil moisture content for the plants in group 1, 2, and 3 respectively.

Statistical analyses of the data showed significant differences in absorption and translocation values brought about by the three different soil moisture levels (Appendix Tables X, XI, and XII). The data suggest that an increase

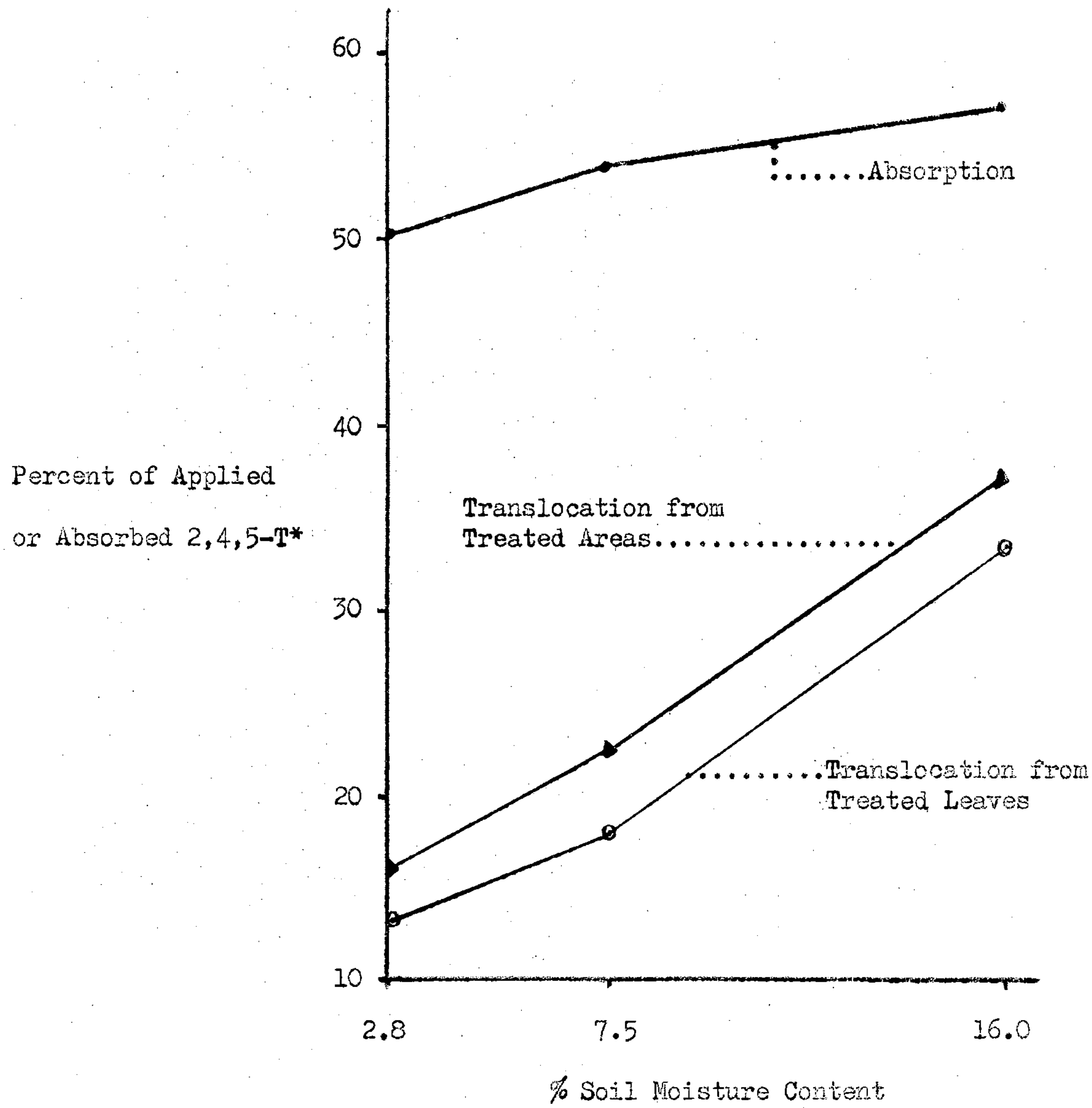

Figure 8. Effect of Soil Moisture Content on Absorption and Translocation of 2,4,5-T* by Blackjack Oak Seedlings. Absorption and Translocation Values are Presented as a Percent of the Total 2,4,5-T* Applied and Absorbed Respectively.

in soil moisture level from 2.8% to 16% has enhanced the rate of translocation considerably more than that of absorption. The decrease in absorbing power of a plant with increasing dryness, according to Loomis (42), could be due to a reduction in growth, accumulation of sugars, and the thickening of the cuticle. To these could be added a lowered general physiological activity of the plant.

The translocation data point out the extreme importance of the plant's internal water status in herbicidal transport. The almost linear relationship between the soil moisture and 2,4,5-T translocation in blackjack oaks obtained in this study supports the experimental results of Pallas (51) who reported a direct relationship between soil moisture percent and the amount of 2,4-D translocated to the epicotyl of bean plants.

The results of the present experiment agree with the stipulation made by Basler et al. (8) that the reported poor kill of blackjack oak during the summer months may be due to a moisture stress within the plant.

3. Study on Plant Sugar Content.

The results on the absorption and subsequent translocation of 2,4,5-T by blackjack oak seedlings are shown in Fig. 9. The data show directly that the depletion of the plant's sugars had a retarding effect on the rate of absorption and translocation of the herbicide. This adverse effect could to some extent be overcome by the addition of sucrose to carbohydrate depleted leaves.

Statistical analyses of the data (Appendix Tables XIII and XVI) did not prove the differences between the absorption values and stem activities to be significant. Differences between the translocation values for dark and dark plus sucrose treatments were also shown to be statistically insignificant. Light treatment, however, caused a significant increase in translocation when compared with that of the dark treatment.

As shown in Fig. 9, there was a consistant increase in stem activity and

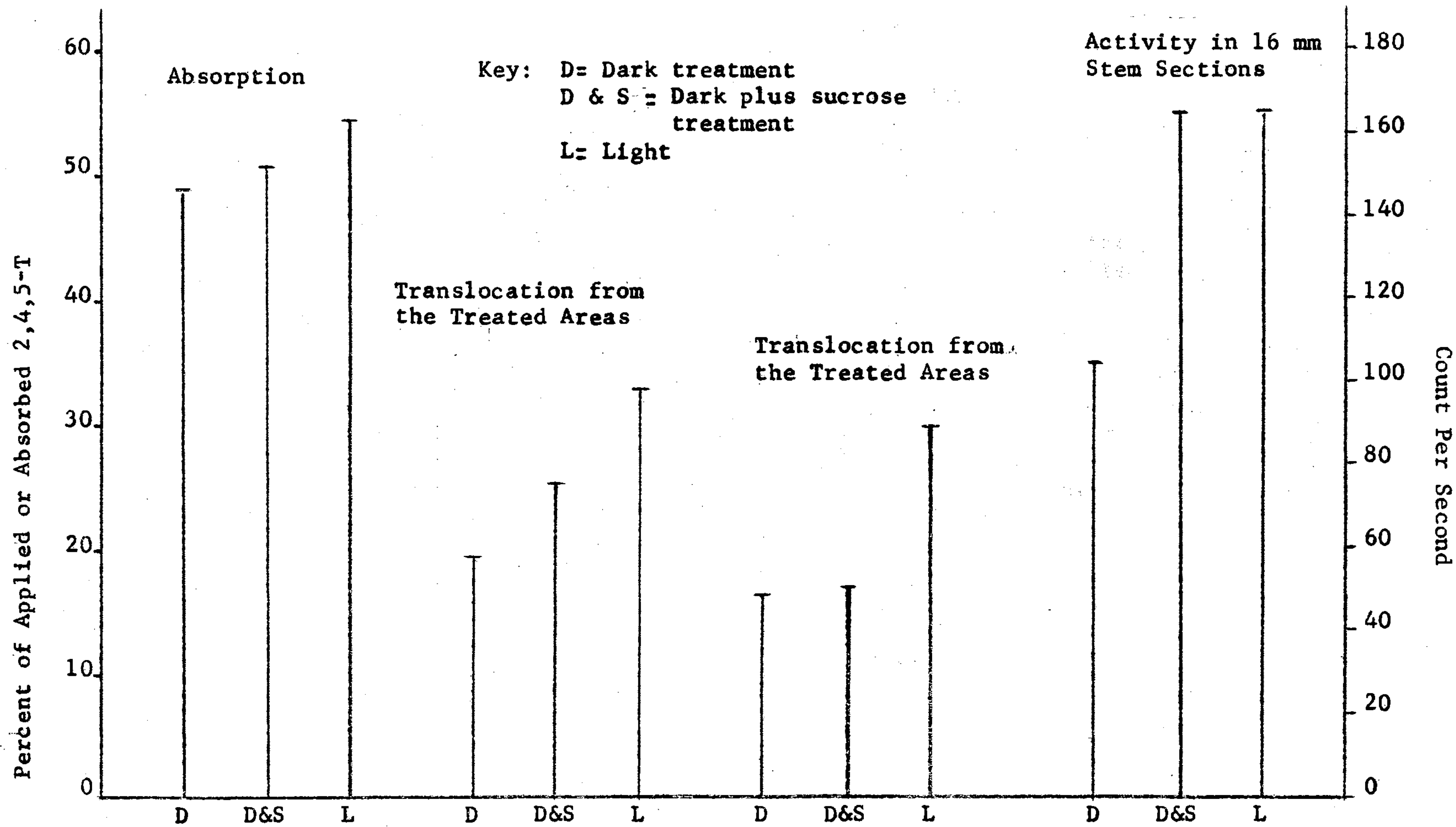

Figure 9. The Effect of Plant Sugar Content on Absorption and Translocation of 2,4,5-T* by Blackjack Oak Seedlings. Absorption and Translocation Values are Presented as a Percent of the Total 2,4,5-T* Applied and Absorbed Respectively.

2,4,5-T* entry and movement in the case of dark plus sucrose treated plants over those of the dark treated plants. This pattern could not be ignored although statistical measures prove it insignificant due to high variations existing among the individual values. The promoting effect of the application of sugars on the movement of the foliar applied compounds in the destarched plants has been demonstrated by many workers (36, 47, 57). Barrier and Loomis (4) reported that although the foliar absorption of 2,4-D and P^{32} was not affected by depletion of carbohydrates, transport from such leaves was greatly reduced or stopped. As in the case of the present experiment, sucrose treatment increased the translocation but not to the level of light treatment.

Light enhances penetration and translocation by stimulating opening of stomata and supporting photosynthesis (18). The effect of sugars on translocation might indicate mass flow in the translocation of herbicides, swept along in the transport pathway of sugars (21). The role of sugars might also be that of providing energy for an active transport mechanism. The greater efficiency of translocation in the presence of light as obtained in this experiment could be due to the fact that light is not only necessary for the production of sugars but also for the generation of energy necessary for active transport.

Metabolism of 2,4,5-T in Blackjack Oak

A graphic presentation of 2,4,5-T breakdown in excised leaves of blackjack oak during a 24 hour period is given in Fig. 10. The data illustrate that blackjack oak converts 2,4,5-T into three major chromatographically different breakdown products throughout the growing season. Toward the end of the season some additional products were also detected (Appendix Table XVII). The values for R_f and percent radioactivity listed in the table are the averages for the four samples of each treatment date. Since purified 2,4,5-T is ether

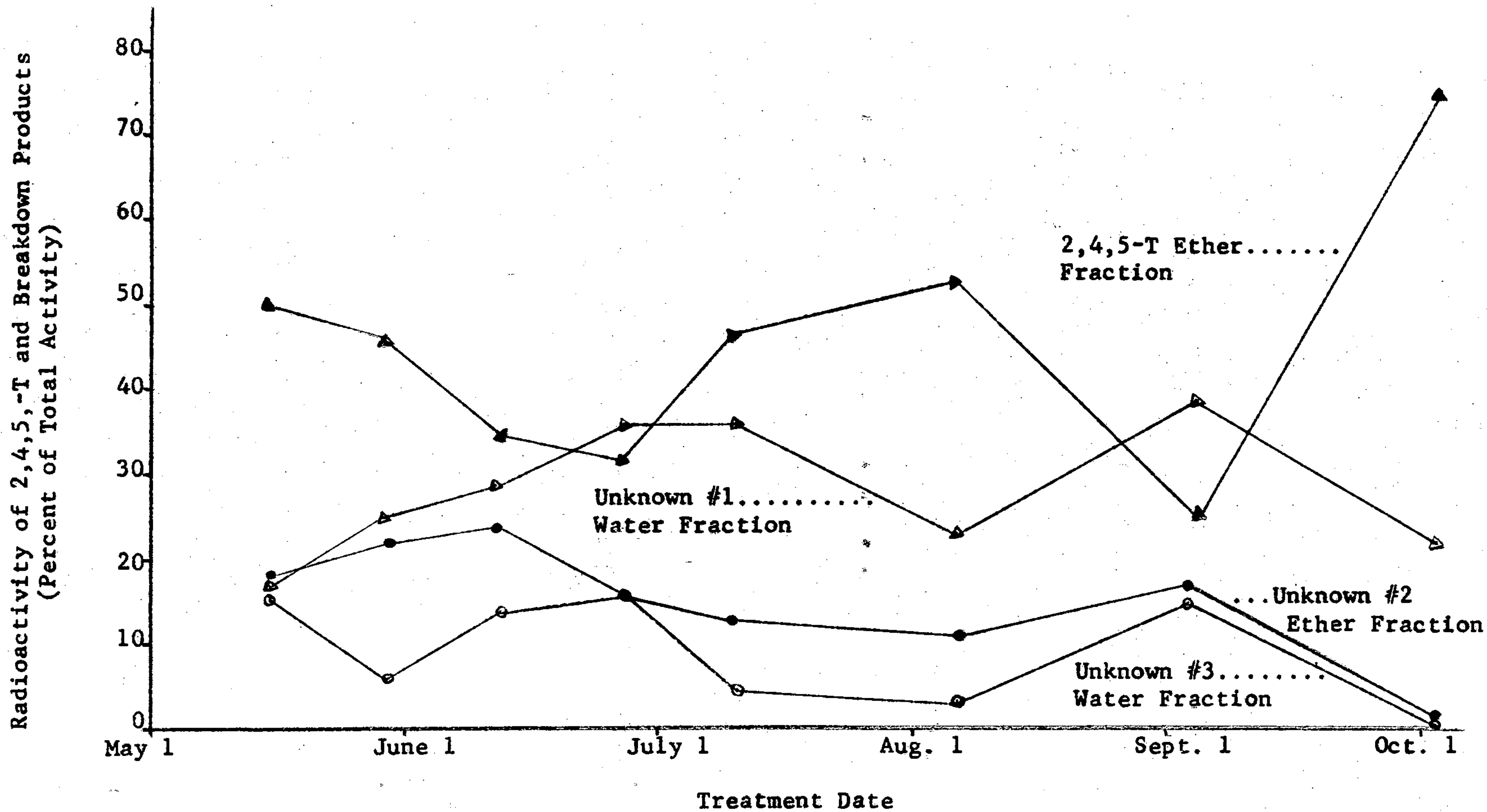

Figure 10. Seasonal variation in the metabolism of 2,4,5,-T by blackjack oak leaves

soluble and was found to have an R_f value of 0.85 when co-chromatographed with leaf extracts, the ether soluble component with an R_f value of 0.84 was considered to be the unaltered 2,4,5-T.

Of the three major breakdown products two were water soluble, designated as unknowns number one and three, and one was ether soluble designated as unknown number two. Unknown number one was found in greater quantities than the other two and showed a definite relationship with unaltered 2,4,5-T. The trend of variation in the production of this unknown compound was found to be reverse of that of unaltered 2,4,5-T throughout the growing season. This kind of relationship between phenoxy herbicides and an unknown detoxification product has been reported by a number of workers. Jaworski and Butts (37) reported that a decrease in concentration of a 2,4-D metabolic product in bean plants was accompanied by a corresponding increase in the concentration of 2,4-D. Morton (50) noticed an increase in the amount of unaltered 2,4,5-T after 72 hours as compared with that of after 24 hours of treatment in mesquite seedlings. He believes this to be partly due to a release of 2,4,5-T from unknown compounds.

The data show a rather extensive degradation of 2,4,5-T in blackjack oak leaves. 2,4,5-T was broken down at a rate of about 50% to 75% during a 24 hour period on all the treatment dates except October. The low rate of breakdown of herbicide late in the season was verified by a subsequent experiment in October, 1964. The percent unchanged 2,4,5-T after a 24 hour metabolism period was 68%. This lowered rate of alteration could be due to a decrease in the plant's metabolic activity this late in the season.

In Fig. 11 the results of the present experiment are compared with those of the experiment on the effectiveness of 2,4,5-T as influenced by time of spraying. The data presented here indicate that differential translocation and metabolism are important mechanisms of resistance to 2,4,5-T in blackjack oak.

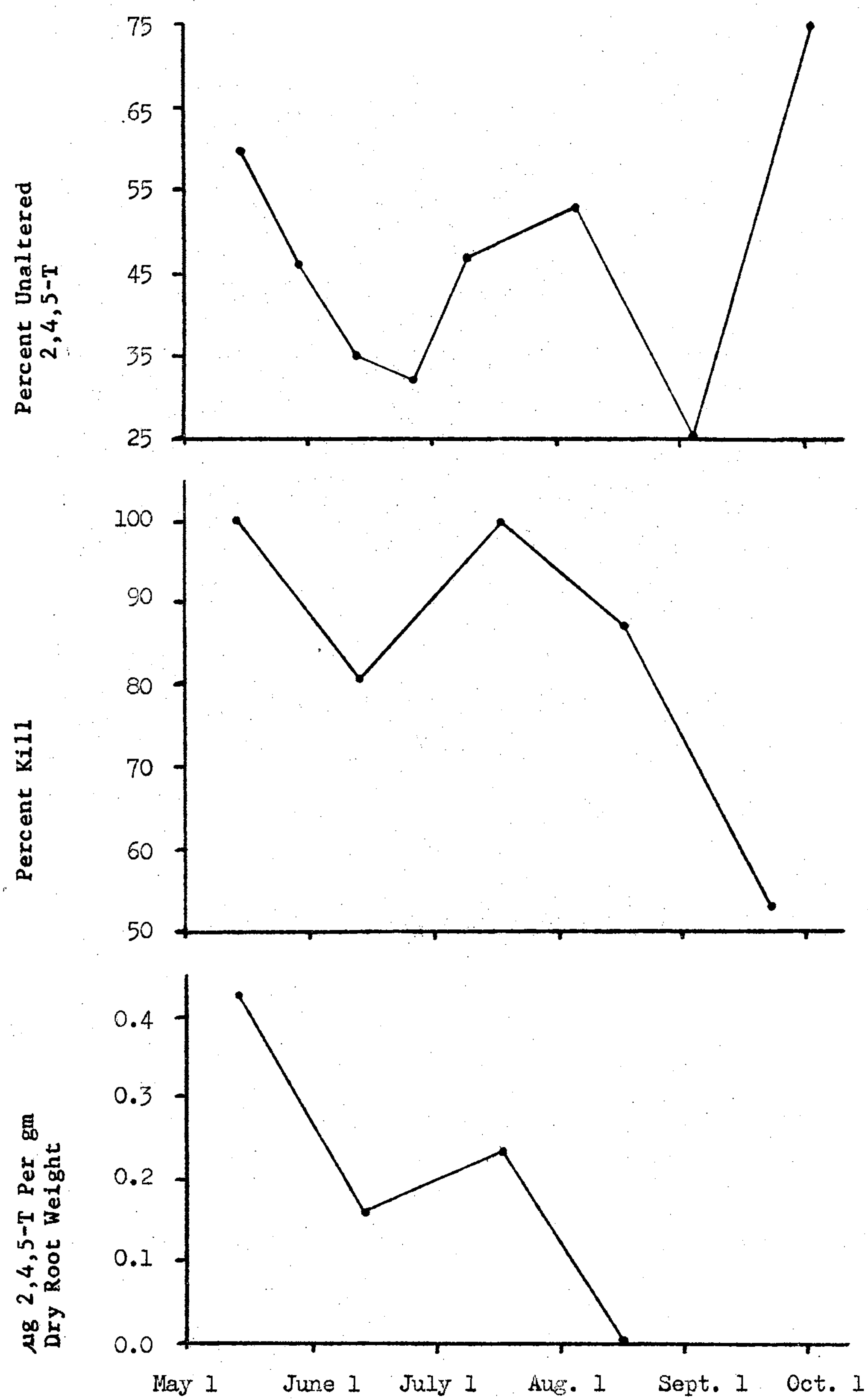

Figure 11. Relationship Among 2,4,5-T Translocation, Metabolism, and Percent Kill of Blackjack Oak.

A low rate of absorption or a low rate of translocation or a high rate of metabolism could either individually or in combination account for a low percent kill of blackjack oak by 2,4,5-T. Fig. 11 suggests that the maximum kill could be obtained when both translocation rate and percent unaltered 2,4,5-T are fairly high.

The results of the study on the rate of 2,4,5-T breakdown in blackjack oak leaves are shown in Fig. 12. About 40% of the 2,4,5-T was broken down within 24 hours, but no further loss occurred during the following 48 hours. It should be mentioned that the rate of 2,4,5-T degradation could have been much higher than indicated by the results. That is, it is entirely possible that the herbicidal breakdown reached its maximum value sometime sooner than 24 hours. It should also be mentioned that this rate of 2,4,5-T breakdown (40% in the first 24 hours) was obtained from blackjack oak leaves at a single treatment date. As was presented above, the seasonal breakdown during a 24 hour period was as high as 75% and as low as 25%. The seasonal average 2,4,5-T breakdown was 55% during 24 hours.

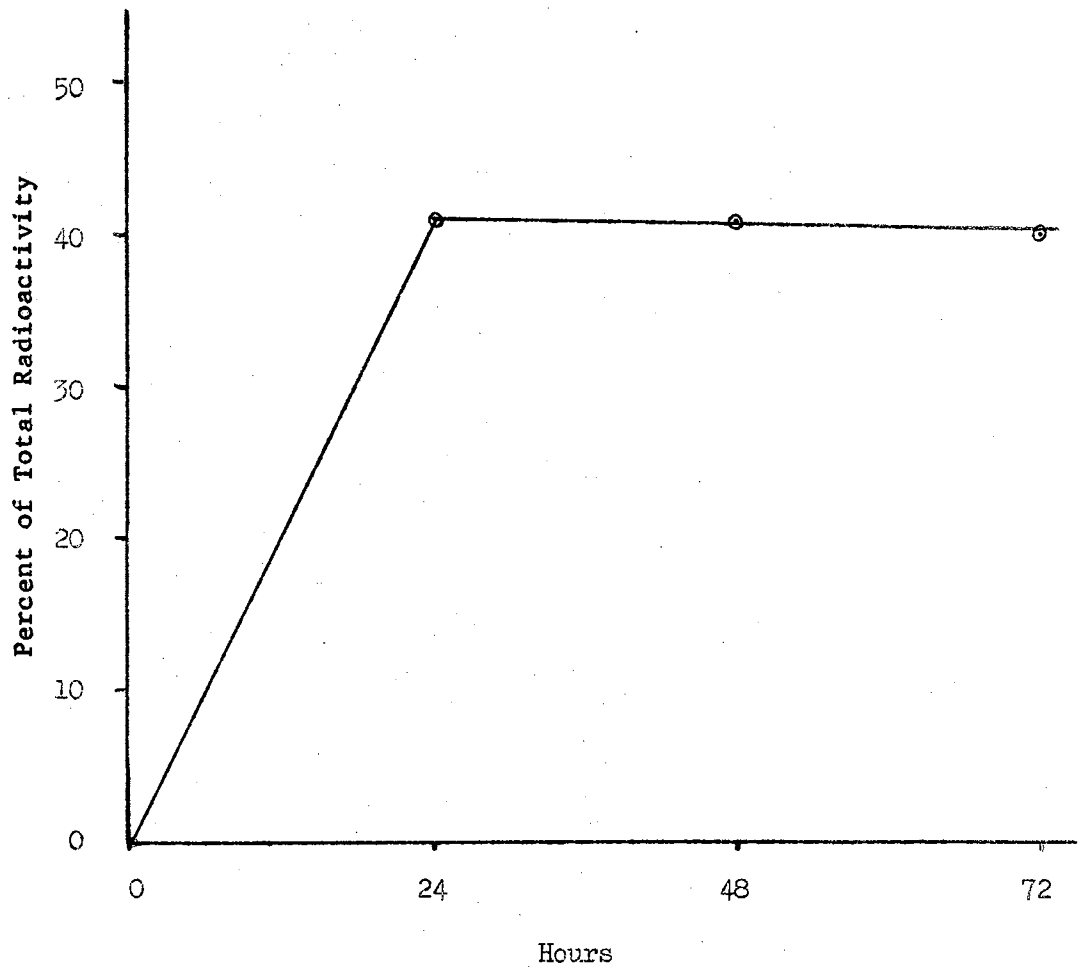

Figure 12. The Rate of 2,4,5-T Breakdown in Excised Leaves of Blackjack Oak.

CHAPTER V

SUMMARY AND CONCLUSIONS

An attempt was made to determine why 2,4,5-T gives variable control of blackjack oak in the field. In the first experiment C^{14}-labeled 2,4,5-T, applied in the form of droplets on the leaves, was used to determine the absorption and translocation of 2,4,5-T in blackjack oak trees during the 1963 and 1964 growing seasons. Radioassays were made with autoradiography and a counting method 24 hours after treatment. Absorption, expressed as a percent of the total applied 2,4,5-T-1-C^{14}, was relatively high early in the season and decreased to a minimum of about 35% in late June of 1963 and late September of 1964. Translocation from the treated area and the entire leaf, when expressed as a percent of the total 2,4,5-T-1-C^{14} absorbed into the leaf, increased to a maximum in June and was followed by a decrease in mid-summer. There was an increase in translocation near the end of the growing season. Translocation was lower in resprouting blackjack oaks than in the original trees while the absorption was about the same in both groups.

In the second experiment, blackjack oak trees were sprayed with 2,4,5-T at monthly intervals during the 1963 growing season. Root samples were taken both at the time of treatment for carbohydrate analysis and three days after spraying for 2,4,5-T bioassay on cotton seedlings. The percentage of kill was determined a year after the last treatment. Variation in 2,4,5-T accumulation in root tissue tended to parallel that of free sugars in the root. The trend in percentage kill followed that of 2,4,5-T translocation. The data suggest that downward transport of 2,4,5-T in blackjack oak corresponds with the movement of assimilates and that the herbicidal application is most effective when large amounts of assimilates are moving downward.

The third experiment concerned effects of dosage, soil moisture, and sugar content of blackjack oak seedlings on the absorption and translocation of 2,4,-5-T-1-C^{14}. The uptake and movement of the herbicide was studied by a counting method. The dosage effect was studied by treating the seedlings with 10 μg, 25 μg, and 50 μg of labeled 2,4,5-T. Absorption percentage was highest for plants treated with 10 μg and lowest for plants treated with 50 μg. The 25 μg dosage gave the highest translocation percentage followed by the 10 μg then the 50 μg dosage.

The effect of soil moisture on herbicidal movement was studied at three soil moisture levels: 2.8%, 7.5%, and 16%. The results indicate an adverse effect of low soil moisture content on the absorption and translocation of 2,4,5-T in blackjack oak seedlings. Both absorption and translocation increased as the soil moisture content increased from 2.8% to 7.5% to 16%. This increase was more pronounced for translocation than for absorption.

For studying the effect of plant sugar content on the uptake and subsequent movement of 2,4,5-T, blackjack oak seedlings were kept in the dark for 18 hours prior to the herbicidal application. Following the treatment the first and second groups were left in the dark and the third group was transferred to the light. At the same time, the first group was treated with sucrose by dipping the tips of the treated leaves into a 10% sucrose solution. Absorption and translocation percentages were consistantly higher in plants treated with light. There was a marked decrease in uptake and transport of 2,4,5-T by the dark treated seedlings. Sucrose treatment increased both absorption and translocation values but not to the level of light treatment.

In the fourth experiment the extent and rate of 2,4,5-T metabolism in detached blackjack oak leaves were investigated. At various treatment dates during the 1963 growing season, 2,4,5-T-1-C^{14} was introduced into the excised leaves through their transpiration streams. Paper chromatography was used to assay the homogenates of the treated leaves.

2,4,5-T metabolism by the leaves varied considerably throughout the growing season. The seasonal average of 2,4,5-T breakdown was about 55% during a 24 hour period. Three major breakdown products were observed throughout the the growing season. The study on the rate of 2,4,5-T breakdown showed that about 40% of the compound was broken down in the first 24 hour period and no further loss occurred during the following 48 hours.

The over-all results of the study reported here indicate that variation in absorption and translocation coupled with that in metabolism of 2,4,5-T could account for variable field control of blackjack oak trees by this herbicide.

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APPENDIX

TABLE I

1963 ABSORPTION DATA AS PERCENT OF THE TOTAL APPLIED 2,4,5-T* AT 24 HOURS AFTER FOLIAR APPLICATION TO BLACKJACK OAK

Date	May 11		May 27		June 13		June 26		July 15		July 30		Aug. 14	
Tree #	Leaf 1	2	Leaf 1	2	Leaf 1	2	Leaf 1	2	Leaf 1	2	Leaf 1	2	Leaf 1	2
1	73.4	69.3	62.9	38.9	51.4	59.1	41.9	40.3	43.1	44.4	46.0	40.3	45.6	50.2
2	65.8	67.2	46.6	63.6	66.4	65.8	55.0	39.6	47.0	56.8	50.6	57.6	38.1	39.4
3	66.0	67.2	49.7	52.7	63.2	67.6	37.5	40.8	52.2	39.5	51.8	49.4	45.6	46.1
4	62.5	64.7	64.4	58.7	39.6	23.9	43.7	29.4	32.8	46.6	36.3	36.8	48.7	45.7
5	65.3	69.6	65.1	47.7	50.4	53.5	34.4	33.5	50.5	38.9	43.1	43.6	49.0	36.4
6	66.5	64.7	50.4	67.3	58.1	62.1	43.3	34.6	33.9	46.1	37.5	40.3	43.8	40.4
Ave.	66.8 a**		55.7 b		55.1 b		39.4 e		44.2 cde		45.3 cd		44.1 cde	

**Any two averages followed by the same letter are not significantly different at the 5% level of probability.

ANALYSIS OF VARIANCE OF THE ABOVE DATA

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F
Treatments	6	6,532.36	1088.7	14.2*
Trees	5	775.81	155.2	
Experimental Error	30	2,306.96	76.9	
Subsampling Error	42	1,753.33	41.7	
Total	83	11,368.46		
CV = 17.5				

*The required F at the 5% level of probability is 2.42.

TABLE II

THE 1963 DATA FOR TRANSLOCATION FROM THE TREATED AREA AS PERCENT OF THE TOTAL 2,4,5-T* ABSORBED AT 24 HOURS AFTER FOLIAR APPLICATION TO BLACKJACK OAK

Date	May 27		June 13		June 26		July 15		July 30		Aug. 14	
Tree #	Leaf 1	2	Leaf 1	2	Leaf 1	2	Leaf 1	2	Leaf 1	2	Leaf 1	2
1	99.9	99.7	99.2	94.5	98.3	95.3	99.3	99.3	96.5	97.0	99.0	98.3
2	99.4	99.9	99.6	99.6	99.2	98.1	94.5	99.3	98.4	99.7	98.6	97.2
3	99.6	98.2	99.4	99.2	98.9	97.8	96.6	98.8	99.5	99.9	97.3	98.8
4	99.7	99.8	99.3	90.8	98.3	96.1	99.0	92.8	98.2	98.8	97.3	98.6
5	99.9	99.8	97.8	99.1	99.1	98.7	99.6	99.4	98.6	99.1	98.1	93.6
6	99.8	99.3	99.4	99.4	98.7	98.3	99.8	99.5	98.5	98.0	98.1	98.0
Ave.	99.6		98.1		98.0		98.2		98.5		97.7	

ANALYSIS OF VARIANCE OF THE ABOVE DATA

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F
Treatments	5	26.47	5.3	2.03*
Trees	5	18.74	3.7	
Experimental Error	25	66.17	2.6	
Subsampling Error	36	105.38	2.9	
Total	71	216.76		
CV = 1.64				

*The required F at the 5% level of probability is 2.60.

TABLE III

1964 ABSORPTION DATA AS PERCENT OF THE TOTAL APPLIED 2,4,5-T* AT 24 HOURS AFTER FOLIAR APPLICATION TO BLACKJACK OAK

Date	May 15		June 24		July 13		Aug. 19		Sept. 24	
Tree #	Leaf 1	Leaf 2	Leaf 1	Leaf 2	Leaf 1	Leaf 2	Leaf 1	Leaf 2	Leaf 1	Leaf 2
1	49.7	59.7	62.6	64.5	60.0	54.1	55.8	54.3	37.6	37.3
2	71.0	62.2	64.4	61.6	45.4	46.5	52.5	52.3	35.6	36.8
3	56.6	61.3	56.5	56.0	53.9	56.1	61.0	60.9	46.4	43.5
4	44.0	55.2	64.4	63.4	48.2	49.2	61.5	65.1	19.6	26.2
5	63.9	68.9	49.8	45.3	62.9	53.0	60.1	58.5	28.6	27.1
6	65.4	59.3	62.3	63.5	61.4	56.6	59.8	56.6	37.0	37.4
Ave.	59.8 a**		59.5 a		53.9 a		58.2 a		34.4 b	

**Any two averages followed by the same letter are not significantly different at the 5% level of probability.

ANALYSIS OF VARIANCE OF THE ABOVE DATA

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F
Treatments	4	5,532.77	1,383.2	16.5*
Trees	5	261.45	52.3	
Experimental Error	20	1,675.14	83.8	
Subsampling Error	30	334.73	11.2	
Total	59	7,804.09		
CV = 17.2				

*The required F at the 5% level of probability is 2.87.

TABLE IV

THE 1964 DATA FOR TRANSLOCATION FROM THE TREATED AREA AS PERCENT OF THE TOTAL 2,4,5-T* ABSORBED AT 24 HOURS AFTER FOLIAR APPLICATION TO BLACKJACK OAK

Date	May 15		June 24		July 13		Aug. 19		Sept. 24	
Tree #	Leaf 1	2	Leaf 1	2	Leaf 1	2	Leaf 1	2	Leaf 1	2
1	40.3	49.1	54.9	54.4	49.8	45.3	61.5	59.8	76.1	80.7
2	52.4	45.7	69.3	67.1	55.9	53.2	59.3	57.7	74.7	72.5
3	40.3	44.8	66.8	60.8	43.1	41.0	57.2	51.2	79.9	77.2
4	40.1	49.4	57.6	59.8	45.6	49.3	61.2	62.4	58.1	65.8
5	48.5	54.5	54.6	47.8	59.3	51.8	54.3	45.5	66.9	67.4
6	54.6	50.0	69.9	71.8	74.7	45.0	47.9	46.8	60.6	70.9
Ave.	47.5 c**		61.2 ab		51.2 bc		55.4 ab		67.2 a	

**Any two averages followed by the same letter are not significantly different at the 5% level of probability.

ANALYSIS OF VARIANCE OF THE ABOVE DATA

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F
Treatments	4	4,058.02	1,014.5	11.6*
Trees	5	275.63	55.1	
Experimental Error	20	1,750.00	87.5	
Subsampling Error	30	843.23	28.1	
Total	59	6,926.88		
CV = 16.3				

*The required F at the 5% level of probability is 2.87.

TABLE V

THE 1964 DATA FOR TRANSLOCATION FROM THE TREATED LEAVES AS PERCENT OF THE TOTAL 2,4,5-T* ABSORBED AT 24 HOURS AFTER FOLIAR APPLICATION TO BLACKJACK OAK

Date	May 15		June 24		July 13		Aug. 19		Sept. 24	
Tree #	Leaf 1	Leaf 2	Leaf 1	Leaf 2	Leaf 1	Leaf 2	Leaf 1	Leaf 2	Leaf 1	Leaf 2
1	40.0	49.0	46.9	45.8	48.0	44.2	57.8	55.7	68.2	66.5
2	52.-	42.3	62.4	64.1	53.7	51.4	54.9	48.4	62.6	68.9
3	40.2	42.5	55.1	57.6	41.7	39.2	18.0	47.7	66.2	58.3
4	39.4	48.6	49.6	54.5	43.8	47.8	55.2	56.9	51.7	57.1
5	47.6	53.9	42.4	35.4	55.3	49.5	42.1	42.9	23.2	5.1
6	53.9	49.0	62.1	68.1	63.6	42.6	46.0	45.2	33.4	13.5
Ave.	46.5		53.7		48.4		47.6		47.9	

ANALYSIS OF VARIANCE OF THE ABOVE DATA

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F
Treatments	4	373.75	93.4	0.3*
Trees	5	1,546.83	309.4	
Experimental Error	20	6,057.69	302.9	
Subsampling Error	30	1,351.37	45.0	
Total	59	9,329.64		
CV = 12.1				

*The required F at the 5% level of probability is 3.55.

TABLE VI

ABSORPTION DATA AS PERCENT OF THE TOTAL APPLIED 2,4,5-T* AT 24 HOURS AFTER FOLIAR APPLICATION TO BLACKJACK OAK SEEDLINGS IN THE DOSAGE STUDY

μg 2,4,5-T* Applied	10	25	50
Seedling Number			
1	58.4	54.4	41.7
2	62.0	55.6	46.5
3	61.3	50.9	44.8
4	61.3	49.8	38.4
5	66.4	59.2	39.2
6	49.4	63.1	34.9
7	58.2	49.6	22.6
8	66.3	58.3	38.8
9	70.7	50.9	57.8
10	63.2	49.9	41.1
Average	61.7 a**	54.2 b	40.6 c

**Any two averages followed by the same letter are not significantly different at the 5% level of probability.

ANALYSIS OF VARIANCE OF THE ABOVE DATA

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F
Treatments	2	2,295.30	1,147.6	28.9*
Trees	9	507.07	56.4	
Error	18	714.34	39.7	
Total	29	3,516.71		
CV = 12.1				

*The required F at the 5% level of probability is 3.55.

TABLE VII

DATA FOR TRANSLOCATION FROM THE TREATED AREA AS PERCENT OF THE TOTAL 2,4,5-T* ABSORBED AT 24 HOURS AFTER FOLIAR APPLICATION TO BLACKJACK OAK IN THE DOSAGE STUDY

µg 2,4,5-T* Applied	10	25	50
Seedling Number			
1	62.2	52.6	24.5
2	45.0	58.6	29.8
3	50.8=	52.2	45.8
4	49.4	49.2	40.6
5	47.1	59.8	37.4
6	63.5	74.3	78.1
7	43.2	63.1	27.9
8	47.9	71.6	39.3
9	60.3	33.3	42.9
10	55.6	53.7	34.3
Average	52.5 a**	58.8 a	40.1 b

**Any two averages followed by the same letter are not significantly different at the 5% level of probability.

ANALYSIS OF VARIANCE OF THE ABOVE DATA

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F
Treatments	2	1,825.46	912.73	11.9*
Trees	9	1,758.04	195.3	
Error	18	1,375.51	76.4	
Total	29	4,959.01		
CV = 12.1				

*The required F at the 5% level of probability is 3.55.

TABLE VIII

DATA FOR TRANSLOCATION FROM THE TREATED LEAVES AS PERCENT OF THE TOTAL 2,4,5-T* ABSORBED AT 24 HOURS AFTER FOLIAR APPLICATION TO BLACKJACK OAK IN THE DOSAGE STUDY

μg 2,4,5-T* Applied	10	25	50
Seedling Number			
1	61.7	48.5	22.9
2	44.5	55.2	27.4
3	50.6	46.9	39.8
4	48.5	40.3	37.8
5	47.0	49.9	33.9
6	62.9	55.8	74.8
7	42.6	59.6	25.3
8	47.7	68.6	37.8
9	59.5	51.4	38.1
10	55.1	44.8	32.0
Average	52.0 a**	52.1 a	37.0 b

**Any two averages followed by the same letter are not significantly different at the 5% level of probability.

ANALYSIS OF VARIANCE OF THE ABOVE DATA

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F
Treatments	2	1,515.08	757.54	8.0*
Trees	9	1,279.18	142.13	
Error	18	1,693.18	94.1	
Total	29	4,487.44		
CV = 20.6				

*The required F at the 5% level of probability is 3.55.

TABLE IX

SOIL MOISTURE CONTENT DETERMINED ON A DRY WEIGHT BASIS IN THE STUDY OF THE EFFECT OF SOIL MOISTURE ON ABSORPTION AND TRANSLOCATION OF 2,4,5-T* IN BLACKJACK OAK SEEDLINGS

Plant Group	1	2	3
Plant Number			
1	3.1	8.0	15.5
2	2.9	7.3	16.0
3	3.2	8.5	15.2
4	2.5	9.6	19.2
5	3.0	6.0	17.1
6	2.9	8.3	15.4
7	2.4	7.1	15.8
8	2.3	5.2	15.8
Average	2.8	7.5	16.0

TABLE X

ABSORPTION DATA AS PERCENT OF THE TOTAL APPLIED 2,4,5-T* AT 24 HOURS AFTER FOLIAR APPLICATION TO BLACKJACK OAK SEEDLINGS IN THE MOISTURE STUDY

Soil Moisture Level	2.8		7.5		16.0	
	Leaf		Leaf		Leaf	
Plant Number	1	2	1	2	1	2
1	53.5	49.9	55.4	50.0	56.6	56.0
2	51.6	50.2	54.2	52.8	57.8	56.2
3	42.0	50.0	52.3	56.3	60.1	52.8
4	49.0	50.9	56.7	54.5	58.2	55.0
5	48.3	51.9	55.9	51.0	55.6	59.3
6	52.6	52.8	53.7	56.1	61.5	61.2
7	51.5	50.2	52.7	55.3	56.0	55.2
8	53.4	43.5	54.7	53.8	58.2	56.0
Average	50.1 c**		54.1 b		57.2 a	

**Any two averages followed by the same letter are not significantly different at the 5% level of probability.

ANALYSIS OF VARIANCE OF THE ABOVE DATA

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Treatments	2	412.28	206.14	49.1*
Seedlings	7	55.56	7.94	
Experimental Error	14	58.86	4.20	
Subsampling Error	24	189.93	7.91	
Total	47	711.63		
CV = 3.8				

*The required F at the 5% level of probability is 3.74.

TABLE XI

DATA FOR TRANSLOCATION FROM THE TREATED AREA AS PERCENT OF THE TOTAL 2,4,5-T* ABSORBED AT 24 HOURS AFTER FOLIAR APPLICATION TO BLACKJACK OAK IN THE MOISTURE STUDY

Soil Moisture Level	2.8		7.5		16.0	
	Leaf		Leaf		Leaf	
Plant Number	1	2	1	2	1	2
1	24.6	21.0	28.0	23.6	38.0	42.3
2	19.3	15.5	23.4	24.9	30.0	43.4
3	7.3	22.9	23.1	28.9	36.2	31.6
4	12.2	7.5	23.9	12.7	35.6	31.0
5	6.4	12.9	22.7	20.2	35.7	44.6
6	14.9	30.8	18.0	22.9	41.1	42.0
7	28.0	14.1	19.3	22.9	34.5	37.8
8	10.2	9.1	24.6	19.2	38.0	32.4
Average	16.0 c**		22.4 b		37.1 a	

**Any two averages followed by the same letter are not significantly different at the 5% level of probability.

ANALYSIS OF VARIANCE OF THE ABOVE DATA

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Treatments	2	3,746.33	1,873.16	77.7*
Seedlings	7	378.54	54.1	
Experimental Error	14	337.2	24.1	
Subsampling Error	24	596.80	24.9	
Total	47	5,058.86		
CV = 19.5				

*The required F at the 5% level of probability is 3.74.

TABLE XII

DATA FOR TRANSLOCATION FROM THE TREATED LEAVES AS PERCENT OF THE TOTAL 2,4,5-T* ABSORBED AT 24 HOURS AFTER FOLIAR APPLICATION TO BLACKJACK OAK IN THE MOISTURE STUDY

Soil Moisture Level	2.8		7.5		16.0	
	Leaf		Leaf		Leaf	
Plant Number	1	2	1	2	1	2
1	19.7	17.0	24.0	18.6	34.2	39.3
2	15.9	11.9	19.4	20.2	26.3	40.1
3	25.4	19.1	18.7	24.2	31.7	26.0
4	8.0	3.3	18.9	7.5	31.6	26.0
5	2.1	6.7	18.5	15.2	34.9	41.5
6	11.4	27.2	14.0	19.2	37.2	38.2
7	24.6	10.1	15.4	19.0	30.2	32.8
8	4.8	4.2	19.4	14.2	34.7	26.8
Average	13.2 b**		17.9 b		33.2 a	

**Any two averages followed by the same letter are not significantly different at the 5% level of probability.

ANALYSIS OF VARIANCE OF THE ABOVE DATA

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Treatments	2	3,403.4	1,751.70	43.9*
Seedlings	7	507.73	72.53	
Experimental Error	14	557.98	39.86	
Subsampling Error	24	614.23	25.59	
Total	47	5,183.34		

CV = 29.5

*The required F at the 5% level of probability is 3.74.

TABLE XIII

ABSORPTION DATA AS PERCENT OF THE TOTAL APPLIED 2,4,5-T* AT 24 HOURS AFTER FOLIAR APPLICATION TO BLACKJACK OAK SEEDLINGS IN THE PLANT SUGAR CONTENT STUDY

Treatment	Dark	Dark + Sucrose	Light
Plant Number			
1	54.5	53.4	61.2
2	34.6	43.2	48.0
3	41.4	38.5	73.4
4	36.6	45.4	32.7
5	70.8	74.8	67.7
6	34.6	53.3	44.1
7	54.6	40.2	50.0
8	56.4	48.3	64.8
9	61.4	59.5	47.9
Average	49.4	50.7	54.4

ANALYSIS OF VARIANCE OF THE ABOVE DATA

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Treatments	2	120.56	60.28	0.7*
Seedlings	8	2,369.85	29.62	
Error	16	1,393.39	87.09	
Total	28	3,883.80		
CV = 18				

*The required F at the 5% level of probability is 3.63.

TABLE XIV

DATA FOR TRANSLOCATION FROM THE TREATED AREA AS PERCENT OF THE TOTAL 2,4,5-T* ABSORBED 24 HOURS AFTER FOLIAR APPLICATION TO BLACKJACK OAK IN THE PLANT SUGAR CONTENT STUDY

Treatment	Dark	Dark + Sucrose	Light
Plant Number			
1	19.8	21.6	30.5
2	30.3	00.9	25.6
3	17.3	42.4	44.1
4	18.7	34.7	36.2
5	37.7	73.2	57.5
6	12.8	24.4	35.7
7	4.1	8.9	16.6
8	19.1	9.4	24.5
9	16.9	12.9	25.5
Average	19.6 b**	25.4 ab	32.9 a

**Any two averages followed by the same letter are not significantly different at the 5% level of probability.

ANALYSIS OF VARIANCE OF THE ABOVE DATA

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Treatments	2	798.14	399.07	4.04*
Seedlings	8	4,322.60	540.32	
Error	16	1,579.57	98.70	
Total	26	6,700.31		
CV = 38.3				

*The required F at the 5% level of probability is 3.63.

TABLE XV

DATA FOR TRANSLOCATION FROM THE TREATED LEAVES AS PERCENT OF THE TOTAL 2,4,5-T* ABSORBED AT 24 HOURS AFTER FOLIAR APPLICATION TO BLACKJACK OAK IN THE PLANT SUGAR CONTENT STUDY

Treatment	Dark	Dark + Sucrose	Light
Plant Number			
1	20.4	18.3	27.3
2	00.2	27.9	22.8
3	40.1	14.6	37.3
4	26.9	17.5	33.2
5	18.7	31.6	56.6
6	16.8	11.4	31.9
7	5.5	2.9	15.1
8	8.4	14.5	20.2
9	10.2	13.8	24.2
Average	16.4 b**	16.9 b	29.8 a

**Any two averages followed by the same letter are not significantly different at the 5% level of probability.

ANALYSIS OF VARIANCE OF THE ABOVE DATA

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Treatments	2	1,046.12	523.06	7.12*
Seedlings	8	1,773.44	221.68	
Error	16	1,175.23	73.45	
Total	26	3,994.79		
CV = 47.2				

*The required F at the 5% level of probability is 3.63.

TABLE XVI

COUNTS PER SECOND IN 16 MM SECTIONS OF STEMS REMOVED FROM BELOW THE TREATED LEAVES IN THE PLANT SUGAR CONTENT STUDY

Treatment	Dark	Dark + Sucrose	Light
Plant Number			
1	242	257	196
2	83	189	101
3	82	72	289
4	193	70	164
5	58	154	222
6	81	145	112
7	73	238	65
8	60	119	96
9	70	64	64
Average	105	165	165

ANALYSIS OF VARIANCE OF THE ABOVE DATA

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Treatments	2	9,950	4,975	1.08*
Seedlings	8	50,717	6,340	
Error	16	73,279	4,580	
Total	26	133,946		
CV = 51.3				

*The required F at the 5% level of probability is 3.63.

TABLE XVII

THE BREAKDOWN OF 2,4,5-T IN EXCISED LEAVES OF BLACKJACK OAK DURING A 24 HOUR TREATMENT

Treatment Date	Percent of total Radioactivity (Average of 4 determinations)						
	Water soluble components				Ether soluble components		
	R_f.33	R_f.68	R_f.85	R_f.92	R_f.92	R_f.84	R_f.30
May 14			17	16	18	50	
May 28			25	6	22	46	
June 11			28	14	24	35	
June 26			36	16	16	32	
July 9			36	5	13	47	
Aug. 5		11	23	3	11	53	
Sept. 3	2	39	39		17	25	
Oct. 2	trace		22		1.5	75	1.5

VITA

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Doctor of Philosophy

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