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FACTORS AFFECTING THE ABSORPTION, TRANSLOCATION, AND TOXICITY OF HERBICIDES ON CREOSOTE BUSH

by

Ervin M. Schmutz

A Dissertation Submitted to the Faculty of the
DEPARTMENT OF WATERSHED MANAGEMENT
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
In the Graduate College
UNIVERSITY OF ARIZONA

1963
I hereby recommend that this dissertation prepared under my direction by Ervin M. Schmutz entitled *Factors affecting the absorption, translocation, and toxicity of herbicides on creosotebush.* be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy.

Dissertation Director

Date

After inspection of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:

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ABSTRACT


A study was initiated in 1957 to determine the problems involved in the chemical control of creosotebush (Larrea tridentata (DC.) Cov.), a major dominant over vast acreages of desert and semidesert rangelands in the southwestern United States and northern Mexico.

Both foliar-spray and radioisotope methods were used to determine the susceptibility of creosotebush to various phenoxy herbicides at different seasons of the year and times of the day and to determine the effects of various environmental, physiological, and chemical factors on herbicide absorption, translocation, and toxicity. Major studies were conducted at both Tombstone and Tucson, Arizona, in areas bordering between typical southern desert shrub and desert grassland with an average annual rainfall of approximately 12 inches. Effects of foliar-spray treatments were measured by evaluation of total-kill and topkill of plants. Radioisotope effects were measured by radioautographs showing relative absorption and translocation. Except for the screening tests in which several formulations were tested, the propylene-glycolbutylether esters of 2,4-dichlorophenoxyacetic acid (2,4-D) and/or 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were used in the foliar-spray studies. Radioisotopes used were C14-labeled acid forms of 2,4-D* and 2,4,5-T*.

Screening tests showed that the trichlorophenoxyacetic formulation was more effective on creosotebush than the trichlorophenoxypropionic and that the low-volatile propyleneglycolbutylether, isooctyl,
and tetrahydrafurfuryl esters of 2,4-D and 2,4,5-T were generally more effective than the pentyl.

Susceptibility of creosotebush to 2,4-D and 2,4,5-T varied widely from year to year and from season to season with highest susceptibility generally occurring 12 to 60 days after the first summer rain of at least 1/2 inch and during above-average rainfall years. Translocation of radioactive 2,4,5-T* coincided with high seasonal toxicity being greatest approximately 30 days after initiation of effective summer rains. This period coincided with the full-flowering to mid-fruiting stage of phenological development and overlapped the period of old-leaf drop. High susceptibility during the period may be explained in part by the generally greater basipetal translocation of radioactive 2,4,5-T* from old leaves, flowers, seeds, and young bark than from young leaves. Seasonal temperatures and relative humidity appeared to influence herbicidal toxicity mainly in an indirect fashion through their effects on plant growth.

Creosotebush susceptibility to 2,4,5-T at different times of the day did not show a consistent pattern. Current and prior environmental and phenological conditions conducive to high midday susceptibility of creosotebush appeared to be a high maximum-minimum night-day relative humidity of ca 90 and 30 percent, respectively; a favorable prior rainy season; 3- to 5-inch-depth soil moisture conditions at or above the wilting coefficient; moderate to heavy cloud conditions; and active leaf growth and flower production.

Diesel oil added to the spray emulsion generally resulted in significant increases in creosotebush kill even at high rates of 12.5 to 25 gallons of oil per acre, but increasing the amount of surfactant in the commercial 2,4,5-T preparation had no consistent effect.
Ammonium phosphate applied 10 to 14 days prior to 2,4,5-T treatment resulted in a slight increase in plant susceptibility to herbicides but fertilizer treatments applied 26 to 32 days prior resulted in a slight decrease.

Sucrose added to the treatment emulsions significantly increased topkill of creosotebush by 2,4,5-T but did not increase translocation of radioactive 2,4,5-T*.

Young plants and sprouts of creosotebush were slightly more susceptible to 2,4,5-T than mature plants. pH of the spray emulsion, gibberellic acid treatments, and soil depth factors had insignificant or inconsistent effects on creosotebush susceptibility.

Translocation of herbicides appeared to be the major problem in the chemical control of creosotebush. The translocation rate of radioactive 2,4,5-T* was relatively slow, 1 to 5 cm per hour maximum. Basipetal movement ceased after 18 to 24 hours, following which the 2,4,5-T* largely disappeared from the tissues of the stem. Absorption of herbicides also appeared to be limiting during certain seasons of the year but probably had little effect during the season of high plant susceptibility.
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CHAPTER I

INTRODUCTION

The Problem

Shrub invasion on rangelands of the western United States is a major problem. This invasion may be a result or a cause of range depletion. In either event, an increase in shrub density is generally correlated with a decline in range productivity and with increased difficulty and cost of handling livestock. Since noxious plants utilize soil moisture, plant nutrients, and sunlight essential for the production of grass, their removal or control is necessary as an initial step toward grass establishment and range improvement.

One of the major shrubs occupying desert and semidesert areas of the West is creosotebush (Larrea tridentata (DC.) Cov.).\(^1\) It is a dominant species or associate dominant on millions of acres in the Mohave, Sonoran, and Chihuahuan deserts of the southwestern United States and northern Mexico (Shreve, 1942, 1951; Dalton, 1961) and has

\(^1\) Scientific terminology follows that of Kearney and Peebles (1960).
invaded extensive areas of adjacent desert grassland (Whitfield and Beutner, 1938; Gardner, 1951; Humphrey and Mehrhoff, 1958; Yang, 1961). Much of this area represents a climax creosotebush vegetative type with temperatures too high and rainfall too low to become productive rangeland. However, areas of former grass-shrub types and desert grasslands now dominated by creosotebush are capable of producing considerable forage for livestock and game. While the creosotebush-dominated area is moderate to low in relative productivity, its large size makes it an important segment of our western grazing land, extensive portions of which can be improved by brush control.

Various mechanical, ecological, pyric, and chemical methods of controlling creosotebush have been only moderately successful. Therefore additional information is needed to develop more effective and economical methods.

**Creosotebush Characteristics and Distribution**

Creosotebush is a member of the Zygophyllaceae or caltrop family. It is typically a much-branched shrub growing 3 to 11 feet in height. Leaves are evergreen, leathery, olive to bright green in color, oppositely-branched, almost stalkless, and pinnately divided into two or more leaflets. Leaf surfaces are resinous, finely glandular, hairy, and with numerous stomata on both surfaces (adaxial 24,000, abaxial 36,000 per square centimeter (Ashby, 1932)). Flowers are bright yellow in
color, hypogynous, short-stalked, and solitary in the leaf axils. The five petals are somewhat clawed at the base and twisted in a fashion resembling the blades of a fan. The ten stamens are each subtended by a laciniately, three-toothed scale adnate to the filament, hence the specific epithet tridentata. The fruit, tipped by a single persistent style, is a spherical, densely-hairy, five-celled capsule which breaks into five one-seeded nutlets at maturity. Stems are olive green in color and supple when young, becoming grey to black and hard and brittle when older. Roots are much-branched and widely spreading (Standley, 1923; Ashby, 1932; Martinez, 1936; U. S. Forest Service, 1937; Benson and Darrow, 1954; Kearney and Peebles, 1960).

Dalton (1961) noted that initial flowering of mature plants in the summer occurred 14 to 18 days after watering and preceded development of new leaves; that plants were in full-flower in 21 days; that seed-set occurred in 31 days and seed-fall in 56 days. Shreve (1951) observed that fruits matured in 6 to 8 weeks.

The maximum age of creosotebush was estimated by Shreve (1951) as probably not exceeding 100 years. He also noted that the age of creosotebush could be estimated by the size of its root crown which in old plants may reach 20 to 30 cm in thickness.

Creosotebush gets its common name from its strong, pungent, creosote-like odor, which is most noticeable after a rain or when the plant is burned or crushed. It is also known locally as greasewood,
chaparral, gobernadoro, hediondilla, and numerous other names (Martinez, 1936; U. S. Forest Service, 1937; Benson and Darrow, 1954; Dalton, 1961). It has also been known by various scientific names, and its distinctness from the morphologically similar South American species (L. divaricata Cav.) is still disputed (Standley, 1923; Benson and Darrow, 1954; Kearney and Peebles, 1960; Dalton, 1961; Yang, 1961). However, Covas (1949) and Darlington and Wylie (1955) reported that L. tridentata (X=13) is a polyploid with 2n=52 and 104 (polysomic) chromosomes as compared to 26 somatic chromosomes for L. divaricata, indicating that they are sibling species. Porter (1961), to clarify the original work on L. tridentata by Covas, explained that the 52 chromosomes represent the normal 2n (tetraploid) condition and that the 104 chromosome counts were made by Covas in the roots of L. tridentata and represent a polysomatic condition apparently caused by bacterial infection.

Nichol (1952) estimated that creosotebush was a dominant or associate dominant over approximately one-fourth of the state of Arizona (17.5 million acres). Duisberg (1952b) estimated that it covered 20 million acres in the United States, extending from West Texas to California. Botkin and Duisberg (1949) showed that creosotebush was a dominant over about 20 percent of the state of New Mexico and reported that it covered 35 million acres in the southwestern United States and northern Mexico. Harshberger (1911), Shantz and Zon (1924), Shreve (1940, 1951), Jaeger (1957), Garcia et al. (1960), and Dalton (1961) published
maps showing its range to be much more extensive in both countries but gave no specific data on acreages.

Creosotebush frequently grows in almost pure, evenly-spaced stands but also occurs as a subdominant intermixed with other shrubs and grasses (Clements, 1920; Shreve, 1942, 1951; Nichol, 1952; Benson and Darrow, 1954; Jaeger, 1957; Yang, 1957; Dalton, 1961). Associated shrubs may include mesquite (Prosopis juliflora), bursage (Franseria deltoidea and F. dumosa), paloverde (Cercidium microphyllum), crucifixion thorns (Koeberlinia, Holacantha, and Canotia spp.), lotebushes (Condalia spp.), burroweed (Haplopappus tenuisectus), brittlebushes (Encelia spp.), jointfir (Ephedra spp.), ironwood (Olneya tesota), ocotillo (Fouquieria splendens), yuccas (Yucca spp.), catclaw (Acacia greggii), whitethorn (Acacia constricta), saltbushes (Atriplex spp.), zinnia (Zinnia pumila), tarbush (Flourensia cernua), ratany (Krameria spp.), snakeweed (Gutierrezia spp.), and various cacti (Opuntia and Cereus spp.). Grasses may include bush muhly (Muhlenbergia porteri), black grama (Bouteloua eriopoda), rothrock grama (B. rothrockii), fluffgrass (Tridens pulchellus), slim tridens (T. muticus), Arizona cottontop (Trichachne californica), various threeawns (Aristida spp.), big galleta (Hilaria rigida) and tobosa grass (H. mutica). Forbs may include filaree (Erodium cicutarium), Indian wheat (Plantago spp.), and globemallow (Sphaeralcea spp.).
Creosotebush grows under a wide variety of rainfall conditions, in regions receiving from 1 to 20 inches mean annual precipitation and having different seasonal patterns (Shreve, 1940, 1951; Dalton, 1961). However, Livingston and Shreve (1921) speculated that prolonged wet periods make conditions of soil aeration injurious to creosotebush and limit its distribution in areas of higher rainfall.

Spaulding (1904) and Runyon (1934, 1936) observed that creosotebush possesses a peculiar mixture of xeric and mesic characteristics that enables it to survive severe drouth conditions, yet makes rapid growth under favorable conditions. Runyon also noted that during drouth the leaves do not wilt; that growth of immature leaves and buds is suspended; that leaf resins may aid in drouth resistance; that young leaves regularly have lower water content and are more drouth resistant than older ones; and that under extreme drouth mature leaves and small twigs may absciss. Furthermore, under favorable moisture conditions, all leaves "greened up"; most immature leaves and buds resumed growth, unless severely stunted; and after a period of growth the old leaves were shed. Ashby (1932) attributed the drouth resistance of creosotebush not to reduced transpiration but to the ability of the protoplasm to endure desiccation without harm. Shreve (1940) further emphasized its adaptability when he noted that no part of North America is too dry for creosotebush, yet well-established seedlings, free from competition, will grow in a soil of constantly high moisture
content at a rate which is roughly 70 times that under adverse conditions. Went (1952, 1955) noted that the amount of rainfall, in combination with plant growth inhibitors secreted by the roots of existing plants, regulated spacing of creosotebush and ultimately only one new bush could grow where another one had died. However, the role of plant growth inhibitors in regulating spacing of creosotebush plants was discredited by Knipe (1960) and Dalton (1961). Mallery (1935) concluded that the low moisture content in the upper soil layers played the most important role in the scanty reproduction of creosotebush near Tucson, Arizona. He found that the generally bare ground under creosotebush was due to the fact that the soil moisture content was below the wilting percentage for most plants nearly all the time. As a result creosotebush seedlings became established only during the rainy season, the greatest number becoming established in disturbed areas where the covering of seeds and moisture absorption was increased and plant competition was reduced.

Temperature appears to be a major factor which limits both growth and distribution of creosotebush. Dalton (1961) observed that vegetative growth was initiated only when soil moisture was available and daily minimum and maximum temperatures exceeded 40 and 80°F (4.4 and 26.7°C), respectively. Livingston and Shreve (1921) and Shreve (1940) reported that creosotebush has a high optimum growing temperature and tolerates high desert temperatures but found that its
northern extension is limited by cold. Shreve (1940) noted that it could not survive six consecutive days of freezing temperatures and that snow is distinctly "hostile" and may flatten the "entire" plant. Rzedowsky and Leal (1958) noted that climate, especially the degree of aridity, was the most important factor determining its southern limit.

Shreve (1940) also noted that the rapid disappearance of creosotebush at the northern and western edges of the desert in Mexico indicates that conditions are not suitable for germination and establishment, and that it is unable to persist in competition with vigorous taller shrubs. Shreve (1951) and Went (1952) noted that creosotebush seeds germinated mainly in July and August following initiation of summer rains, indicating a dependence on high temperature for germination. This was substantiated by Dalton (1961) who found that the optimum temperature for germination of creosotebush seed was 35° C. Went and Westergaard (1949) also noted that creosotebush seeds germinated in Death Valley as late as October if rains were followed by minimum temperatures of 15 to 16° C but not in November followed by temperatures of 8 to 10° C. However, Went (1955) later concluded that growing conditions after germination were the main factors controlling establishment rather than germination itself.

Creosotebush grows on a wide variety of soils including shallow calcareous soils underlain by caliche; gravelly slopes and mesas; volcanic hills; sandy soils and dune areas; and deep, moderately-permeable,
fine-textured, caliche-free soils. It is not adapted to highly alkaline, granitic, saline, or poorly-drained, fine-textured soils (Shantz and Zon, 1924; Shreve and Mallery, 1933; U. S. Forest Service, 1937; Shreve, 1940, 1951; Yang, 1950, 1957).

Its altitudinal range varies from below sea level in Death Valley up to 4,000 and 5,000 feet in the United States and up to 8,600 feet in Mexico (Shreve, 1940, 1951; Benson and Darrow, 1954; Kearney and Peebles, 1960).

Creosotebush is classed as worthless for livestock forage at all seasons of the year (U. S. Forest Service, 1937) and may not even be grazed by jackrabbits, although they reportedly sharpen their teeth on the hard stems (Jaeger, 1948). Griffiths (1904) quoted reports that the plant was poisonous to sheep but this was disputed by Crawford (1908). Duisberg (1952b) prepared a livestock feed from creosotebush and found it equivalent to alfalfa in protein value but the feed had to be mixed with alfalfa hay initially before livestock would eat it. Russell (1908), Standley (1923), Martinez (1936, 1944), Rigby (1959), and Kearney and Peebles (1960) reported extensive use of the plant by Indians, Mexicans, and early American pioneers for medicine and food. Rigby labeled it the "desert drugstore." Other prospective uses are resins for varnishes, the antioxidant nordihydroguaiaretic acid (NDGA), pressed building-board, and various other chemurgic uses (Botkin and Duisberg, 1949; Duisberg, 1951, 1952a, b; UNESCO, 1960). The U. S. Forest
Service (1937) classed it as an efficient soil protector and stabilizer but reported that it was not a good soil builder and that it competed with better plants on sites where it occurs abundantly.

In spite of its varied uses, the plant has little economic value and is classed as a noxious plant on rangelands.

**Previous Herbicide Studies on Creosotebush**

Numerous studies have been made on the effect of herbicides on southern Arizona shrubs but only one has been reported on creosotebush. A preliminary report by Schmutz et al. (1957) on a study begun in 1954 showed that season-of-the-year factors markedly influenced the susceptibility of creosotebush to phenoxy herbicides. Highest kills were obtained on plants sprayed during August and September, approximately 30 days after the initial summer rain of at least one-half inch. A secondary period of susceptibility occurred in the spring during the moisture-variable months of March, April, and May. Lowest kills were noted on dormant plants sprayed during the cool winter months. Creosotebush was found to be more resistant to herbicides than tarbush or whitethorn and was slightly more susceptible to propyleneglycol-butylether esters of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)\(^1\) than of 2,4-dichlorophenoxyacetic acid (2,4-D).

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\(^{1}\) Terminology follows that of the Weed Society of America, 1962.
Based on these preliminary findings, and similar investigations on other plants, this study was initiated on creosotebush in 1957 to determine the effect of various kinds and rates of herbicides on plant kill; the effect of different season-of-year and time-of-day factors on plant susceptibility to herbicides; the influence of surfactant and oil-carrier components and pH of the spray emulsion on absorption and translocation of herbicides; the relationship of plant herbicidal response to phenological development and plant age, anatomy, and physiology; and the effect of hormonal stimulants, photosynthate additives, soil fertilizers, and soil depth on herbicidal activity.
CHAPTER II

THE PHENOXY HERBICIDES

This chapter contains a general review of the literature on the characteristics and mode of action of the phenoxy herbicides. It is designed to contribute to a general understanding of the problems involved in this study and to aid in the interpretation of the results. Literature specific to the individual phases of the study will be included in the appropriate chapters.

The effectiveness of the foliar-applied, phenoxy herbicides is dependent on many physical, physiological, and biochemical factors. These factors may include: (1) the kind of formulation of the herbicide; (2) absorption into the plant and migration to the vascular tissues; (3) translocation; (4) loss of herbicide in the spray mixture on or inside the plant; (5) toxic action in the plant; (6) delayed effects of herbicide stored in the plant or in the soil; and (7) the morphological and physiological characteristics and condition of the plant as affected by genetic and environmental factors.
Formulations

The announcement of the use of 2,4-D and 2,4,5-T as selective, hormone-like herbicides in 1944 by Hamner and Tukey (1944a, b), Mitchell and Hamner (1944), and Marth and Mitchell (1944) opened the door to a flood of new herbicides. However, these original phenoxy herbicides and their sister compounds continue to be some of the most effective herbicides for shrub control. Also, the fact that 2,4-D is more effective on some shrubs and 2,4,5-T on others has resulted in the use of mixtures of the two as brush killers (Robbins et al., 1952; U. S. Agricultural Research Service, 1958; British Weed Control Council, 1960).

The related compound, 2-(2,4,5-trichlorophenoxy) propionic acid (silvex), which resembles 2,4,5-T structurally except that the acid is propionic instead of acetic, is effective in the control of some plants that are resistant to both 2,4-D and 2,4,5-T.

These herbicides are made up into various formulations to improve performance and for convenience in handling and application. The acids of the phenoxy compounds are nonpolar and generally only slightly soluble in water and may precipitate in hard water; so the polar, water-soluble amine, sodium, and ammonium salts or the nonpolar oil-soluble ester forms are generally used (Klingman, 1961). The polar salts are more readily absorbed through the roots than the nonpolar acids and esters; the esters and acids are more soluble through the
leaves (Klingman, 1961). Through the leaves, absorption of the esters is most rapid, the amine salt intermediate, and the sodium salt slowest (Hauser, 1955).

The esters, methyl through octyl, are more volatile than the acid, salt, or amine forms at normal temperatures and generally decrease in volatility with increasing length of side chain (Marth and Mitchell, 1949; Baskin and Walker, 1953; Hitchcock et al., 1953). However, volatility and physiological activity of herbicides may increase as either temperature or duration of exposure increases. For example, at 90 to 120° F the normally low-volatile tetrahydrofurfuryl and propylene-glycolbutylether esters become volatile, but the amine salts remain low in volatility (Baskin and Walker, 1953; Hitchcock et al., 1953). The non-volatile salts and volatile esters are both effective on susceptible herbaceous plants, but the emulsifiable acids and esters are more effective on heavily cutinized woody plants (Crafts, 1953, 1956b). On woody plants the low-volatile, longer-chain esters are generally more effective than the short-chain volatile esters because, having heavier and slower acting molecules, they enter the leaves in an orderly manner causing less contact injury; the oil-like characteristics of the heavy esters resist drying on the leaf surface; and being less volatile, they penetrate the plant over a longer period of time (King and Kramer, 1951); Robbins et al., 1952; Crafts, 1953, 1956b, 1960, 1961a; U. S. Agricultural Research Service, 1958). An ether linkage in the molecule may further reduce volatility.
Also, the oil-like, low-volatile, heavy esters are soluble in oil and when applied in an oil-water emulsion may penetrate the leaf three ways—as a gas through the stomata, in the oil-water phase through the stomata, and in the oil phase through the cuticle (Currier and Dybing, 1959; Klingman, 1961).

The inclusion of humectants, such as the glycols or glycerine, also helps to reduce drying and keeps the formulation in a liquid state longer and in closer contact with the cuticle for greater absorption (Crafts, 1961a; Klingman, 1961). On the other hand, inclusion of aromatic solvents and oils may result in highly toxic effects which result in rapid burning of the foliage, thus inhibiting the uptake and movement of the herbicides (Crafts, 1961a). Crafts (1956a) found that increasing the length of the ester on the side chain decreased polarity, increased lipoid solubility, and increased penetration. He noted that this may adversely affect translocation due to greater adsorption of the herbicide in the cuticle, greater difficulty in translocating the heavier molecules, lower water solubility in the phloem, and greater contact injury to the phloem.

The emulsified acids, which combine balanced solubility, orderly penetration, continued migration to the phloem, optimum pickup and translocation in the phloem, minimum contact injury, stable spray mixture, and syrupy films for indefinite absorption, are receiving new attention (Crafts, 1956b). Pelleted formulations of many herbicides are
also becoming increasingly popular because they are easy to apply and are more effective in some situations. Formulations are also being made to include two or more mixtures of herbicides in order to obtain additive or synergistic responses through interactive effects on absorption, translocation, or toxicity; through inhibition of degradation; and/or by broadening the spectrum of weed population controlled (Crafts, 1961a).

Absorption

Most of the work on absorption and translocation of phenoxy herbicides has been done with 2,4-D rather than 2,4,5-T. However, comparative studies have shown that activity of these compounds is similar except that in some cases absorption and translocation of 2,4,5-T may be somewhat less than 2,4-D (Linder et al., 1950; Thimann, 1951; Muir and Hansch, 1953; Crafts, 1956a; Weintraub et al., 1956; Yamaguchi and Crafts, 1959; Leonard and Yeates, 1960). On the other hand, Leonard and Crafts (1956) reported 2,4,5-T was a better penetrating agent than 2,4-D.

Absorption of herbicides is reported to involve not only the physical adsorption and diffusion through the stomata or cuticle and cell wall but also to include an active metabolic step through the plasma membrane (Loomis, 1955; Crafts, 1956b; Van Overbeek, 1956; Leonard, 1958; Shaw et al., 1960). However, absorption is not dependent on carbohydrate
reserves (Hauser, 1955; Barrier and Loomis, 1957). The cuticle layer is composed primarily of cutin and cutinwax imbedded in the outer pectin and cellulose layers of the epidermal cells and is lipoidal and nonpolar in nature (more so in the outer than inner layers) (Van Overbeek, 1956; Orgell, 1957; Schieferstein, 1957). As a result of these characteristics, absorption through the cuticle may be slow and continuing and is dependent on the polarity and lipophilic nature of the herbicide formulation (Crafts, 1953). However, as long as the spray solution of phenoxy acids remains a liquid on the leaf, there is probably some movement inward along the aqueous route as well, but this may be relatively small if drying is rapid (Crafts, 1961a).

The use of surfactants increases the rate of foliar absorption by reducing interfacial tension and solubilizing the wax- and oil-like substances of the cuticle and cell wall (Van Overbeek, 1956; Klingman, 1961). This may increase absorption and toxicity of the sodium and amine salts more than five times (Staniforth and Loomis, 1949).

Increases in temperature, if not excessive, may result in more rapid absorption of herbicides (Rice, 1948; Hauser, 1955; Van Overbeek, 1956; Barrier and Loomis, 1957) and being a chemical process in part may, within biological limits, double with each increase of 10° C or

1 Solubilization was defined as the incorporation of foreign molecules in the colloidal micelles of surface-active substances (Van Overbeek and Blondeau, 1954).
$17^\circ$ F (Klingman, 1961). Temperature effects may include increased rate of diffusion, lowered viscosity, acceleration of photosynthesis, more rapid phloem translocation, protoplasmic streaming, and growth (Currier and Dybing, 1959). However, excessive temperatures may reduce the period of absorption by crystallization, volatization, and evaporation of the herbicides (Rice, 1948).

The exact role and importance of stomata in the absorption of herbicides under field conditions has been much debated and many workers claim that it does not occur or that it is insignificant (Weaver and DeRose, 1946; Norman et al., 1950; Van Overbeek, 1956; Currier and Dybing, 1959; Crafts, 1961a). This debate results from the fact that stomata may be only on the underside of the leaf or may be closed at the time of spraying due to the effects of light, temperature, relative humidity, water balance, nutrient supply, or other factors. In addition, the herbicide itself may cause closure of stomata (Bradbury and Ennis, 1952). However, Dybing and Currier (1959) reported that aqueous solutions enter mainly by the way of open stomata and are excluded when stomata are closed. Skoss (1955) found that regardless of the nature of the sprayed substance, stomata act as a major portal of entry. Pallas (1960) noted that absorption and translocation of 2,4-D was greatest when stomata were open and humidity and temperatures were high. Leonard and Crafts (1956) reported that absorption was greatest in leaves with stomata on both leaf surfaces. Currier and Dybing (1959)
concluded that on the basis of all available information both the stomata and cuticle may be involved in absorption with the stomatal component varying widely due to a complex of factors influencing stomatal opening. They noted that stomatal entry of aqueous solutions are especially dependent on surfactant factors; that stomatal uptake is rapid when it occurs; that stomatal size may directly increase the amount of penetration; and that light promotes penetration directly by stimulating stomatal opening. If stomatal opening is important then the inclusion of a proper chemical compound in the herbicide formulation to stimulate stomatal opening and increase absorption offers an interesting possibility (Crafts, 1961a).

Other morphological characteristics of the plant which influence distribution, retention, and uptake of herbicides include plant form; leaf shape, position, density, hairs, cuticle, and margin; exposure of growing points; presence of hydathodes and lenticels; necrosis; and root form, shape, size, density, and distribution (Norman et al., 1950; Ennis et al., 1952; Currier and Dybing, 1959; Shaw et al., 1960). In general, application to the lower surface of the leaf results in more rapid absorption due both to thinner cuticle and more numerous stomata (Ennis et al., 1952; Weintraub et al., 1954b; Currier and Dybing, 1959).

Rice (1948) reported that absorption was greatest in the dark, and that low and medium light had no effect. However, Currier and Dybing (1959) reported that this response could be due to other factors
such as high humidity or lower temperature. Weintraub et al. (1954b) found that light had no effect on absorption of 2,4-D. Uptake is also dependent on the concentration of the herbicide and the area of the plant exposed (Hauser, 1955; Bach and Fellig, 1961). Absorption of the major part of the herbicide appears to take place rapidly, generally within 1 to 4 hours (Day, 1952).

Translocation

Once compounds such as 2,4-D reach the living cells they are rapidly absorbed into the symplast and migrate toward and into the vascular channels (Crafts, 1953, 1961a; Yamaguchi and Crafts, 1959). Subsequent translocation of the herbicide from the leaves generally takes place in the phloem and is greatest during those periods or seasons of the year when plant food materials are being actively transported to other parts of the plant (Mitchell and Brown, 1946; Weaver and DeRose, 1946; Rice, 1948; Linder et al., 1949, 1950; Rohrbaugh and Rice, 1949, 1956; Weintraub and Brown, 1950; Crafts, 1951, 1953, 1956b, 1960, 1961a, b; Mitchell, 1951; Bonner and Galston, 1952; Day, 1952; Mitchell et al., 1953; Hay and Thimann, 1956b; Leonard and Crafts, 1956; Barrier and Loomis, 1957; Clor and Crafts, 1957; Crafts and Yamaguchi, 1958; Morre and Rogers, 1960; Pallas, 1960). Translocation of various forms of 2,4-D and 2,4,5-T was related to conditions favoring translocation of photosynthate regardless of whether the growth
regulator was mixed with alcohol, kerosene, Varsol, or motor oil (Linder et al., 1949).

Translocation of phenoxy herbicides in the phloem may be up (acropetal) or down (basipetal), the direction varying according to growth stage, concentration of substance applied, and technique of application (Weaver and DeRose, 1946; Weintraub and Brown, 1950; Van Overbeek, 1956; Crafts and Yamaguchi, 1958; Hull, 1960; Crafts, 1961b). Movement may also occur laterally from phloem to xylem or vice versa in the symplast or apoplast of the ray parenchyma cells (Yamaguchi and Crafts, 1959) and subsequently may move upward in the xylem (Hay, 1956a, b; Van Overbeek, 1956; Hull, 1960). The rate of herbicide translocation in the phloem is generally reported at 10 to 100 cm per hour but rates vary from 1 cm per day to over 300 cm per hour, depending upon complex and interrelated morphological, physiological, biochemical, and physical factors (Weintraub and Brown, 1950; Bonner and Galston, 1952; Day, 1952; Crafts, 1953; Hay, 1956a; Van Overbeek, 1956; Hull, 1960; Zimmerman, 1960).

Translocation of 2,4-D from the soil upward occurs in the xylem and follows the movement of water and soil nutrients in the transpiration stream (Mitchell, 1951; Hay, 1956b; Yamaguchi and Crafts, 1959; Crafts, 1961b; Klingman, 1961). Translocation up or down is favored by soil moisture sufficient to produce rapid plant growth and is slowed
or stopped by dry soils (Crafts, 1956b; Leonard and Crafts, 1956; Yamaguchi and Crafts, 1959; Klingman, 1961).

Reports vary on the effect that herbicide formulation has on translocation, but in general the acids translocate best followed by the salts and then the esters (Linder et al., 1949; Weintraub and Brown, 1950; Robbins et al., 1952; Hull, 1956).

Reports on the chemical form in which phenoxy herbicides are translocated and are active are somewhat varied. Crafts (1960) reported that, in the case of isopropyl esters of 2,4-D, the 2,4-D moves across the mesophyll and into the phloem as an ion or free acid. Pallas (1960) noted that the bulk of the 2,4-D is translocated as free 2,4-D and that movement is polarized. Morre and Rogers (1960) noted a lag in curvature response of about two hours after treatment with esters indicating that hydrolysis must occur before translocation. Shaw et al. (1960) reported that conceivably some herbicides are translocated in a degraded or metabolized form but others seem to be absorbed, translocated, and accumulated at the site of action as the original molecule. Hull (1960), after reviewing current literature on translocation of foliar-applied herbicides, concluded that an increasing number of recent studies indicate that a significant part of most organic substances are transported in the same molecular form as applied. Hagen et al. (1949) and Linder et al. (1950) reported that herbicide activity results from the acid form of 2,4-D and that the salts and esters must be hydrolyzed
before they become active. Audus (1949) reported that the inhibitory activity of 2,4-D is proportional to the logarithm of the concentration of undissociated 2,4-D molecule rather than the free acid, indicating that the molecule acts in the undissociated form. Thimann (1951) postulated that hydrolysis of the esters may be necessary for transport but not for primary activity as the esters may be active per se. Veldstra (1953) concluded that there is no direct evidence that conversion of the derivative to the parent acid is a prerequisite for auxin activity.

The method of herbicidal transportation has been much debated. Several observers reviewed by Crafts (1951) suggested that the herbicides are carried along in the photosynthate stream by a simple diffusion process. However, an analysis by Hay and Thimann (1956) indicates that a metabolic component is involved in which the sugars act as a source of energy for the transport mechanism. Reasons cited for this conclusion include: (1) movement of 2,4-D in the dark is not greatly stimulated by concentrations of mannitol, arabinose, or urea; (2) low temperatures reduce translocation to a greater degree than is normal for a simple diffusion process; (3) a mixture of sucrose, hexoses, and their phosphates are found in leaf cells while sieve tubes contain only sucrose, indicating a metabolic transfer; (4) palisade cells can be plasmolyzed in the phloem sap, indicating both a metabolic transfer and a reverse gradient; and (5) movements of dyes are independent of changes in turgor pressure. Other investigators whose work indicates
that translocation of photosynthate, and consequently herbicides, involves a metabolic or secretory step (probably between the leaf parenchyma cells and the phloem vessels) include Leonard (1939), Loomis (1945, 1955), Wanner (1952), Van Overbeek (1956), Barrier and Loomis (1957), Esau et al. (1957) and Zimmerman (1960).

The effect of contact injury by the herbicide to the foliage and its effect on absorption and translocation must also be considered. Loomis (1955) concluded that phloem injury was the major cause of lack of 2,4-D translocation in plants. Leonard and Crafts (1956) noted that contact injury varied with species, age of leaves, surface of leaf treated, presence of stomata, air temperature, addition or presence of surfactant, herbicidal formulation, and rate of application.

As already noted, more rapid entry of the lighter esters of 2,4-D and 2,4,5-T may result in more contact injury and failure to translocate (Leonard, 1958; Crafts, 1960). Also excess dosage above the "optimum" herbicide concentration may cause visible injury to the foliage and/or disruption of the transport mechanism within a relatively short time (usually less than 24 hours) and stop further chemical distribution (Crafts, 1956b, 1960, 1961a; Hay, 1956a, b; Hay and Thimann, 1956b; Walker et al., 1959). Contact injury due to rapid absorption of herbicides is usually greater in young leaves before cuticular development and when the herbicide is applied to the underside of old leaves where the cuticle is thinner and the number of stomata greater (Leonard and
Crafts, 1956). Similarly, the addition of surfactants or oil may increase contact injury through increased absorption of the herbicide, but may also cause direct injury to the tissues (Robbins et al., 1952; Hull, 1956; Leonard and Crafts, 1956).

Temperature was found by Leonard and Yeates (1960) to influence contact injury by slowing translocation at low temperatures so that higher dosage could be applied without damage; in fact, increased dosage may compensate for reduced translocation. At higher temperatures equal dosage may cause contact injury and restrict uptake and movement of the herbicide by destroying the transport mechanism. However, where injury was not serious a rise in temperature markedly increased absorption and translocation (Pallas, 1960; Leonard and Yeates, 1960). The U. S. Agricultural Research Service (1958) noted that moderate temperatures ranging from 70 to 85° F were favorable for application of most herbicides, that lower temperatures reduced plant growth and retarded herbicidal activity, that higher temperatures increased volatility losses and the possibility of injury to adjacent crops, and that above 95° F all herbicidal spray treatments were generally avoided.

Hay (1956b) postulated that because of injury to the transport mechanism phytotoxic quantities of 2,4-D or 2,4,5-T probably do not move downward through the living tissues of the stem (in the phloem) of the marabu shrub (Dichrostachys nutans). Because of this
poor translocation he stressed the importance of basal application with repeat applications for good kill.

The effect of relative humidity on absorption and translocation appears to be variable. As already mentioned, Pallas (1960) found that the uptake and translocation of 2,4-D was increased by humidity and that the humidity effect was correlated with the degree of stomatal opening. Leonard and Crafts (1956) reported that for effective results relative humidity must be above 20 percent. U. S. Agricultural Research Service (1958) noted that moderate to high relative humidity increased the effectiveness of most foliar applications of herbicide by reducing losses of spray due to evaporation and aiding in the absorption of the chemicals by the leaves. They also noted that the disadvantages of low humidity could be overcome in part by the addition of oil in the spray mixture. However, Leonard (1961) found that when relative humidity was high the acid form of 2,4,5-T was absorbed and translocated poorly and there was no appreciable difference between 95 and 70 percent relative humidity; but at or near saturation the absorption and translocation of both ester and acid forms of 2,4,5-T was markedly increased.

Fate of Herbicides

Loss of herbicides on and in plants may occur in many ways. Salts and acids of phenoxy herbicides may be precipitated in the spray mixture even before application (Klingman, 1961). After application
the herbicides may dry out and crystallize on the leaf surface (Leonard, 1958), may be lost through volatilization (Day et al., 1959), may be destroyed by photo-oxidation (Carrol, 1949; Hansen and Buchholtz, 1952; Evans and Smith, 1954), or rain may wash the herbicide from the leaf (U. S. Agricultural Research Service, 1958). Loss of herbicides from rain varies with the formulation, rate of application, temperature, humidity, and susceptibility of the plants. The oil-like esters or acids in an oil carrier tend to resist washing from the plant even though rain occurs soon afterward; salts are readily washed from the plant (Weaver et al., 1946). Klingman (1961) estimated that in most cases a rain-free period of 6 to 12 hours is adequate for effective weed control.

After absorption in the plant the fate of herbicides is determined by many factors including morphological characteristics, physiological processes, biochemical properties of the protoplasm, and various environmental factors that affect growth (Shaw et al., 1960). Studies with radioactive 2,4-D showed that losses may occur through adsorption in the cuticle, accumulation in the vacuoles of cells of the leaf and stem or by chemical binding in the sieve tubes and other cells (Crafts, 1953, 1956b, 1959, 1961a, b; Hay, 1956b; Leonard and Crafts, 1956; Clor and Crafts, 1957; Crafts and Yamaguchi, 1958). Herbicides may even be lost through leakage from the roots (Clor and Crafts, 1957; Crafts, 1961b).
After absorption in the living cells of the leaf, the herbicides may be metabolized and given off as \(^{14}\text{O}_2\) (Holley et al., 1950; Fang et al., 1951; Weintraub et al., 1952a, 1956; Blackman, 1956; Crafts, 1956a; Bach and Fellig, 1961). Crafts noted that this loss occurred gradually after 24 hours. However, the amount lost as \(^{14}\text{O}_2\) is estimated to be relatively small (less than 17 percent) (Holley et al., 1950; Fang et al., 1951; Weintraub et al., 1952a; Blackman, 1956; Shaw et al., 1960; Bach and Fellig, 1961).

Metabolism and loss of herbicide activity in phenoxy herbicides may also occur through minor alterations in the structure of the molecule, partial degradation or hydrolysis of the side chains, breakdown into unknown products, formation of nontoxic complexes with other substances, and conjugation of the herbicides or its metabolites with plant substrates (Holley et al., 1950; Fang et al., 1951; Holley, 1952; Jaworski and Butts, 1952; Weintraub et al., 1952b; Weintraub, 1953; Jaworski et al., 1955; Blackman, 1956; Butts and Fang, 1956; Hay, 1956b; Hay and Thimann, 1956a, b; Leonard and Crafts, 1956; Crafts and Yamaguchi, 1958; Fang, 1958; Morre and Rogers, 1960; Pallas, 1960; Shaw et al., 1960; Bach and Fellig, 1961). Degradation of 2,4-D in the plant begins soon after its absorption in the plant (Blackman, 1956; Hay and Thimann, 1956a; Ashton, 1958, 1959; Fang, 1958). As much as one-half may be lost in one day and 65 to 80 percent in five to seven days (Holley et al.,
Because of this generally rapid breakdown of 2,4-D and related compounds, it has been reported that only a small amount of herbicide activity remains in the plant after a few months. Reports of prolonged activity were explained as being due to prior injury to dormant buds or immature leaves rather than to 2,4-D residues in the plant (Eames, 1950; Tukey, 1950; Tullis and Davis, 1950). However, Walker et al. (1959) with chromatograph tests showed that there was very little breakdown of 2,4,5-T salts in oak and sweetgum; Crafts (1961a) noted that phenoxy herbicides may accumulate in young parenchyma cells and result in prolonged activity; Weintraub et al. (1954a) reported that some 2,4-D remained active in the buds of cherry trees for a period of up to five months. Emrick and Leonard (1954) found that delayed kill of most live oak sprouts from spray applications of 2,4-D amine occurred between 2 and 3 years after treatment; and Leonard (1957) noted herbicide activity (verified by bioassay) for 3 to 5 years after phenoxy herbicide concentrates were applied to cuts of sprouting tree species. Emrick and Leonard found that the 2,4-D and 2,4,5-T herbicides were bound in the wood and later released and drawn into the shoots during the process of transpiration. On the other hand, toxicity of some herbicides may be increased or nonphytotoxic compounds may be made toxic.
by metabolism in plants (Synerholm and Zimmerman, 1947; Wain and Wightman, 1954; Wain, 1955; Crafts, 1960; Morre and Rogers, 1960).

Because of the losses of 2,4-D (and 2,4,5-T) resulting from various causes, because of disruption of the transport mechanism due to contact injury and other effects, because of better methods of measuring translocation, and for other reasons, there is an increasing number of reports indicating that most of the 2,4-D applied to and/or absorbed by the leaves of plants remains in the leaves at or near the point of application and only a small amount (1 to 15 percent) is translocated out of the leaves (Hay, 1956b; Clor and Crafts, 1957; Fang, 1958; Crafts, 1959, 1961b). Another factor receiving increasing attention is the narrow threshold concentration for maximum herbicide effect (Hull, 1961). High concentrations of herbicide injure the phloem and block translocation; at low concentrations lethal quantities are simply not translocated.

**Herbicidal Action**

A review of the literature makes it apparent that there is no general theory of action for hormonal herbicides (Koepfli et al., 1938; Veldstra and Booij, 1949; Thimann, 1951; McRae et al., 1953; Muir and Hansch, 1953, 1955; Veldstra, 1953; Weintraub, 1953; Loomis, 1955; Wain, 1958; Woodford et al., 1958; Audus, 1959; Galston and Purves, 1960). These reports indicate that many mechanisms may be
involved. Suggested mechanisms of toxic action include respiratory depletion of carbohydrates and starvation of the plant, blockage of photosynthesis, abnormal growth proliferation, disorganization or crushing of the phloem, production of abnormal or toxic cell metabolites, protein hydrolysis or coagulation, abnormal phosphatase activity, unpolarized cell division, increased cell permeability and leakage of contents, derangement of various other metabolic and physiological processes (Norman et al., 1950; Mitchell, 1951; Weintraub, 1953; Leopold, 1955; Loomis, 1955; Butts and Fang, 1956; Crafts, 1956c, 1961a; Currier, 1956; Meyer and Anderson, 1956; Woodford et al., 1958; Andus, 1959; Galston and Purves, 1960; Shaw et al., 1960).

Surficially visible symptoms of phenoxy herbicidal toxicity may include malformations, marked deficiency-cell formation, yellowing or reddening of leaves, gall formation and other tumorous growths, proliferation of adventitious roots, tissue collapse, and death (Robbins et al., 1952; Crafts, 1961a). Death may occur within a few days, weeks, months, or may even take years (Emrick and Leonard, 1954; Leonard and Crafts, 1956; Leonard, 1957; Crafts, 1961a). Because of this delay Klingman (1961) suggested waiting until the next growing season before attempting an evaluation of effects.

The relative importance of various physiological factors in determining herbicidal specificity for different classes and species of plants has been discussed by various authors. Wain (1955, 1958) and
Shaw et al. (1960) stressed the importance of differential metabolism of herbicides (or potential herbicides) by plants. Butts and Fang (1956) reported that differential absorption and translocation were primary factors contributing to the selective action of herbicides. Weintraub et al. (1956), after comprehensive studies with a number of herbicides on both broadleaf and cereal plants, concluded that, compared to absorption and metabolic inactivation, translocation played the major (but not exclusive) role in determining species or compound specificity. Other factors affecting herbicidal selectivity may include kind and concentration of herbicide formulation; carrier and surfactant effect; time of application (both seasonal and time of day); plant growth habit; physiological and morphological differences in plants; age and stage of development in plants (Norman et al., 1950; Ahlgren et al., 1951; Ennis et al., 1952; Loomis, 1955; Currier, 1956; Leonard and Crafts, 1956; Crafts, 1961b; Rediske, 1961).
CHAPTER III

STUDY AREAS

The study was conducted mainly in two areas, one near Tombstone and one near Tucson, Arizona. Major phases of the study were conducted in the Tombstone area during 1957 and 1958 and in the Tucson area from 1959 to 1962. Both areas were located where vegetative, slope, and soil conditions were uniform.

Tombstone Study Area

Description

The Tombstone study area is located in the Chihuahuan Desert 8 miles south of Tombstone on the former Pete Keller Ranch (Figure 1).

The type is described by Benson and Darrow (1954) as an isolated island of Chihuahuan Desert flora within the desert grassland which occurs at an elevation normally suitable for the development of a desert grassland type and which is almost entirely restricted to limestone hills and shallow favorable soils developed from limestones. Shantz and Zon (1924) and Nichol (1952) divided the area between desert (mes-
Figure 1. General view of the Tombstone study area located 8 miles south of Tombstone, Arizona.
quite) grassland and creosotebush-southern desert shrub. Darrow (1944) mapped the area as desert shrub grassland.

**Topography and Soils**

The area is located in Section 23, T21S, R22E, Gila and Salt River Base and Meridian. It is in a broad section of the upper San Pedro Valley on a gentle, west-facing upland slope at an elevation of approximately 4,200 feet. The soil is a shallow, grayish-brown, gravelly-loam phase of the Cave series underlain with a hard caliche layer at 6 to 10 inches under the surface. It was formed from mixed alluvium strongly influenced by limestone and is highly calcareous. Occasional small rocks appear on the surface and are mixed throughout the profile. Slopes average from 1 to 2 percent.

**Climate**

The climate of the area is similar to that at Tombstone. Summers are moderately hot and winters are mild. Temperatures seldom go above 100° F or below 10° F and temperatures below zero are unknown. The frost-free period extends from about the last of March to the last of November (Sellers, 1960).

Rainfall closely approximates that of the Y-Lightning Ranch and Fairbank, Arizona, weather stations, which are similarly situated in

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1 Personal communication from Dr. Stanley W. Buol, Department of Agricultural Chemistry and Soils, University of Arizona, Tucson.
the valley and at approximately the same elevation. Interpolations from these stations indicate that the average annual rainfall on the study site is approximately 11.84 inches (Table 1). Monthly patterns indicate that approximately 60 percent of this precipitation falls during the three warm-season months of July, August, and September, and less than 20 percent falls during the three wettest cool-season months of December, January, and February. Comparisons also indicate that annual rainfall was below average during 1960, 3 to 6 inches above average during 1958 and 1959, and approximately average in 1957 and 1961.

**Vegetation**

Various reports from early expeditions through the San Pedro Valley describe an abundance of grama grass in the hills; unbroken plains covered with a uniform growth of upland grama but with not a tree or shrub visible; in the more arid localities a thick "chapporal" of "mezquit" and its thorny associates; and parts of the river bottom covered with very high grass and small "mezquit" trees resembling orchards (Bartlet, 1854; Parry, 1859; Cooke, 1878; Cooke et al., 1938).

At present the study area is dominated mainly by shrubs with only a few relict grasses remaining. Creosotebush, whitethorn, and tarbush are the dominant shrubs. Relict grasses include bush muhly, black grama, and spike dropseed (*Sporobolus contractus*).
Table 1. Rainfall records from Y-Lightning Ranch and Fairbank, Arizona, weather stations and estimated mean annual rainfall at the Tombstone study area.\(^1\)

<table>
<thead>
<tr>
<th>Location</th>
<th>'Year'</th>
<th>Rainfall by months</th>
<th>'Annual Rainfall'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y-Lightning Ranch, elevation 4,480 feet, 12 miles SW of study site</td>
<td>1957</td>
<td>1.15</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>1958</td>
<td>0.15</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>1959</td>
<td>0.00</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>1960</td>
<td>2.982</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>1961</td>
<td>0.68</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>22-yr. mean</td>
<td>0.84</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>1940-61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fairbank, Arizona, elevation 3,860 feet, 9 miles NW of study site</td>
<td>1957</td>
<td>1.33</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>1958</td>
<td>0.00</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>1959</td>
<td>0.00</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>1960</td>
<td>1.37</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>1961</td>
<td>1.27</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>49-yr. mean</td>
<td>0.65</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>1910-61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study site, elevation 4,200 feet, 8 miles SW of Tombstone, Arizona</td>
<td>Estimated mean(^3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Data obtained or calculated from records reported by U. S. Weather Bureau (1957-61) and Sellers (1960).
2. Records missing, estimated monthly rainfall.
3. Estimate based on altitude and rainfall relationships with adjacent stations.
Reports cited by Griffiths (1901) indicate that the major change in vegetation in this area occurred between 1880 and 1900.

**Tucson Study Area**

**Description**

The Tucson study area is located in the southern desert shrub type 15 miles southeast of Tucson near the abandoned railroad stop of Esmond (Figure 2). The area is in the upper edge of the southern desert shrub bordering the desert grassland. It was mapped as creosotebush desert by Shantz and Zon (1924), as creosotebush-saltbush desert by Nichol (1952), and as Arizona Sonoran Desert by Benson and Darrow (1954).

**Topography and Soils**

The area is located in Section 25, T15S, R15E, Gila and Salt River Base and Meridian. It is in the lower alluvial outwash slopes of the Rincon Mountains on a gentle, west-facing upland slope at an elevation of 3,000 feet. The soil is a moderately deep to shallow, reddish-brown, sandy-loam member of the Continental series developed from dissected alluvium.\(^1\) It is similar to that of the Tombstone study area but is slightly deeper and has a soft caliche layer. The slope is approximately 1 percent.

\(^1\) Personal communications from Dr. Stanley W. Buol, Department of Agricultural Chemistry and Soils, University of Arizona, Tucson.
Figure 2. General view of the Tucson study area located 15 miles southeast of Tucson, Arizona.
Climate

The climate of the area is similar to that of Tucson. Temperatures commonly exceed 100° F in the summer but rarely drop below 10° F in the winter. The frost-free period generally extends from the middle of March to the middle of November (Sellers, 1960).

Estimated mean annual rainfall at the Esmond site is 11.5 inches (Table 2), slightly less than that at the Tombstone study area. However, a smaller proportion of the precipitation, 50 percent of the total, falls during the rainy-season months of July, August, and September (Sellers, 1960). Records from three stations near the study area show that rainfall was approximately average during the years 1959 and 1961 and 2 to 3 inches below average in 1960 and 1962 (Table 2).

Vegetation

The vegetation of the area consists of almost pure stands of creosotebush with only occasional associated shrubs of mesquite, whitethorn, prickly pear, and cholla cacti. Since the area is less than one-half mile from livestock water and has a long history of heavy grazing, very few understory grasses or weeds of any kind remain. Those remaining consist mainly of zinnia, coldenia (Coldenia canescens), and fluffgrass.

No records of past vegetative conditions on the site are available. However, some information is available from a similar area in
Table 2. Monthly and annual rainfall during the study period and estimated mean annual rainfall at the Tucson study area.\(^1\)

<table>
<thead>
<tr>
<th>Year</th>
<th>Rainfall by months</th>
<th>Annual rain-</th>
</tr>
</thead>
<tbody>
<tr>
<td>1959</td>
<td>0</td>
<td>0.34</td>
</tr>
<tr>
<td>1960</td>
<td>2.23</td>
<td>0.88</td>
</tr>
<tr>
<td>1961</td>
<td>1.24</td>
<td>0.01</td>
</tr>
<tr>
<td>1962</td>
<td>1.00</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Estimated mean\(^2\) 11.50

\(^1\) Data from Institute of Water Utilization, Arizona Agricultural Experiment Station, Tucson, Arizona.

\(^2\) Estimated from Greene (1959) altitude-precipitation curve for southeastern Arizona.
the Sonoran Desert, approximately 20 miles west of the study site. An analysis of this area, which had been protected for 50 years, indicated that even though shrub composition may have been similar the density of palatable shrubs and perennial grasses was greater originally than at present (Blydenstein et al., 1957).
CHAPTER IV

EXPERIMENTAL PROCEDURES

In this study both foliar-spray and radioisotope methods were used to treat plants with herbicides. Treatments were made in the field under natural conditions.

**Foliar-spray Methods**

Foliar-spray methods were used to determine differences in the susceptibility of creosotebush to various formulations of herbicides; differences in seasonal and time-of-day susceptibility of plants to herbicides; the effects of different carrier formulations, photosynthate additives, and pH levels on toxic effects of herbicides; differences in susceptibility of different-aged plants and sprouts to herbicides; and the effects of soil-depth factors on the toxic effects of herbicides. Herbicides studied were various ester formulations of phenoxy herbicides.  

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1 The author is indebted to the following companies for herbicides furnished: Dow Chemical Co., Midland, Michigan; California Spray-Chemical Corp., Richmond, California; Chipman Chemical, Inc., Palo Alto, California; Thompson Chemicals Corp., St. Louis, Missouri.
Application Methods

Foliar treatments of herbicides were made by spraying selected plants with the herbicide emulsion from a two-gallon Hudson hand-sprayer. Generally, the spray emulsion consisted of the herbicide in a 2-percent (by weight) diesel oil-water emulsion. The herbicide and oil were mixed together, then the water was added and mixed by vigorous shaking. The emulsion was kept mixed by shaking at intervals during the spraying operation. The spray was applied from a teejet nozzle with either a 6502 or 8002 tip and at a pressure of 40 to 50 pounds per square inch (psi). The plants were sprayed from at least two directions in an attempt to wet all plant surfaces uniformly. The amount of herbicide in the mixture was calculated in terms of pounds of acid equivalent (ae) per acre and varied with the treatment involved.

Except for the screening tests in which several formulations were used, only the propyleneglycolbutylether esters of 2,4-D and 2,4,5-T were used in the foliar-applied herbicide treatments.

Measurements and Evaluation

The treatment plots were 25x100 feet in size, except on the fertilized areas where 10x100 foot plots were used. Plants to be treated were located along a 100-foot chain stretched between two stakes centrally located at the ends of each plot; the chain thus dissecting the plot longitudinally. In each plot either 10 or 20 medium-sized, vigorous
plants were located and recorded by measured distances along the chain and at estimated distances to the right or left of the chain. Both plots and plants were randomly selected for treatment.

In the foliar studies a minimum of 10 plants were sprayed for each treatment. However, in some tests not made in a series, a total of 20 or 40 plants were sprayed for each treatment.

Check-plot treatments were made for comparison as needed. This and previous studies showed that natural death loss of medium-aged creosotebush plants, such as used in this study, was virtually nonexistent. Also, the 2-percent diesel oil-water emulsion sprayed on plants in the check plots had negligible effects on creosotebush.

Each plant was relocated by the same method for treatment evaluation. In general, it was necessary to wait 16 to 18 months for final evaluation of treatment effects. Since treatment effects varied widely with season of year and between seasons, it was necessary to evaluate treatments by two methods. Where total-kill was substantial, plants were recorded as dead or alive and the percentage total-kill was calculated. Where total-kill was low or negligible, effects of treatment were evaluated by percentage topkill. To determine percentage topkill, treated plants were placed in 10-percent injury classes, based on the relative percentage of the top killed, and averaged.
Radioisotope Methods

Radioautographs of radioactive 2,4,5-T* and 2,4-D* were used to measure the absorption of herbicide through various parts of the creosotebush plant and to determine the rate and direction of its translocation in the plant at various times of the day and seasons of the year. In addition, it was used to measure the effect of photosynthate additive in the treatment mixture. Relative absorption and translocation results from the radioisotope studies were used to interpret variations in plant response to herbicides obtained in the hand-spray studies. Radioisotopes used were the acid forms of 2,4-D-2-C\textsuperscript{14} and 2,4,5-T-1-C\textsuperscript{14}.

Radioisotope Treatment Procedures

The procedures used to treat the plants with radioactive herbicides and to radioautograph the results are modifications of the methods used by Leonard and Crafts (1956) and Yamaguchi and Crafts (1958).

The radioactive herbicide was applied to the various parts of the plant in a stock solution. The treatment varied slightly with the herbicide used. The treatment dose for 2,4,5-T* consisted of 45 micrograms (\(\mu g\)) of 2,4,5-T* with 0.1 microcuries (\(\mu c\)) of radioactivity, dissolved in a 1:1 solution of ethyl alcohol and water and with 0.1 percent

\[1\] The asterisk on 2,4,5-T or 2,4-D is used to indicate the radioactive form of the herbicide.
nonionic surfactant (liquid Joy) added. The stock solution was made up to contain 22.5 µg of 2,4,5-T* and 0.05 µc of radioactivity per 0.01 ml of solution so that 0.02 ml of solution could be used per treatment. This dosage was suggested by Yamaguchi and Crafts (1958) to provide adequate activity and penetration for herbicides which produce only minor injury and formative effects and have relatively low penetrability. The treatment dose for 2,4-D* was similarly prepared and contained the same radioactivity per dose but contained only one-half the concentration of 2,4-D*, 22.1 µg of 2,4-D*, and 0.1 µc of radioactivity per 0.02 ml dose. The lower concentration of 2,4-D was used to reduce contact injury, which is higher from 2,4-D than 2,4,5-T, and still provide adequate penetration. Plants selected for treatment were medium-aged, vigorous plants 2 to 3 feet in height. Each treatment was replicated 2 or 3 times and/or repeated for verification of results. Check plants were mounted as needed for comparison.

The radioactive herbicides were applied to a small side branch which was approximately 5 to 7 cm long, contained at least 5 pairs of leaflets, and intersected the main branch 6 to 10 cm below its apex. This procedure was followed to indicate upward as well as downward movement and still treat young actively growing tissues. The treated branch was marked by a small cloth flag fastened to the main stem just above the axil of the treated branch. Ground markings were used to identify treated bushes.
The treatment dose was applied to approximately five pairs of leaflets. Immediately following application of the stock solution, an additional 0.02 ml of 2-percent diesel oil-water emulsion was applied to the same leaves to reduce drying of the herbicide and facilitate entry of the herbicide into the leaf. This procedure was followed since the diesel oil would not go into solution with the stock solution and make uniform applications possible. Both the stock solution and the oil-water emulsion were applied with 0.1 ml micro-pipettes graduated in 0.01 or 0.02 ml divisions. The pipettes were operated by mouth, with cotton string inserted in the barrel for safety, or by a rubber bulb fastened on the end of the pipette.

The general treatment procedure was to apply the radioactive herbicide and then allow 24 hours for absorption and translocation before mounting. After the 24-hour period the treated plants were dug up and the treated branch mounted on one side of 14x17-inch heavy paper folders. Two mounting procedures were used. In the first procedure, five treated pairs of leaflets were mounted, one leaflet being wiped free of radioisotope with 95 percent ethyl alcohol just prior to mounting and the other unwiped. After the treated leaflets were mounted the terminal leaves and bark sections of the main branch and root were mounted. The bark sections were cut at 5 cm intervals along the main stem and root and were cut approximately one cm long. Thus, the samples showed translocation at 6 cm intervals. The bark samples were prepared by
cutting a slice of bark and wood from the stem with a scalpel, peeling the bark from the wood at the cambial layer, and trimming the section into 1 cm lengths. The sections varied from 1 to 5 mm wide depending on the size of the stem or root from which they were taken. This method was subject to some error if translocation was greatly one-sided down the side on which the treated branch was attached, and if this area was missed in collecting the bark sample. The sections were then glued to the mounting paper with the cambium and phloem layers exposed for radioautographing. Sections above the treated branch and root sections were marked to facilitate later measurements. The samples dried rapidly on the sheets, and the radioisotope was confined to the cut sections. Usually four plants were mounted on one-half of one side of the folder. After each plant was mounted, a plastic ruler was thumb-tacked with its flat side pressing the sections to prevent curling, disturbance, or contamination of the sections. When mounting was completed and the bulk of the moisture evaporated, the rulers were removed and the folder folded to enclose the mounts and prevent radioactive contamination. The folders were then placed in 8 1/2x14-inch plant presses made of 1/2-inch plywood with cardboard spacers and further air-dried for 5 to 7 days before radioautographing.

The second mounting procedure was the same as the first except that the whole apical branch of the main stem and the treated side-branch was trimmed and mounted as one piece. The trimming included thinning
of dense branches and leaves and slicing one side of the main branches to expose the phloem. Subsequent bark sections of the stem and root were then taken as above. A sample mount is shown in Figure 3.

In the studies to determine differences in absorption by various tissues, treatments were applied to the bark, flowers, fruits, old leaves, or young leaves of the terminal portion of the main branch. After treatment the complete terminal portion of the plant was mounted, and then bark sections were taken below at the regular intervals. Only downward translocation was measured.

Rubber gloves were worn for protection and instruments were cleaned with alcohol and water after use on each plant part. The mounting paper was thumb-tacked to a 14x17-inch plywood board during the mounting procedure, and the half of the folder not used for mounting the specimens was covered with aluminum foil and used as a work table.

Radioautograph Method

The dried plant mounts were radioautographed with Kodak Medical X-ray, duplitized, blue brand film in special 14x17-inch presses made of 1/2-inch plywood. In order to obtain good contact between the samples and the film and to prevent contamination between samples, the presses were made up of successive layers in the following order: a plywood back lined on the inside with aluminum foil or two layers of disposable film wrapping paper; the X-ray film; the plant mounts,
Figure 3. Leaf- and bark-sample mounts of creosotebush plants treated with C$^{14}$-labeled 2, 4, 5-T*.
opened and facing the film; a 1/2-inch thick layer of sponge rubber; a
double layer of paperboard with aluminum foil between; repeat layers
of sponge rubber, plant sample, X-ray film, wrapping paper or foil,
and plywood backing. When assembled the press was strapped together
firmly, placed in a double-layered black plastic bag, and strapped
again. The film was exposed for 30 days and then removed, developed
and fixed in X-ray developer and fixer (Figure 4).

Radioautograph Measurements

Absorption

Absorption of the radioactive herbicide was estimated qualita-
atively by two methods. The first method used was a comparison of the
relative degree of radioactivity shown by the radioautographs of wiped
leaves treated at different times. This method was indicative only since
it was not known how much of the radioactive material was driven into
the leaf by alcohol absorption during the wiping process.

The second procedure was the indirect method of estimating the
amount of absorption based on the relative translocation of the radio-
isotope. This method was also indicative only since it presupposes that
whenever absorption takes place conditions are also suitable for trans-
location.
Figure 4. Radioautograph of plant mounts of creosotebush treated with $\text{C}^{14}$-labeled 2,4,5-T*. Plants were treated with 0.1 $\mu$c of radioactivity, treatment time was 24 hours, and the sample sheet was exposed to the film for 30 days.
Translocation

Translocation of the radioactive herbicide was determined from the radioautographs by observing the occurrence of the radioactive tracer in the various plant sections. Upward translocation was determined as the greatest distance the tracer occurred above the point of intersection of the treated branch with the main branch. It was computed from the mounted segments showing radioactivity or measured directly where the apical branch was mounted. The method of mounting generally limited measurements of upward translocation to about 6 cm. Downward translocation was computed by measuring the distance from the treated leaves of the side branch to the point of intersection with the main branch plus the accumulative distances between mounted segments showing radioactivity. Partial translocation beyond the last plant segment showing radioactivity was estimated based on the degree of radioactivity shown by the radioautograph of the last section.

Statistical Analyses

In general, the t-test was used at the 5- and 1-percent levels to test the significance of treatments (Snedecor, 1959). However, because of the limited replication of treatments possible in a study of this scope, trends, indicated by the 10-percent level of probability, were evaluated.
CHAPTER V

SCREENING AND RATE TESTS WITH PHENOXY HERBICIDES

Screening and rate tests were conducted with various phenoxy herbicides to determine the formulations of herbicides and rates of application most effective on creosotebush. All treatments were made by foliar-spray methods. Symptoms of phenoxy herbicide effects on creosotebush were observed on plants treated in both the screening and rate tests.

Most of the literature applicable to this section was reviewed in Chapter II.

Methods of Study

Screening Tests

In the screening tests, formulations of 2,4-D and 2,4,5-T were applied at rates of approximately two and eight pounds per acre ae. Ten to 30 plants were sprayed for each treatment. Applications were made when plants were highly susceptible to treatment during the month of August in 1957, 1958, and 1959.
Rate Tests

In the rate tests, concentrations of propyleneglycolbutylether esters of 2,4,5-T, at rates from 1/4 to 8 pounds per acre, were applied during August and September in 1958, 1959, and 1961. A minimum of 10 plants were sprayed for each treatment.

Results of Screening Tests on Creosotebush

Screening tests with foliar-applied phenoxy herbicides on creosotebush showed differences between both (1) the different kinds of herbicides, when applied at the same rates and during the same season of the year, and (2) the same herbicide when applied at the same rates but at different seasons of the year.

A comparison of the data in Table 3 shows that when applied during the same season of the year, the trichlorophenoxyacetic formulation was more effective on creosotebush than the trichlorophenoxypropionic. Also, the low-volatile propyleneglycolbutylether, isooyctyl, and tetrahydradurfuryl esters of 2,4-D and 2,4,5-T were generally more effective than the pentyl. The greater effectiveness of the low-volatile esters corresponds to the findings of King and Kramer (1951) and Crafts (1960, 1961a) and suggests that absorption and/or translocation in creosotebush occurred slowly and continued over a long period of time, minimizing contact injury and maximizing translocation of toxic amounts of herbicide.
Table 3. Relative toxicity of phenoxy herbicides applied to creosotebush in August of three different years.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Formulation</th>
<th>1957</th>
<th>1958</th>
<th>1959</th>
</tr>
</thead>
<tbody>
<tr>
<td>2, 4-D</td>
<td>Propyleneglycolbutylether esters</td>
<td>10</td>
<td>70</td>
<td>90</td>
</tr>
<tr>
<td>2, 4, 5-T</td>
<td>Propyleneglycolbutylether esters</td>
<td>23</td>
<td>67</td>
<td>100</td>
</tr>
<tr>
<td>2, 4-D-2, 4, 5-T</td>
<td>50-50 mixture propyleneglycolbutylether esters</td>
<td>7</td>
<td>63</td>
<td>100</td>
</tr>
<tr>
<td>Silvex</td>
<td>Propyleneglycolbutylether esters</td>
<td>7</td>
<td>37</td>
<td>80</td>
</tr>
<tr>
<td>2, 4-D-2, 4, 5-TP</td>
<td>50-50 mixture propyleneglycolbutylether esters</td>
<td>3</td>
<td>30</td>
<td>90</td>
</tr>
<tr>
<td>2, 4, 5-T</td>
<td>Pentyl ester</td>
<td>0</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>2, 4-D-2, 4, 5-T</td>
<td>24-23 mixture pentyl esters</td>
<td>5</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>2, 4-D</td>
<td>Isooctyl ester</td>
<td>30</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>2, 4, 5-T</td>
<td>Isooctyl ester</td>
<td>30</td>
<td>45</td>
<td>40</td>
</tr>
<tr>
<td>2, 4-D-2, 4, 5-T</td>
<td>33-32 mixture isooctyl esters</td>
<td>0</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>2, 4-D</td>
<td>Isooctyl ester</td>
<td>20</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>2, 4, 5-T</td>
<td>Isooctyl ester</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>2, 4, 5-T</td>
<td>Tetrahydrofurfuryl ester</td>
<td>7</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>
Where it was possible to make year-to-year comparisons, the data showed that the percentages of total-kill from herbicide treatments made in 1958 were generally about twice as high as those in 1959 and many times higher than those in 1957. Factors contributing to the marked variation in yearly toxicity will be discussed under seasonal susceptibility.

Results of Rate Tests with 2,4,5-T

An analysis of the rate studies with 2,4,5-T showed wide year-to-year differences in the susceptibility of creosotebush to the same herbicide (Figure 5). In 1958, an apparently favorable year, 100 percent total-kill resulted from as low as 1 pound of 2,4,5-T per acre. In 1959 and 1961 the same rate produced only 55 and 0 percent, respectively. Similarly, total-kill from the 8-pound rate during the same 3 years was 100, 80, and 40 percent, respectively.

Since average annual precipitation was 5 inches above average in 1958 and average in 1959 and 1961, the higher rainfall and associated environmental conditions apparently contributed to this difference. However, there was little difference in the stage of phenological development of the plants on the three spray dates.
Figure 5. Total-kill of creosotebush at various rates of 2,4,5-T applied during periods of high plant susceptibility.
Symptoms of Phenoxy Herbicides on Creosotebush

The surficially visible effects of phenoxy herbicides on creosotebush were not spectacular. In general, treated leaves remained normal in shape but went through a series of color changes which varied somewhat with the rate of treatment. On plots treated with moderate to high rates of herbicides the leaves turned greenish-yellow or greenish-brown in about five days. In 5 to 10 days the leaves had become light brown or tan in color, in 10 to 20 days they had turned dark brown, and after 28 to 30 days they had begun to drop from the plants. On plants treated at low rates of application the yellow-green color of the leaves often persisted for several months and the colored leaves were often intermixed with normal appearing green ones. Sparse to dense regrowth of leaves occasionally occurred on the branches, but other formative effects were not surficially evident. On plants treated with light to moderate rates, basal sprouts often appeared in 15 to 25 days after treatment. During subsequent growing seasons, these sprouts often turned yellow and died, apparently as a result of the herbicide. Death of these sprouts occurred as late as 12 to 15 months after treatment. Typical effects of treatment and resprouts are shown in Figure 6.
Figure 6. Effects of 2,4,5-T on creosotebush. Upper photo shows a creosotebush plant approximately three weeks after treatment; center photo shows regrowth on a branch approximately one year after treatment; lower photo shows basal sprouts approximately one year after treatment.
CHAPTER VI

SEASONAL SUSCEPTIBILITY OF CREOSOTEBUSH

This phase of the study was designed to define more accurately the season of highest susceptibility of creosotebush to herbicides and to study the factors influencing this susceptibility. Both foliar-spray and radioisotope methods of treatment were employed to study absorption, translocation, and toxic effects of the herbicides.

Methods of Study

Foliar-spray Methods

Foliar applications of herbicides were made at monthly and semimonthly intervals on both study areas. At the Tombstone site, applications of propylene glycol butylether esters of 2, 4-D and 2, 4, 5-T and 50-50 mixtures of the two were made at rates of 2 and 8 pounds per acre from July, 1957, through September, 1958. At the Tucson site only 2, 4, 5-T was applied at the 8-pound rate from December, 1958, through November, 1959. Applications were made at approximately the same period during the day on each date, i.e., between 11 am and
3 pm. Ten plants were sprayed per treatment in the studies at the Tombstone site and 20 plants each at the Tucson site.

In an attempt to delineate the important factors influencing seasonal susceptibility, records were kept on climatic and edaphic factors and observations made on the phenological development of creosotebush. Because of the remoteness of the Tombstone area and difficulties involved in keeping accurate and continuous records, climatic data from the nearest available Weather Bureau or private stations were used. Daily rainfall totals were obtained from the temporary weather station on the Keller Ranch located one mile west of the study site; daily maximum and minimum temperatures were obtained from Tombstone; and daily maximum and minimum humidity data were interpolated from Tucson humidity records adjusted with fragmentary data from the Apache Powder Company Plant Weather Station near St. David.

At the Tucson area, temperature and humidity data along with rainfall data mentioned earlier were obtained on the site through the cooperative efforts of the Institute of Water Utilization of the University of Arizona. Soil moisture data were obtained on spray dates by the gravimetric method. Three samples were taken on each date from randomly selected locations at the 3- to 5-inch depth. Data were collected from this depth because it has been observed from personal experience that moisture at this level provides the best measure of rainfall effective for plant growth under arid and semiarid southwestern
conditions. This zone is shallow enough to be wet by effective summer storms yet deep enough to escape rapid desiccation by evaporation. Phenological observations on leaf, flower, and seed development were made on spray dates and at other periodic intervals throughout the season.

Radioisotope Studies

Three series of radioisotope tests were conducted concurrently with the hand-spray treatments at the Tucson site. The first series of radioisotope tests was designed to determine the seasonal pattern of absorption and translocation in creosotebush and to determine if there was a correlation between absorption and translocation as measured by the radioisotope method and total-kill as measured by the foliar-spray method. Plants were treated with 2,4,5-T* on the same dates as the seasonal foliar-spray treatments and mounted for radioautographing the following day. Three treated-plants and a check-plant were mounted on each date.

The second series of radioisotope tests was run during the 1959 and 1961 seasons of greatest susceptibility to determine the rate and pattern of translocation in creosotebush and to serve as a check on the 24-hour translocation exposure time used in the major studies. A series of plants was treated with 2,4,5-T* and then subsequently mounted at increasing hourly intervals the first day, then at increasing
daily intervals up to 60 days. One plant was mounted for each test during the first 3 hours after which 2 plants were mounted at each subsequent time interval.

A third series of radioisotope tests was made on various plant organs and tissues to determine differences, if any, in the absorption and subsequent translocation of herbicides through new or old leaves, flowers, seeds, or young bark. Repeated tests were made in 1960 and 1961 at the Tucson site during the late-flower-early-seed stage of growth.

Factors Affecting Seasonal Toxicity of Herbicides

Variations in plant susceptibility to herbicides during different seasons of the year have long been recognized. In general, there is maximum susceptibility to herbicides during the flush season of growth and when translocation of food reserves to below ground parts is most rapid. This period of susceptibility usually occurs when twigs and roots are making rapid growth, when leaves are abundant and have reached full development, before cuticular development is sufficiently advanced to markedly affect absorption of herbicides, and during the budding-prebloom state of some species or between flower initiation and full flowering of others (Ahlgren et al., 1951; Robbins et al., 1952; Crafts, 1953, 1956b; Young, 1954; Leopold, 1955; Leonard and Crafts, 1956; Leonard and Carlson, 1958; U. S. Agricultural Research Service,
1958; Klingman, 1961). These stages of plant development usually occur soon after initiation of plant growth during a period of favorable seasonal rainfall or stored soil moisture and following a period of growth dormancy due to a cold or dry season. As a result, the effects of herbicides are frequently influenced by various environmental factors such as rainfall, temperature, relative humidity, or level of soil moisture and may be correlated with certain morphological and physiological changes associated with different stages of phenological development in the plant. In addition, these factors interact with each other so that the relationships become very complex. In the creosotebush study, the effects and interaction of these factors were no less complex. However, certain definite relationships were found to exist. These relationships are shown during parts of three years in Figure 7.

Environmental Factors

Precipitation and Temperature Effects

Precipitation during the study period followed the normal pattern with the major concentration occurring during mid and late summer and the rest scattered through the fall, winter, and spring months.

Temperatures ranged from lows of about 8° C (18° F) in the winter up to highs of about 41° C (106° F) in summer. Differences between daily maximum and minimum temperatures were about 16 to 20° C and varied about the same amount throughout the year.
FIGURE 7- RELATIONSHIPS BETWEEN SEASONAL CHANGES IN ENVIRONMENTAL AND PHENOLOGICAL FACTORS AND THE RESULTS OF FOLIAR-SPRAY AND RADIOISOTOPE HERBICIDAL TREATMENTS MADE ON CREOSOTEBUSH DURING THREE GROWING SEASONS.
One of the most obvious relationships shown by this study is the effect of summer rainfall on plant susceptibility to herbicides. In general, highest plant kill occurred 12 to 60 days after the first summer rain of at least 1/2 inch. The peak and length of the period of high susceptibility varied with the amount and distribution of the rain.

In addition to the summer period of susceptibility to herbicides, similar periods of lesser magnitude occurred at almost any time of the year when plant growth conditions were favorable. However, the most consistent period of secondary susceptibility occurred in the spring.

During the spring the effect of significant rainfall on plant susceptibility varied with temperature. During the cold-season months of February and March, 1958, there was little immediate response to rainfall. However, as soon as temperatures approximated the 4.4-26.7° C minimum-maximum found by Dalton (1961) to be necessary for growth of creosotebush, there was also a response to herbicides. This corresponds to the findings of other workers who found that, in general, at temperatures below 5° C, 2,4-D may remain in the plant but exert little influence; between 5 and 20° C the effects slowly increased with temperature, while between 20 and 35° C translocation and the effects of 2,4-D increased rapidly (Hamner and Tukey, 1944b; Marth and Davis, 1944; Kelly, 1949; Young, 1954; Hauser, 1955; Pallas, 1960). However, Brown and Weintraub (1952) noted that growth repression was constant at temperatures between 22 and 34° C, indicating that no temperature-
limited process was involved in 2,4-D inhibition within this tempera-
ture range.

Another peak period of growth and toxicity was noted in April
and May of 1959, even though there had been practically no rainfall the
previous winter and soil moisture at the 3- and 5-inch depth was low
(less than 3 percent). However, plant-kill at the 8-pound rate was only
about 60 percent as compared to 90 and 100 percent during the previous
spring when soil moisture was higher. On the other hand, good rains
during the fall months of October and November produced moderate
increases in plant response to herbicides even though temperatures
were borderline or below the minimum for creosotebush growth.

Since the growth responses in the spring occurred regardless of
wide differences in soil moisture, spring growth may have been influ-
enced more by internal seasonal perioicity factors than by moisture
level or other external environmental influences (Kramer, 1943).

**Soil Moisture Effects**

During the summer the soil moisture pattern followed and was
sensitive to the rainfall (Figure 7). During the winter after rains
occurred, although moisture accumulated, there was no response to
herbicides until spring. As already noted, this response to herbicides
was highly variable depending at least in part on the moisture level in
the soil and the moisture storage in plant tissues. This correlates with
the work of Young (1954), Basler et al. (1961), and Pallas and Williams
(1962) who found that translocation (but not necessarily absorption), and consequently the effectiveness of 2,4-D, can be greatly reduced (50 percent or more) by moderate decreases in soil moisture. Basler et al. also observed that when the relative turgidity of plants falls below 80 percent only trace amounts of 2,4-D were translocated. They noted that the reduced translocation was due to changes in metabolism or to cellular structure and composition, rather than a direct result of water tension, since plants regained turgidity in 1 to 2 hours after watering, but the ability to translocate 2,4-D was not regained until 12 to 24 hours afterward. In addition, Pallas and Williams (1962) noted that soil-moisture stress reduced translocation by decreasing the effective area (vacuole and protoplasm) of the phloem due to cell contraction; and Skoss (1955) found that water stress increased the wax content of the cuticle which in turn reduced its permeability to aqueous solutions. However, the production of photosynthate also may have had an effect on translocation of herbicides in creosotebush since Mallery (1935) noted an inverse correlation between the moisture level of the soil and the osmotic concentration of the expressed sap of the leaves.

An examination of the data in this study indicates that, in general, soil moisture at the 3- to 5-inch depth must be above 5 percent before herbicidal activity may be effective. This is below the wilting coefficient of 6 to 6.9 percent calculated for similar soils by Mallery
(1935), and Yang and Lowe (1956) and is considerably below the wilting coefficient of 7.9 estimated for these soils in the present study.\footnote{The wilting coefficient was calculated from the moisture equivalent by the method of Briggs and Shantz (1912).}

**Humidity Effects**

Relative humidity has been reported to influence herbicide toxicity through its effects on moisture stress in the leaf, stomatal opening, drying of spray deposits, and cuticular permeability. Leonard and Crafts (1956) noted that a relative humidity above 20 percent was important for the penetration of 2,4-D. Pallas (1960) stated that more 2,4-D was absorbed and translocated at high, 70 to 74 percent, humidity than at low, 34 to 48 percent, humidity. As already noted this effect was correlated with the degree of stomatal opening. Leonard (1961) found no difference in absorption between 70 and 95 percent humidity, but at or near saturation there was a marked increase. Hopp and Linder (1946) found that low humidity caused the spray solution to dry rapidly and thereby reduced absorption. However, they found that the drying effects on spray deposits could be reduced and the penetration time extended by the addition of humectants such as carbowax and glycerine. Low humidity also affects the cuticle directly causing it to harden thereby reducing the penetrability to water (Lee and Priestley, 1924).

Relative humidity, as indicated by the data on specific spray dates in this study, was generally high during periods of high
susceptibility to herbicides and seldom dropped below the critical 20-
percent level drawn by Leonard (Figure 7). Effects of relative humidity
also appear to depend on temperature since high relative humidity in
winter had little influence on plant response to herbicide. It appears
that, under conditions similar to those in this experiment, relative
humidity may have a marked effect on the activity of herbicides only at
very low levels. Lack of effect at the higher levels may be due in part
to the humectant effect of diesel oil used in the carrier.

**Phenological Effects on Plant Susceptibility to Herbicides**

In general, when plants initiate growth the young leaves have
high absorptive capacity for herbicides but present a relatively small
absorbing surface. As growth progresses the leaf absorbing surface
increases but the absorptive ability per unit area decreases due to
various morphological and physiological changes. This was confirmed
by Fang et al. (1951), Mitchell (1951), and Weintraub et al. (1954b) who
noted that young leaves absorbed 2,4-D faster than older leaves.
Schieferstein (1957) found that there was a decrease in the rate of pene-
tration and an increase in the time-lag of penetration with increasing
thickness of cuticle as well as with increasing age of leaves. Schiefer-
stein and Loomis (1956) postulated that possibly an immature zone
around the margins of the expanding epidermal cells was largely respon-
sible for the greater susceptibility of the young leaves. Mueller and
Loomis (1954) noted that older leaves were more easily wetted with solutions containing surfactant but were more difficult to kill with hormone-type herbicides, suggesting changes in the cuticle which compensate for the loss of external wax which flakes off with age. However, decreased penetration of herbicides into mature leaves appears to result mainly from increasing thickness of cuticle which is deposited continuously until the leaf reaches maturity (Skoss, 1955), and increasing internal cuticular wax content, which also increases with age of leaf in most species (Kurtz, 1950; Skoss, 1955). Weintraub et al. (1954b) noted that after maturity absorption of 2,4-D by bean leaves remained constant until senility (yellowing) when the absorption rate dropped.

During the summer period of growth in both 1958 and 1959, transformation of creosotebush leaves from the drab gray-green color of the dry season to the dark green of the growing season occurred in just a few days, as mature leaves greened up and immature leaves and dormant buds resumed growth (Figure 7). Flowers began to appear about two weeks after the first rain, reached a peak about four weeks, and were nearly gone after six weeks. Seeds began to form 7 to 10 days after flowers appeared but did not reach maturity until late October and early November, about three months after growth was initiated. Old leaves turned yellow and most were shed 30 to 40 days after the new growth was initiated, but some hung on for two months.
The highest susceptibility of creosotebush to herbicides during the summer growth period occurred between the full-flower and mid-fruiting stage. This was also a period of active leaf growth and overlapped the period of old leaf drop. The response of creosotebush to herbicides was normal in part in that it occurred during periods of active leaf growth but was unusual in that it occurred during periods of active flowering and fruiting.

Radioisotope tests, which were made to determine if there was differential absorption and subsequent translocation of herbicide from various plant tissues or organs, showed that differential absorption and/or translocation may have been a contributing factor to the unusual response. Translocation of 2,4,5-T* was generally greater when the herbicide was applied to old leaves, flowers, seeds, and young bark than when applied to young leaves (Table 4). While the number of samples were too small for adequate statistical analysis, if real these differences could explain in part the greater absorption, translocation, and toxicity of herbicides during the active flowering and fruiting stage.

Seasonal Effects on Translocation of Herbicides

Results of the 1959 seasonal study with 2,4,5-T* showed that no translocation occurred during 24-hour tests made in the relatively dry winter and spring months, but translocation did occur during the summer
Table 4. Maximum translocation of $^{14}$C-labeled 2, 4, 5-T* from different plant tissues of creosotebush during a 24-hour period when applied in the season of high plant susceptibility.

<table>
<thead>
<tr>
<th>Date of application</th>
<th>Translocation of 2, 4, 5-T* from various tissues</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upward</td>
<td>Downward</td>
</tr>
<tr>
<td></td>
<td>'Young' 'Old' 'Young' 'Old' 'Flowers' 'Seeds' 'Young bark'</td>
<td></td>
</tr>
<tr>
<td>Aug. 29, 1960</td>
<td>0.0 6.0 3.0 3.0</td>
<td>4.5 6.0 0.0 0.0</td>
</tr>
<tr>
<td>Aug. 30, 1960</td>
<td>6.0 4.5 0.0 3.0</td>
<td>0.0 6.0 0.0 3.0</td>
</tr>
<tr>
<td></td>
<td>6.0± 5.0 15.0 6.0</td>
<td>6.0± 9.0 9.0 3.0</td>
</tr>
<tr>
<td>Avg. 1960</td>
<td>3.8 6.1 4.5 3.0</td>
<td></td>
</tr>
<tr>
<td>Relative translocation</td>
<td>100 160 100 67</td>
<td></td>
</tr>
<tr>
<td>Sept. 6, 1961</td>
<td>5.0 11.0 7.0 8.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Sept. 12, 1961</td>
<td>3.0 11.0 6.0 2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Avg. 1961</td>
<td>4.0 11.0 6.5 5.0</td>
<td>8.5</td>
</tr>
<tr>
<td>Relative translocation</td>
<td>100 275 162 125</td>
<td>212</td>
</tr>
</tbody>
</table>
growing season. Maximum translocation coincided with the summer period of high susceptibility to herbicides, reaching a peak 30 days after the start of effective rains (Figure 7).

These data show that the rate of translocation of 2,4,5-T* in creosotebush was extremely low, the maximum rates varying from 1 to 5 cm per hour. This relatively slow rate of translocation was confirmed by field observation where it was noted that leaves on the tips or basal parts of branches which were missed during the spraying operation remained green for several months after the sprayed parts had turned brown.

The slow rate of translocation in creosotebush was further confirmed by the two series of tests made during the periods of high herbicidal activity in the summers of 1959 and 1961. These tests showed that the average rate of translocation varied from 1 to 5 cm per hour during the first 24-hour period after treatment (Figure 8). These rates are in sharp contrast to the normal rates of translocation for 2,4-D in the phloem of 10 to 100 cm (4 to 40 inches) per hour reported by previous workers. This slow rate of translocation may have been due to an inherently slow rate of translocation in creosotebush or to partial or complete plugging of the vascular tissues caused by herbicidal injury.

These studies also showed that in creosotebush the maximum distance of 2,4,5-T* translocation occurred 18 to 24 hours after treatment, varying somewhat with season. This contrasts with 1- to 3-week
Figure 8. Translocation of radioactive 2, 4, 5-T* from creosote-bush leaves during various intervals of time after treatment.
AUGUST 17, 1959

PERIOD OF TRANSLOCATION

SEPTMBER 7, 1961
periods found optimum for autographing translocation in California chaparral species by Leonard and Crafts (1956). After 18 to 24 hours the radioautographs of creosotebush showed a marked reduction in downward translocation and apparent loss of 2,4,5-T*. Cessation of downward movement appears to have been due to disruption of the transport mechanism by 2,4,5-T* injury (Hay, 1956b; Hay and Thimann, 1956b; Leonard and Crafts, 1956). Loss of 2,4,5-T* may have been due to transfer of the herbicide from the phloem into the xylem and its movement into the apical regions of the plant where it was utilized, stored, or metabolized; or to conversion of the C$^{14}$ in 2,4,5-T* to C$^{14}$O$_2$ and slow diffusion of the C$^{14}$O$_2$ into the atmosphere (Holley et al., 1950; Fang et al., 1951; Weintraub et al., 1952a; Blackman, 1956; Crafts, 1956a; Leonard and Crafts, 1956; Crafts and Yamaguchi, 1958).

**Seasonal Effects on Absorption of Herbicides**

The results of the translocation tests may be used to indicate absorption. During the periods of highest translocation, absorption undoubtedly was also taking place. However, limited absorption may have been responsible, in part, for the slow rate of translocation which occurred.

Results of the leaf-wiping method of measuring absorption, although not conclusive, showed that absorption was greater during the
periods of peak susceptibility than during periods of growth dormancy (Figure 9). Therefore, absorption may be an important factor in limiting the toxic effects of herbicides to creosotebush. However, this appears unlikely in view of (1) the generally favorable environmental conditions for absorption, at least during the periods of highest plant susceptibility; (2) the abundance of stomata on both leaf surfaces, which facilitates absorption in addition to cuticular absorption; and (3) the use of oil and surfactant in the spray mixture, which aids in both cuticular and stomatal absorption.
Figure 9. Relative penetration of radioactive 2,4,5-T* into leaflets of creosotebush at different seasons of the year. Leaflets on the left show treatment concentration; those on the right were wiped with ethyl alcohol and indicate relative absorption of the herbicide.
CHAPTER VII

TIME-OF-DAY SUSCEPTIBILITY OF CREOSOTEBUSH

This phase of the study was designed to determine if diurnal regimes of various ecological and physiological factors influenced the susceptibility of creosotebush to herbicides. Foliar-spray methods were used to study the effects of temperature, humidity, and physiological regimes. Radioisotope methods were designed to determine if there was a correlation between the pattern of translocation and daily climatic regimes, physiological changes, or susceptibility to herbicides.

Methods of Study

Foliar-spray Treatments

Foliar-spray herbicidal treatments were made at 2-hour intervals during the day and at 4-hour intervals during the night to determine the diurnal period of greatest susceptibility to herbicides. Tests were made during the peak summer period of susceptibility and during the spring and fall to determine seasonal differences.
Rates of 2,4,5-T application were varied according to seasonal susceptibility and both topkill and total-kill readings were made on the treated shrubs. Ten plants were treated at each rate at each treatment time.

Temperature and humidity measurements were made at the time of application for the Tombstone area and taken from Esmond hygrothermograph charts at hourly intervals for the Tucson area.

Soil moisture was measured at the 3- to 5-inch level and rainfall, cloud, wind, and phenological conditions were observed on each date.

Radioisotope Treatments

Radioisotope tests were conducted to determine the daily pattern of translocation of radioisotopes in creosotebush during the 1961 and 1962 seasons of greatest susceptibility. Two plants were treated at each 2-hour interval during the day and 4-hour interval during the night. Plants were mounted 24 hours after treatment with 2,4,5-T* and radio-autographed for 30 days.

Time-of-day Factors Affecting Plant Susceptibility to Herbicides

Interrelated environmental and physiological factors which may affect the absorption, translocation, and toxicity of herbicides through daily fluctuations include: rate of photosynthetic activity and resulting
translocation, light intensity, CO₂ supply, temperature, relative humidity, and soil moisture. Of these, Mitchell (1951) considered that light and CO₂ supply were the main factors affecting photosynthesis and subsequent translocation.

As already reported, numerous workers have shown that translocation of photosynthates is dependent on the buildup of photosynthate at the source and that translocation of herbicides downward and upward through the phloem parallels the movement of these materials.

Various hypotheses proposed for phloem transport of solutes, none of which are entirely satisfactory, have been reviewed by Curtis (1935), Crafts (1951, 1961b), Esau et al. (1957), and Zimmerman (1960). These include (1) mass or pressure flow along turgor gradients, (2) mass flow activated by parenchyma "pump" cells, (3) transport of solutes in the sieve tube along protoplasmic interfaces, (4) accelerated solute movement in sieve tubes resulting from cytoplasmic movement or flow, and (5) independent solute movement resulting from unknown active transfer processes, possibly enzymatic in nature which occur in the sieve-element cytoplasm.

The daily course of photosynthetic activity, as measured by CO₂ assimilation in alfalfa, wheat, and sugar cane rises rapidly from no photosynthesis in the morning before sunup to a broad maximum during the middle of the day, then falls off rapidly during the late afternoon to none after sundown (Thomas and Hill, 1937; Ashton, 1956). High
photosynthesis generally occurs from 9 or 10 am to 3 or 5 pm under high light and adequate moisture. Mallery (1935), measuring the osmotic concentration of the expressed sap of leaves, observed a similar pattern in creosotebush. The osmotic concentration was highest from 9 am to 1 or 3 pm and the lowest from 9 pm to 6 am. This pattern was strongly influenced by the energy flow from the sun. Mallery noted that continuous cloudy weather lowered the osmotic pressure of the cell sap of creosotebush owing to a decrease in the photosynthate rate.

Thomas and Hill (1957) found that under heavy cloud conditions there was a reduction in the rate of photosynthesis in alfalfa and wheat to 20 to 50 percent of maximum. However, they also observed that light intensities above 52 percent of normal maximum did not increase the rate of photosynthesis appreciably. They also noted that the temperature curve followed a pattern similar to the photosynthetic curve but with a lag of 3 to 4 hours. Manning (1938) found that rising temperatures increased the rate of photosynthesis in Nitzschia at high light intensities but had no influence at low light intensities. His data showed that at low light intensities maximum photosynthesis was reached at 30° C; at high light intensities maximum photosynthesis was reached at 35° C and declined sharply at 40° C. Ashton (1956) noted that moisture tension decreased photosynthesis in sugar cane even at high light intensities to about 20 to 50 percent of full rate. However, the decrease was not at a uniform rate, the decrease increasing as the wilting point was approached.
In addition to their effect on photosynthesis, fluctuations in light, temperature, and relative humidity may influence absorption of herbicides and consequently their translocation through drying effects on the spray emulsion, regulation of stomatal opening, and by their effects on various physical and chemical processes. Stomatal opening, in particular, may be affected by daily variations in climatic and physiological regimes, but effects vary widely with species (Loftfield, 1921; Maximov, 1938). Wilson (1948) noted that when the other factors were kept constant increases in temperature, relative humidity or light intensity generally increased stomatal opening.

From the above findings it would appear logical to conclude that any factors which influence photosynthesis may also influence the relative translocation and effectiveness of herbicides applied at different times of the day. This observation is substantiated by the work of Morre and Rogers (1960) who found that the translocation of 2,4-D was proportional to light intensity, indicating a dependence on carbohydrate transport, and that highest herbicide activity was reached 8 to 10 hours after exposure to light which was continued for 18 hours. Based on their study, it would appear that herbicide applications made at midday or early afternoon would result in maximum translocation and highest herbicidal activity. However, during this period there may be counteracting effects from other factors which more than offset the favorable conditions for translocation.
Results of Time-of-day Studies

The results of eight different time-of-day, foliar-spray, and radioisotope studies made on creosotebush at various seasons of the year did not show a consistent, well-defined pattern of daily susceptibility (Figures 10 and 11). The spray rate in August, 1958, was excessive for the favorable year. In August, 1959, results followed the photosynthesis-translocation pattern with greatest total-kill occurring when the herbicide was applied at midday and early afternoon. In October, 1960, total-kill was slightly greater from applications made at midday and early afternoon than from those made in the early morning and late afternoon. However, total-kill at midday was not significantly different than from the 4 am or 8 pm treatments. In the April, May, and September, 1961, tests, application rates were too low to produce total-kill and topkill was estimated. None of the 1961 tests showed significant treatment differences related to time-of-day.

The radioisotope test made in September, 1961, showed that maximum translocation, both upward and downward, occurred in mid-afternoon, again correlated with the photosynthate-translocation pattern. However, the test in August, 1962, showed greatest downward translocation in early morning and late afternoon and little difference in upward translocation.
TEMPERATURE - DEGREES C
RELATIVE HUMIDITY - PERCENT
2,4,5-T INJURY TO CREOSOTEBUSH - PERCENT

Figure 10: Relationship of time of day, temperature, relative humidity, and 2,4,5-T to topkill and total kill.
Figure 11. Twenty-four-hour translocation of radioactive herbicides from creosotebush leaves treated at different times of the day.
In an attempt to explain these variable results on the basis of favorable or counteracting environmental influences which may have affected absorption, translocation, or toxic effects, an analysis was made of daily temperature and relative humidity trends; related rainfall, soil moisture, cloud, and wind conditions; and stage of phenological development (Figure 10, Table 5). Environmental and phenological conditions conducive to high susceptibility of creosotebush to herbicides applied at midday appear to be a high maximum-minimum, night-day relative humidity of ca 90 and 30 percent, respectively; a favorable prior rainy season; current soil moisture conditions above 6.7 percent at 3- to 5-inch depth (at or above the wilting coefficient); moderate to heavy cloud cover; and active leaf growth and flower production. Environmental and phenological conditions conducive to low herbicide toxicity appear to be a maximum-minimum, night-day relative humidity of less than 55 and 25 percent, respectively; low rainfall during the preceding 30 days; low soil moisture, both preceding and at the time of spraying; a scarcity of clouds; and low plant growth or dormancy. Factors which seemed to have little effect under the existing conditions were temperature and wind.

A similar radioisotope study by McRae (1962) on tarbush showed that translocation of radioactive 2,4-D*, which correlated with herbicide toxicity, reached high levels at midday on both days studied. However, he noted that a cloud cover in the early afternoon of one day caused a
Table 5. Environmental and phenological conditions of creosotebush related to time-of-day treatments.

<table>
<thead>
<tr>
<th>Spray date</th>
<th>Rainfall</th>
<th>Soil moisture</th>
<th>Cloud cover</th>
<th>Wind</th>
<th>Relative humidity</th>
<th>Temperature</th>
<th>Phenological development</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/19-20/59</td>
<td>good 4.19</td>
<td>medium 6.7</td>
<td>cloud. cloud. calm</td>
<td>calm</td>
<td>88 29 32 19</td>
<td>Many new leaves forming. Old leaves shedding. Late-flower, early-seed stage.</td>
<td></td>
</tr>
<tr>
<td>10/10-11/60</td>
<td>poor 0.73</td>
<td>medium 9.4</td>
<td>clear windy calm</td>
<td>calm</td>
<td>92 28 24 11</td>
<td>Leaves greening up. No flowers. Old fruits present.</td>
<td></td>
</tr>
<tr>
<td>4/6/61</td>
<td>poor 0.17</td>
<td>dry 0.1</td>
<td>few cloud. windy calm</td>
<td>44 19 27 13</td>
<td>Leaves green. No flowers. No fruits.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/5-6/61</td>
<td>poor 0.00</td>
<td>dry 0.1</td>
<td>clear windy calm</td>
<td>30 16 24 9</td>
<td>Leaves dry. No flowers. No fruits.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/7-8/61</td>
<td>fair 2.40</td>
<td>dry 0.1</td>
<td>clear breezy calm</td>
<td>53 22 33 21</td>
<td>New and mature leaves green. Few flowers. Many seeds maturing.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/21/61</td>
<td>fair 1.95</td>
<td>dry 0.1</td>
<td>clear mild breeze calm</td>
<td>54 20 29 14</td>
<td>New and old leaves present. Past flower stage. Abundant fruits.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/20-21/62</td>
<td>fair 2.30</td>
<td>low 4.2</td>
<td>cloud. mild breeze calm</td>
<td>53 22 36 26</td>
<td>New and old leaves green. Late-flower stage. Few fruits.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Days after temperature favorable for spring growth.
reduction in translocation. On both days the minimum daily relative humidity remained above 30 percent, maximum night-time relative humidity varied between 60 and 70 percent, prior rainfall and soil moisture conditions were favorable, and plants were actively growing.

In both the creosotebush and tarbush studies it appears that no single factor alone is responsible for controlling absorption and translocation of herbicides, but rather that favorable conditions are the result of a combination of interacting factors. For example, a marked change in one factor may have little effect unless accompanied by changes in other factors. This principle may be illustrated by the low absorption and translocation which occurred under high night-time humidity but low light and temperature conditions as contrasted with the high activity which occurred under the lower (but above minimal) daytime humidity accompanied by high light and temperature conditions. However, the high night-time relative humidity may have had a conditioning effect on the physiology of the plant necessary for high absorption and translocation during the day.

As a further consideration, any factor which becomes limiting may have a critical and even dominating effect. This could be illustrated by an extremely low humidity, such as 5 percent, which could prevent absorption and consequently translocation, or low temperatures, such as 5°C, which could stop translocation.
CHAPTER VIII

CARRIER COMPONENT EFFECTS ON HERBICIDAL TOXICITY

Formulation and preparation of herbicides for the selective control of weeds has become increasingly complex in recent years. This has resulted not only from the introduction of a wide variety of new organic and inorganic herbicides but also from the use of various solvents, cosolvents, surfactants, emulsifiers, humectants, and other carriers, surface-active agents and/or adjuvants. As a result, herbicides must be evaluated not only for their general characteristics but also for their effectiveness and selectivity with various additives.

Two components of the carrier emulsion—surfactant and oil—were studied to determine their influence on the final herbicidal effect on plants.

Methods of Study

Surfactant Treatments

The effect of surfactant on the toxicity of propyleneglycolbutyl-ether esters of 2,4,5-T was studied using liquid Joy, a nonionic detergent. Since the herbicide solution already had some surfactant present,
only the effects of added amounts, up to 1 percent, were studied. Four series of foliar-spray tests were made during the years 1958-61. Ten plants were sprayed for each treatment.

Oil-carrier Treatments

The effect of oil-carrier concentration on the toxicity of propyleneglycolbutylether esters of 2,4,5-T was studied using diesel oil. Concentrations were varied from none up to 25 gallons per acre (gpa) and were applied with and without water and with and without 2,4,5-T in the emulsion. Plots of 10 plants each were used for each treatment, and tests were run on four dates from 1959-61.

The diesel oil used in these experiments was No. 1 grade with a boiling range of 343-524° F, a gravity American Petroleum Institute (API) rating of 42.4°, and flash point of 135° F.

Surfactant Effects

Characteristics and Effects of Surfactants

Surfactants (surface-active agents) are probably the most universal additives to herbicidal formulations. They are added to provide emulsification, dispersion, solubilization, wetting, spreading, and adhering properties to the spray mixture (Hitchcock and Zimmerman, 1948; Schwartz and Perry, 1949; Furmidge, 1959a; Jansen et al., 1961). Substances may act as surfactants when they possess both a polar group, which is attracted to hydrophilic substances (water, salts,
and etc.) and a nonpolar group (hydrocarbon chain or ring) which is attracted to lipophilic or hydrophobic substances (oils, fats, and waxes). The surface-active agent then orients itself between the interfaces of the polar and nonpolar substances, modifying surface tension and bringing the two substances into intimate contact (Schwartz and Perry, 1949; Freed, 1958; Furmidge, 1959a; Klingman, 1961). However, not every substance which lowers the surface tension of water will serve as a surfactant for any herbicide; the surfactant must be chosen to suit the herbicide and the particular problem (Freed, 1958; Freed and Montgomery, 1958; Jansen et al., 1961). For example, Gertsch (1953) noted that the surfactant facilitated the penetration of 2,4-D by acting as a solvent more than as a spreading agent.

Surfactants may be classed as ionic or nonionic. Nonionic substances ionize little or not at all in water, are nonelectrolytic, and are usually chemically stable in acid, neutral, or alkaline media. Ionic surfactants ionize in an aqueous medium and are classed as anionic (negatively charged surface-active ions predominate), cationic (positively charged surface-active ions predominate), or ampholytic (those cationic in acid media and anionic in basic media) (Schwartz and Perry, 1949; Freed, 1958; Union Carbide Chemicals Co., 1961). In general, cationic surfactants are rated considerably less effective than anionic and nonionic surfactants (Weintraub et al., 1954b; Loomis, 1955). As a result, most emulsifiers used in herbicides are mixtures of anionic
and nonionic substances blended to fit field conditions; anionic agents working best in cold and soft water, nonionic best in warm and hard water (Freed, 1958; Klingman, 1961).

The spreading and wetting qualities of liquids and the effectiveness of surfactants to reduce surface tension and interfacial tension\(^1\) have been determined by measuring the contact angle of a drop of the liquid with the solid substrate surface (Ebeling, 1939; Fogg, 1948; Jansen et al., 1961). In general, the lower the contact angle the lower the surface tension and vice versa. Fogg (1948) found that contact angle may be affected by the chemical nature of the solid surface and its roughness, position of the leaf on the plant, and diurnal changes such as wilting of the leaf. He noted that contact angle may change as much as 30° due to diurnal variations.

The addition of surfactants to spray mixtures has increased the absorption, translocation, and toxic effects of herbicides as much as 1400 percent (Staniforth and Loomis, 1949; Ennis, 1951; Ennis et al., 1952; Weintraub et al., 1954b; Crafts, 1956a; Hull, 1956; Barrier and Loomis, 1957; Freed, 1958; Yamaguchi and Crafts, 1958; Jansen et al., 1961).

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1 Jansen et al. (1961) defined surface tension as the molecular tension that occurs at the liquid-gas interface and interfacial tension as that at the liquid-liquid or liquid-solid interfaces.
The increased toxicity of herbicides resulting from the addition of surfactants may be due to the increased uptake and effectiveness of herbicides resulting from one or more of the following: (1) increased wetting of the leaves by the spray and less bounce-off and runoff, (2) removal of air films between spray and leaf surface, (3) reduction of interfacial tension between relatively polar and apolar submicroscopic regions of the cuticle, (4) increased entry through the stomata, (5) increased permeability of the plasma membrane through solubilization, stimulation or incipient toxicity, (6) facilitation of movement in the cell wall in the region of the wall-cytoplasm interface, (7) action as co-solvents, (8) interaction directly with the herbicide in some manner, and/or (9) action as secondary humectants (Hitchcock and Zimmerman, 1948; Crafts, 1959, 1960; Currier and Dybing, 1959; Jansen et al., 1961; Klingman, 1961).

Surfactant adjuvants may aid the penetration of 2,4-D regardless of whether it is present as an acid, ester, or salt (Hitchcock and Zimmerman, 1948). However, an "excess" of surfactant may result in runoff (Crafts, 1961a) or promote rapid entry of herbicides which may result in increased contact injury and reduced translocation and toxicity of some herbicides in some plants (Hull, 1956).

Surfactants may also influence the synthesis, formulation, concentration, packaging, marketing, and mixing of herbicides (Jansen et al., 1961) and by equalizing the absorption of salts and esters they may
reduce the selectivity of foliar herbicides (Staniforth and Loomis, 1949; Hauser, 1955; Jansen et al., 1961; Klingman, 1961).

In addition to their effects on the phytotoxicity of herbicides, surfactants may possess inherent toxic properties of their own (Hitchcock and Zimmerman, 1948; Gast and Early, 1956; Furmidge, 1959a, b, c; Jansen et al., 1961). Direct toxicity of the surfactant results from solubilizing the cell wall which permits the cell sap to leak into the intercellular spaces; from protein precipitation and denaturization; and from inactivation of enzymes, viruses, and toxins (Putnam, 1948; Furmidge, 1959c).

The degree of phytotoxicity of the surfactant and its effect on herbicidal toxicity varies from the marked increases in toxic effects noted above to none or may even result in growth stimulation depending on such factors as composition and concentration of the surfactant, the age of the leaf, the variety or species of plant, leaf pubescence and cuticle formation, leaf angle and leaf surface, droplet size and composition, nutritional status, weather, a number of physico-chemical factors, and the interaction of the surfactant phytotoxicity with the herbicidal phytotoxicity (Ennis et al., 1952; Gast and Early, 1956; Blackman et al., 1958; Furmidge, 1959b; Jansen et al., 1961).

Phytotoxicity of surfactants is governed by the ionic charge, by physical size of molecules or ions, and by their chemical nature. Surfactant concentrations up to 0.1 percent have been most effective in
reducing surface and interfacial tension but have little effect at higher concentrations (Ebeling, 1939; Gertsch, 1953; Young, 1954; Crafts, 1956a; Leonard, 1958; Currier and Dybing, 1959; Jansen et al., 1961). However, the adjuvant effects of surfactants on herbicide toxicity generally result from concentrations above 0.1 percent indicating that their biological effectiveness is correlated with properties other than their surface-active characteristics (Staniforth and Loomis, 1949; Furmidge, 1959b; Crafts, 1961a; Jansen et al., 1961). This dual role may be due to changes in properties of the surfactant itself since at low concentrations (below 0.1 percent) they act as simple ions; at higher concentrations as colloidal micelles or aggregates (Putnam, 1948; Schwartz and Perry, 1949; Jansen et al., 1961). Because of this Jansen et al. postulated that the biological activity of surfactants may be due to colloidal-system effects which may include changes in the relative conductivity, electrophoretic, and osmotic properties of the solution. Surfactant effects may also vary with concentration of the herbicide (Young, 1954; Leonard, 1958).

**Surfactant-herbicidal Effects on Creosotebush**

The effects of added surfactants on toxicity of 2,4,5-T to creosotebush varied with herbicidal rate (Figure 12). At the 8-pound rate in 1958 there was a slight but not significant decrease in total-kill at surfactant rates above 0.1 percent. At the 1-pound rate in 1959 and 1961
Figure 12. Effect of added surfactant (liquid Joy) on 2,4,5-T toxicity to creosotebush.
effects were erratic. At the 1/2-pound rate in 1959 the added surfactant resulted in an increase in toxicity from 50-percent total-kill at less than 0.1 percent surfactant concentration to 80-percent total-kill at 1.0 percent concentration. While this was not significant at the 5-percent level of significance the trend corresponded to the phytotoxic effects of surfactant noted above by Staniforth and Loomis (1949), Furmidge (1959b), Crafts (1961a), and Jansen et al. (1961). The adjuvant effect of the surfactant on the light herbicidal application also may be due in part to increased absorption of the herbicide without an accompanying blockage of translocation which might result from contact injury at higher concentrations of herbicide. These effects support the work of Leonard (1958) who found that at light rates of application (500 to 1,000 ppm) the toxic effects of the triethanolamine salt of 2,4-D increased progressively as the concentration of Tween 20 was increased from 125 to 32,000 ppm.

The lack of a more marked response to surfactant additions may have been due to the supply of surfactant already present in the commercial preparation which was sufficient for "normal" effects on surface tension. Therefore, added effects were probably due to the toxic effects of the surfactant.
Oil-carrier Effects

Characteristics and Effects of Oil Components

Various oils may also act both as herbicides per se and as adjuvants to the effectiveness of herbicides. Their toxicity to plants varies considerably depending on characteristics of both the oils and the plants treated. Oils act as contact herbicides and may be used either as selective or as general weed killers. General contact oils are heavy oils having a gravity API (American Petroleum Institute) Baume scale (hydrometer) reading maximum of 32° and with flash points above 180° F; most selective oils are light weight oils having a gravity API of approximately 42° and flash points above 100° F (Bell and Norem, 1950).

Aromatic oils (ring or cyclic unsaturated hydrocarbons) appear to be most toxic to plants, the naphthenes (saturated cycloparaffins) and olefins (unsaturated, straight- or branch-chain hydrocarbons) are intermediate, and the paraffins (saturated, straight-chain hydrocarbons) are the least toxic (Crafts and Reiber, 1948; Havis, 1948; Bell and Norem, 1950; Leonard and Harris, 1952). To be effective phytotoxicants, oils must contain at least 25 percent unsaturated (sulfonatable) and aromatic compounds. However, toxicity depends on the nature of the unsaturates as well as the amount present (Bailey and Smith, 1951). In general, aromatics make up 40 to 100 percent of the mixture in general contact herbicides and 10 to 20 percent in selective herbicides (Bell and Norem,
1950). The toxicity of aromatic compounds may be increased as one or more side chains are added until the molecular weight of the side chain or chains equal that of the aromatic portion (Bell and Norem, 1950).

In general, hydrocarbons within the boiling range of 150 to 275°C (302 to 527°F) are most toxic to plants (Havis, 1948). Lower boiling hydrocarbons may evaporate before plant injury occurs and higher boiling hydrocarbons are too viscous to enter the plant (Bell and Norem, 1950). Bell and Norem added that, in general, oils with viscosity ratings of 35 to 50 seconds are most satisfactory for weed control, that oils with boiling points between 300 and 400°F act as selective sprays, while those with boiling points between 350 and 700°F are general weed killers.

Oils are relatively nonpolar and have low surface tension (Crafts and Reiber, 1948; Robbins et al., 1952), so much so that surfactants have little effect on the surface tension of oils (Ebeling, 1939).

Highly refined oils, such as Stoddard solvents or Varsol, petroleum spirits, mineral spirits, kerosene or high grade fuel oils, have low contact injury and may be used for selective weed control. On the other hand, the low grade fuel oils, the crude diesel oils, and shale oils are more toxic and may be used as general contact herbicides. They may be made even more toxic by fortification with pentachlorophenol (PCP) or 4,6-dinitro-o-sec-butylphenol (DNBP) (Klingman, 1961). However, when diluted, the relative toxicity of certain hydrocarbons may
change greatly or may even be reversed depending on whether the diluent is air, water, or paraffin oil (Currier, 1951; Currier and Peoples, 1954).

Oils may penetrate into plants through stomates, thin cuticle, epidermis, bark, and even through injured roots but may penetrate slowly or not at all through thick cuticle, the rate depending on surface tension and viscosity (Ginsberg, 1931; Crafts and Reiber, 1948; Minshall and Helson, 1949; Norman et al., 1950; Currier, 1951; Leonard and Harris, 1952; Klingman, 1961).

Oil toxicity may be acute or chronic. Acute toxicity is characterized by rapid burning of the leaves and stems, immediately or within 24 hours and death within 48 hours; chronic injury by yellowing, rolling, curling, and stunting of leaves and eventual death (Young, 1935b; Crafts and Reiber, 1948; Norman et al., 1950; Currier, 1951). Both types may include wilting and translucent coloring of leaves. Oil toxicity appears to result from accumulation of the oil in the cell lipids resulting in cytolytic action, solubilizing of the cell membrane with a rapid increase in permeability, sap leakage into the intercellular spaces, and destruction of the living cell (Crafts and Reiber, 1948; Van Overbeek and Blondeau, 1954; Crafts, 1961a). Other effects may include temporary or permanent stoppage of photosynthesis, increased respiration in some plants, with no effect or slower respiration in others, and decreased transpiration (Minshall and Helson, 1949; Klingman, 1961).
Solubilization may be the mechanism for both acute and chronic toxicity with the only difference being the time factor (Van Overbeek and Blondeau, 1954). The character of the cell wall and the outer plasma membrane appears to be the critical structure in the susceptibility and tolerance of plants to oils, and consequently the selectivity of oils (Crafts and Reiber, 1948; Currier, 1951; Currier and Peoples, 1954).

Most workers report that oils move principally in the intercellular spaces by capillarity, that oils do not penetrate living protoplasm, that translocation is not associated with the xylem or phloem, and that movement may be in any direction (Rohrbaugh, 1934; Young, 1935a, b; Minshall and Helson, 1949; Bell and Norem, 1950; Norman et al., 1950; Currier, 1951; Rice and Rohrbaugh, 1953; Van Overbeek and Blondeau, 1954; Loomis, 1955; Klingman, 1961).

Most of these authors report that translocation distances may be relatively short, only a few cells in depth. However, Minshall and Helson (1949) reported that oils may move from the leaves in the intercellular spaces of the parenchyma tissue in the midrib and petiole of the leaves to the intercellular spaces of the phloem parenchyma of the dandelion root and that movement occurs at the rate of 4 to 5 cm per hour. Similar rates, 4 cm per hour, were observed in bean plants by Rice and Rohrbaugh (1953) for kerosene mixed with 2,4-D.

Depending on their physical and chemical properties, light oils may readily evaporate from the surface of plants or from the
intercellular spaces within a few hours to a few weeks while heavy oils may remain in the plant for from six months to the normal lifetime of the plant (Rohrbaugh, 1934; Young, 1935b; Minshall and Helson, 1949).

The use of oils as adjuvants to herbicide toxicity, particularly in aqueous sprays, has been noted by various workers (Ennis, 1946, 1951; Gertsch, 1953; Leonard and Crafts, 1956; Van Overbeek, 1956; Leonard, 1958; U. S. Agricultural Research Service, 1958). Oils may increase herbicidal effectiveness by increasing the uniformity of herbicide spread on the leaf, deterring losses of herbicide from the leaf by rains, delaying drying out of the herbicide, improving contact with the lipophilic layers on the epidermal wall, and acting as a carrier to increase absorption and translocation of the herbicide in the plant (Bell and Norem, 1950). In addition, oils may reduce volatilization of herbicides and injury to adjacent crops (Marth and Mitchell, 1949). However, the use of highly toxic oils or solvents in the mixture may reduce herbicidal effectiveness by causing contact burning of the leaves, leaf abscission, or injury to the phloem with consequent reductions in herbicide absorption and translocation (Crafts and Reiber, 1948; Bell and Norem, 1950; Hay, 1956a; Hull, 1956; Behrens, 1957; Leonard, 1958; Crafts, 1961a). Contact injury may be particularly detrimental on brush and trees, where extensive translocation is essential, and in drier regions where rainfall may not be sufficient to carry chemicals into the soil (Crafts, 1961a).
Oils and surfactants used as adjuvants may react differently on various herbicides. For example, Gertsch (1953) noted that surface-acting agents were chiefly responsible for increasing the effectiveness of triethanolamine salt of 2,4-D but that the oil, not the detergent, was the agent increasing the effectiveness of the oil-soluble polypropylene-glycolbutylether esters of 2,4-D. Also, Gertsch found that with propyleneglycolbutylether esters of 2,4-D, inhibition was greater the higher the relatively nontoxic oil content of the emulsion indicating that effects were due to factors other than the toxicity of the oil.

Comparative studies on the relative effects of surfactant and oil additives in water showed that water alone was generally least effective, water-surfactant mixtures intermediate, and water-surfactant-oil mixtures were most effective in increasing the absorption and toxic effects of 2,4-D herbicides (Gertsch, 1953; McRae, 1962).

**Oil-carrier Effects on Creosotebush**

Results of the oil-carrier studies on creosotebush showed that diesel oil added to the water-surfactant-herbicide emulsion generally resulted in significant increases in plant kill even at high oil rates of 12.5 and 25 gpa (Figure 13). This is in contrast to reduced injury from similar rates of oil on mesquite reported by Behrens (1957). This effect appears to be due mainly to oil effects in increasing the penetration and translocation of the herbicide since diesel oil alone resulted in
Figure 13. Effect of diesel oil, with and without water, on the toxicity of 2,4,5-T to creosotebush.
little or no increase in total-kill even at 25 gpa. The addition of diesel oil to the herbicide-surfactant mixture without water had no significant effect on the total-kill of creosotebush at the rates tested, further substantiating the low toxicity of the oil. The low toxicity of the diesel oil per se to creosotebush may have been due to the relatively high grade of diesel oil used. However, the difference in effectivity may have been related to changes in the relative effects of oils when diluted with air versus dilution with water, effects noted by Currier (1951) and Currier and Peoples (1954). However unintentional, it appears that creosotebush was well named since it is highly oil-tolerant.

The oil tests show some interesting effects from low rates of application. In 1959 at both the 1/2- and 1-pound herbicidal applications the 1/4-gallon applications of diesel oil resulted in sharp increases in herbicide toxicity over no diesel oil followed by a sharp decrease at the 1/2-gallon rate. These changes were followed by gradually increasing toxic effects from 2,4,5-T at the higher rates of diesel oil. The 1/4-gallon rate was not included in the 1960 and 1961 tests, but it appears that a similar effect could have occurred during these years as evidenced by the kill pattern at the 8-pound rate of herbicidal application in 1960. From these results it appears that the diesel oil may have produced adjuvant effects on the absorption of 2,4,5-T at the 1/4-gallon rate; that contact injury to the phloem may have occurred from the more rapidly absorbed 2,4,5-T at the 1/2-gallon diesel oil rate, blocking
translocation of the herbicide; and that the heavier rates of diesel oil may have circumvented blockage of the phloem by facilitating movement of the herbicide in the intercellular spaces. The conclusion that the diesel oil facilitated movement of the herbicide in the intercellular spaces is substantiated by the radioactive phases of this study which show that (1) the rate of herbicidal movement in creosotebush falls within the rate of oil movement in intercellular spaces (less than 5 cm per hour) rather than normal phloem transport (10 to 100 cm per hour), and (2) that the cessation of translocation of 2, 4, 5-T in creosotebush (18-24 hours) coincides with the time in which light oils would normally volatilize into the intercellular spaces and "transpire" from the plant.
CHAPTER IX

EFFECT OF pH OF THE HERBICIDAL EMULSION

This phase of the study was made to determine if changes in the pH of the spray emulsion would affect the final toxicity of the herbicide through interim effects on the absorption, translocation, or chemical reaction of the herbicide.

Method of Study

The effect of the pH of the spray emulsion on the toxicity of 2,4,5-T was tested during the summers of 1958, 1959, and 1961. The pH of the regular spray emulsion varied between pH 5.5 and 6.5, depending on the alkalinity of the water used; the pH of 2,4,5-T approximated 6.0 and diesel oil 3.5. The pH of the solution was changed to pH 3, 5, 7, and 9 by the addition of a concentrated solution of sodium tetraborate or borax (Na$_2$B$_4$O$_7$·10H$_2$O) to increase alkalinity and by adding hydrochloric acid (HCl) to increase acidity. Two herbicide rates were applied at each pH and 20 plants were sprayed at each rate.
Previous Studies on pH Effects

The effect of pH on the absorption of herbicides by plants varies with addition of various surfactants, cations, anions, and buffers; and with herbicidal rate, formulation, and conditions of treatment (Lucas et al., 1948; Crafts, 1956a; Van Overbeek, 1956; Orgell, 1957; Orgell and Weintraub, 1957; Szabo and Buchholtz, 1961).

Early work with arsenical spray solutions (Kennedy and Crafts, 1927; Crafts, 1933a, b) showed that rapid penetration was dependent on the killing action of strong acids or bases which quickly killed the leaves or roots and increased permeability of the cells. Penetration by modern growth-regulating herbicides is dependent on both physical and metabolic processes which may or may not be affected by the pH of the herbicide mixture. Studies by Crafts (1956a) showed that the activity of 2,4-D was greatly reduced when the pH of the solution was below pH 2.0. This he attributed to phloem injury which hindered transport. It was also found that the activity of phenoxy herbicides (which act as weak acids) was generally greatest when applied in a solution with a pH of 2.0 to 6.0 and that activity decreased with increasing pH, particularly in the 7 to 10 range (Hamner et al., 1947; Lucas et al., 1948; Audus, 1949; Norman et al., 1950; Brian and Rideal, 1952; Blackman and Robertson-Cuninghame, 1953; Crafts, 1953, 1956a; Weintraub et al., 1954b; Hauser, 1955; Blackman, 1956; Van Overbeek,
1956; Orgell, 1957; Orgell and Weintraub, 1957; Wedding and Erickson, 1957). These studies were generally carried out under conditions of high humidity or saturated atmosphere.

Reinhold (1954), in studies with indole-3-acetic acid (IAA), noted a similar effect and concluded that pH affected the physical processes, which are akin to diffusion, but not the metabolic processes, and that the decline in rate with increasing pH was due to changes in the ionization of the cytoplasmic proteins and protein complexes. Reinhold reported that the anion, not the undissociated molecule, was involved in absorption. However, Blackman (1956) showed that although there was some absorption of 2,4-D acid as a dissociated ion, most of it occurred as an undissociated molecule.

Brian and Ridael (1952), on studies with 2-methyl-4-chlorophenoxyacetic acid (MCPA), concluded that the effect of pH at physiological acidities must largely result from an effect on the protoplasmic membranes of the plant rather than on the ionization of the penetrating chemical. Van Overbeek (1956) reported that increased acidity not only represses ionization of applied chemicals but also the acid residues of the cuticle itself. This in turn reduces the negative charge in the cuticle and makes it more permeable.

Orgell (1957) gives a detailed explanation of the effects of pH on sorption (a term used to include both adsorption on the interface of the cuticle and absorption into the bulk of the cuticle):
The effects of pH on the sorption of acid and basic substances can be interpreted by assuming that the cuticle surface is semi-lipoidal and weakly acidic. At low pH values (below pH 5) the cuticle would be relatively uncharged. Acid substances (e.g., acid dyes, 2,4-D acid, and the dinitrophenols) would be relatively undissociated and their sorption would depend upon their interfacial activity, or capacity to form proton or Van der Waals' bonds with the cuticle surface, as well as their solubility in certain phases of the cuticle. A basic substance (e.g., a quaternary ammonium dye) would be positively charged and relatively polar at a low pH, and hence sorption to the relatively unionized cuticle would not be great. At higher pH values (above pH 6) both cuticle and acid substances would be negatively charged and electrostatic repulsion could hinder or prevent sorption. On the other hand, a basic quaternary ammonium dye would remain positively charged and electrostatic attraction would result in very pronounced sorption similar to salt formation. With respect to these properties, the cuticle resembles a semi-lipoidal cation exchange membrane.

In brief, he concluded that acid substances were sorbed best at acid pH values and basic substances best at basic pH values. However, Orgell pointed out that the effects of various factors on sorption do not necessarily parallel their effects on penetration (distinguished from sorption as movement completely through the cuticle).

Wedding and Erickson (1957) noted that penetration of 2,4-D was mainly in the molecular form. They found that the permeability of Chlorella cells to 2,4-D molecules was 800 to 1,000 times as great as for anions at the normal cell pH of 5.3 to 5.8.
Results of the pH studies on creosotebush showed no significant effect of pH on total-kill (Figure 14). Since the pH effect is largely an effect on adsorption and absorption, the nondifferential response appears to be the result of the modifying effects of the surfactant, oil carrier, and propyleneglycolbutylether esters of 2,4,5-T. Apparently, at the low pH values the anionic surfactant hindered absorption of 2,4,5-T but had no effect at the high pH values, thus tending to equalize absorption. This conforms to the findings of Orgell (1957) who noted that both anionic and cationic surfactants hindered sorption of acid substances at low pH values but at high pH the cationic surfactant increased sorption and the anionic surfactant had no effect. The effect of the diesel oil in the emulsion would be to solubilize the membranes and increase cell permeability, thereby reducing the differential effect of pH on absorption. Similarly, the use of the heavy aliphatic esters of 2,4,5-T (which have both water and fat solubility) would increase cell permeability and reduce pH effects. In addition, the increased concentration of the spray (due to rapid evaporation of water from the spray solution after its application on the leaves) may further reduce the effect of pH on absorption.

Another factor which apparently had an effect was the choice of borax to prepare the alkaline pH concentrations. The borate ion was
Figure 14. Effect of spray-emulsion pH on 2, 4, 5-T toxicity to creosotebush.
reported by Robbins et al. (1952) to be toxic to plants and thus may have increased plant kill at the high pH concentrations further equalizing pH effects on the absorption of 2,4,5-T. This conclusion is supported by Crafts (1956a) who found that 2,4-D solutions buffered with borate continued to produce high stem curvatures in bean plants at high pH levels.
CHAPTER X

HORMONAL, FERTILIZER, AND PHOTOSYNTHATE STUDIES

The purpose of these phases of the study was to determine if growth stimulation or greater concentrations of photosynthate would increase the toxic effects of herbicides in creosotebush. Gibberellic acid and ammonium phosphate fertilizer were used as growth stimulants, and sucrose was sprayed on the leaves to increase photosynthate concentration.

Methods of Study

Hormonal Treatments

The effect of the hormonal plant-growth stimulant gibberellic acid, a tetracyclic dihydroxylactonic acid \( \text{C}_{19}\text{H}_{22}\text{O}_{6} \), on the toxicity of propyleneglycolbutylether esters of 2,4,5-T was studied during the season of peak herbicidal activity in August, 1958 and 1959. Potassium gibberellate \( \text{C}_{19}\text{H}_{21}\text{O}_{6}\text{K} \), at the rate of 20 gm per acre, was applied 10 and 20 days prior and simultaneously with 2,4,5-T treatments.

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1 The author is indebted to Merck and Co., Inc., Rahway, New Jersey, for furnishing the potassium salt of gibberellic acid used in this study.
Prior applications of gibberellate were made in water. Two percent diesel oil, 0.1 percent Joy, or both were added to aid in wetting the leaves and in the absorption of the salt. In the treatments made simultaneously with the herbicide, the gibberellate was added directly to the spray emulsion—which included both diesel oil and surfactant. Plots of 10 plants were sprayed for each treatment. Concurrently with the herbicidal treatments on the gibberellate-treated plots, nongibberellate-treated plots were sprayed with herbicide for comparison.

**Fertilizer Treatments**

The fertilizer ammonium phosphate (16-20-0) was applied at the rate of 200 pounds per acre on 10x100-foot plots at various intervals prior to herbicidal treatments. Treatments were made in two series in 1958 and 1959. The fertilizer was divided into two parts and broadcast by hand to insure even distribution. Each group of previously fertilized plots and their respective nonfertilized check plots were treated with propyleneglycolbutylether esters of 2,4,5-T on the same day to validate treatment comparisons. Effects were evaluated on 20 plants per treatment in 1958 and 10 plants per treatment in 1959.

**Photosynthate Treatments**

Sucrose was added to the spray emulsion to make a 7 percent by weight sugar solution which was then applied directly on the plants. Two
rates of 2, 4, 5-T were used and each rate was applied to 20 plants. Treatments were made in September, 1961, at the Tucson study area.

Also, applications of sucrose were made to the leaves simultaneously with the radioactive 2, 4, 5-T* to determine if photosynthate additions affected the absorption and translocation of the herbicide in creosotebush. Treatments were made on 4 plants in September, 1961.

**Hormonal Effects**

**Review of Gibberellic Acid Interactions with Herbicides**

As already noted, numerous studies have shown that phenoxy herbicides are generally most effective on rapidly growing plants. Gibberellic acid has been found to be a dramatic growth stimulant (Stowe and Yamaki, 1957, 1959; Wittwer and Bukovac, 1957, 1958; Merck and Co., Inc., 1958; Stodola, 1958; Steward and Shantz, 1959; Phinney and West, 1960; Stuart and Cathey, 1961). These reports indicate that the major effect of the gibberellins is to increase cell elongation and possibly linear cell division. This results in taller plants (and in cereals greater leaf length) that may or may not increase vegetative dry matter. Gibberellins may also induce bolting and earlier flowering, but not necessarily more or larger flowers; seed maturity may be hastened but seed yield may be decreased; roots are generally reduced in length, weight and number; startling growth increases in dwarfs may be produced; and cold, seed, and bud dormancies may be counteracted.
Because of these growth effects, studies have been made to determine if there are interactions between the gibberellins and (1) other auxins, or (2) the various growth-regulating herbicides.

Extensive reviews have been made of studies on the interactions between gibberellins and other auxins (Stowe and Yamaki, 1957, 1959; Steward and Shantz, 1959; Van Overbeek, 1959; Galston and Purves, 1960; Phinney and West, 1960; Stuart and Cathey, 1961). These reviewers reported studies indicating that interactions of gibberellins and other auxins varied from inhibitory, to noninteraction, to synergism. In general, they concluded that gibberellic acid is one of a number of native regulators all of which may interact through closely related but distinct systems to control the growth and differentiation of the plant.

Several studies have been reported on interactions between gibberellic acid and the phenoxy herbicides. Hauser (1959) noted that gibberellic acid applied a week before or after treatment with 2,4-D or 2,2-dichloropropionic acid (dalapon) reduced the growth of nutgrass. In the case of dalapon and gibberellic acid the reduction was more than additive indicating synergism. Clor et al. (1958) found that interaction of 2,4-D and gibberellic acid varied with the rate of 2,4-D application. Their study indicated that in amounts of 2,4-D greater than 1 μg per plant, inhibition of gibberellic acid-induced growth in bean plants by 2,4-D was proportional to 2,4-D dosage. The effectiveness of gibberellic acid lasted for at least three weeks. When lower dosages of 2,4-D
(1 µg or less) were applied the two compounds acted synergistically and promoted shoot elongation of cotton seedlings and petiole elongation of bean leaves. The deformative effects of 2,4-D at the higher levels were not affected by increased gibberellic acid concentration. On the other hand, gibberellic acid promoted the bean epicotyl curvature which developed as a result of 2,4-D action on cell elongation.

These results agree with the findings of Van Overbeek (1959) who concluded that if a particular phase of growth is principally controlled by other naturally occurring auxins, the effect of gibberellic acid will be synergistic. If, on the other hand, the growth is controlled by gibberellic acid, the auxins will antagonize the gibberellic-acid-induced growth. Stuart and Cathey (1961) confirmed this stating that, for the most part, applied gibberellin does not establish new growth patterns but accelerates those in progress.

Both Hauser and Clor et al. found that interaction effects were greater when the gibberellic acid was applied several days before herbicidal treatment. Ashton (1958, 1959) also reported that gibberellic acid, applied 24 hours before treatment by 2,4-D* increased the effects of 2,4-D* in bean plants, as evidence by callus formation. Furthermore, the gibberellic acid resulted in three times more 2,4-D* being retained in the plants 5 days after treatment, which he attributed to increased translocation or decreased 2,4-D* breakdown or both. This
latter conclusion was substantiated by Basler (1959) who found that gibberellic acid stimulated translocation of 2,4-D in bean plants.

**Gibberellic Acid-2,4,5-T Effects on Creosotebush**

Results of the gibberellic acid-2,4,5-T study on creosotebush varied, in part, from the above studies. No significant interaction was found between potassium gibberellate and 2,4,5-T as measured by total plant kill, regardless of method of application or time of treatment (Figure 15). However, since no interaction occurred, these results do tend to confirm the findings of Clor et al. (1958) who found that at high rates of 2,4-D application some herbicide effects were not affected by gibberellic acid concentration.

**Fertilizer Effects**

**Review of Fertilizer Interactions with Herbicides**

The growth-promoting effects of fertilizers on plants are too well documented to be reviewed here. On the other hand, very few studies have been made on the effect of fertilizer-induced growth stimulation on hormonal-type herbicides. Rohrbaugh and Rice (1956) and Rice and Rohrbaugh (1958) found that phosphorus or potassium additions enhanced the effect of 2,4-D as measured by stem curvature and translocation of radioactive 2,4-D*. Both translocation and stem curvature were greater when the fertilizer were applied 3 days before 2,4-D
Figure 15. Effects of prior and simultaneous applications of potassium gibberellate applied with various combinations of carrier and surfactant on the susceptibility of creosotebush to 2,4,5-T.
treatment than when applied 8 hours before, at the time of treatment, or 24 hours afterward.

**Fertilizer-2, 4, 5-T Effects on Creosotebush**

Fertilizer treatments applied to plots 10 days prior to 2, 4, 5-T treatments increased total-kill of creosotebush (Figure 16), but differences were significant only at the 10-percent level. Plants treated 26 to 32 days prior to herbicidal treatment showed decreased susceptibility, but again differences were statistically significant only at the 10-percent level. These results suggest (1) that a relationship may exist between growth mechanisms affected by both the fertilizers and by the herbicides, or (2) that fertilizer stimulation may have in some way increased the absorption, translocation, and/or toxic effects of the herbicide.

A growth-mechanism effect may have resulted from combined growth stimulation first by the fertilizer and later by the 2, 4, 5-T. These effects may have resulted in more rapid growth proliferation and crushing of the phloem. This possibility was not investigated.

Results of this study appear to support the theory that increased plant kill was due to fertilizer effects which increased absorption and translocation of herbicides. It was observed that the fertilizer treatments increased the growth and vigor of existing leaves on the creosotebush plants but did not noticeably stimulate initiation of new leaves.
Figure 16. Effects of prior ammonium phosphate fertilizer treatments on the susceptibility of creosotebush to 2,4,5-T.
Based on the studies reported earlier (Table 4), absorption through these actively growing mature leaves would be high. In addition, the active mature leaves probably resulted in high photosynthate production and transport which in turn increased the translocation of herbicides to the vital parts of the plant. These effects would be greatest after several days growth and would coincide with the increased plant kill 7 to 10 days after fertilization. Later, when leaves were more mature and less active in photosynthate production, the absorption and translocation of herbicides would decrease. This might explain the decreased plant kill which occurred after 26 to 32 days. Also concurrent physiological changes in the plant may have had an effect on translocation. Further studies on the relationship of photosynthate translocation of herbicidal effects are reported in the next section.

Photosynthate-additive Effects

Review of Photosynthate-additive Effects on Herbicides

As already mentioned, numerous studies have shown that translocation of certain foliar-applied herbicides follows the assimilate stream. Studies have also shown that the application of sugars and other substances to the leaves of plants affected translocation of herbicides (Rohrbaugh and Rice, 1949; Weintraub and Brown, 1950; Mitchell et al., 1953; Hay and Thimann, 1956b; Van Overbeek, 1956; Barrier and Loomis, 1957). In general, these studies showed that sugars were
effective in promoting translocation of herbicides only when applied to plants grown in the dark or at low light intensities. On the other hand, sugars applied in the light generally did not enhance translocation of herbicides and in one case (Mitchell et al., 1953) sugars decreased translocation of 2,4-D by 75 percent. This decrease was attributed to dilution of the 2,4-D by the sugar applied.

Translocation of sugars, and therefore possibly the translocation of herbicides in plants grown in the dark, was accelerated by the addition of boron to the sugar solution (Gauch and Dugger, 1953; Mitchell et al., 1953), was increased by the addition of gibberellic acid (Alvim, 1960), and was doubled by addition of surfactant (Weintraub and Brown, 1950). It was also noted that there was no difference in uptake (absorption and translocation) of sugar between two high relative humidites, 65 and 95 percent (Went and Carter, 1948), that translocation was optimum at temperatures between 20 and 30°C and was reduced or blocked at higher (ca 40°C) or lower (ca 5°C) temperatures (Went and Carter, 1948; Swanson and Bohning, 1951; Bohning et al., 1953; Hay and Thimann, 1956b). Hay and Thimann (1956b) also recorded that pretreatment with 2,4-D blocked sugar transport, confirming that 2,4-D damages the phloem. Weintraub and Brown (1950) found that buffering the sugar solution at pH 4.6 or 5.7 had no effect on translocation of sugars but transport was reduced to one-half at pH 6.6 and completely abolished at pH 8.8.
Transport of the various growth regulators, including IAA, 2,4-D, and 2,4,5-T, was about equally accelerated by sugar additions (Weintraub and Brown, 1950). Although sugars increased translocation of herbicides they reportedly have little or no effect on absorption of herbicides (Hay and Thimann, 1956b; Barrier and Loomis, 1957).

Sugars found most effective in promoting translocation of herbicides were sucrose, glucose, maltose, and fructose; least effective were lactose and galactose (Rohrbaugh and Rice, 1949; Weintraub and Brown, 1950; Mitchell et al., 1953). Concentrations of sugar solutions used for foliar application varied from 5 to 10 percent. Went and Carter (1948) observed that 10-percent concentrations of sucrose sprayed on tomato leaves at high temperature and humidity produced no apparent injury. This study did indicate, however, that leaves sprayed with sugar at low temperatures and in sunlight became stiff, turned yellowish, and developed hyponasty and that plant growth was reduced, indicating a lack of sugar uptake.

Photosynthate-2,4,5-T Effects on Creosotebush

Additions of the photosynthate sucrose had variable effects on the toxicity of 2,4,5-T to creosotebush. In the foliar-spray studies, the sucrose produced a significant increase in topkill at the 1/2-pound rate of herbicide per acre and a highly significant increase at the 2-pound rate (Figure 17). In contrast, in the radioisotope studies the
Figure 17. Effect of sucrose additive in the spray emulsion on 2,4,5-T toxicity to creosotebush under daylight conditions.
addition of sucrose resulted in an insignificant decrease in translocation of 2,4,5-T* (Table 6).

The nonsignificant effect on translocation from sucrose additions in the light confirms the findings of previous investigators, especially those of Mitchell et al. (1953) who noted a decrease in translocation due to additions of sugar. The effect on translocation also indicates that the significant increases in topkill of creosotebush from 2,4,5-T due to sucrose additions were not a result of increased translocation of the herbicide. Also, considering previous reports by Hay and Thimann (1956b) and Barrier and Loomis (1957), it is unlikely that the higher topkill resulting from sucrose addition was due to increased herbicide absorption. Therefore, it appears that some unknown physiological or chemical process in the plant was affected by the sucrose additions which in turn increased the toxic effects of the herbicide.
Table 6. Effect of sucrose applications to creosotebush leaves on 24-hour absorption and translocation of radioactive 2,4,5-T* under daylight conditions.

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<th>Date of application</th>
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<tr>
<td></td>
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<td>Relative effect</td>
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CHAPTER XI

AGE-OF-PLANT AND REGROWTH STUDIES

The purpose of this phase of the study was to determine if young plants or sprouts of mature plants were more susceptible than mature plants to the toxic effects of herbicides.

Methods of Study

Age-of-plant Studies

The relative herbicidal susceptibility of young and mature plants of creosotebush was studied in both the Tombstone and Tucson areas during the years 1958 and 1959. Vigorous plants, 3 to 6 feet in height, were selected as mature plants, and well-established plants 1 to 2 feet in height were classed as young plants. A representative sample of the sprayed plants showed that the age of the mature plants, as determined by annual-ring analysis, was approximately 22 years and the young plants 14 years.\(^1\) Both classes of plants were selected and treated in

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\(^1\) The author is indebted to Mr. Thomas P. Harlan of the Laboratory of Tree Ring Research at the University of Arizona for age determinations of sprayed plants.
the same plots. Twenty plants were sprayed for each treatment in 1958 and 10 plants per treatment in 1959.

**Regrowth Studies**

The study on regrowth susceptibility was made on an area pitted with an eccentric-disc plow and reseeded to mixed grasses. The area is located 5 miles west of Tombstone. Mature plants whose tops had been completely broken off by the pitting operation and which were regrowing from the base were classed as sprouters. Mature plants on the adjacent unpitted area were sprayed for comparison. Sprouts were 2 to 3 years old. The reseeding operation was unsuccessful so there was no difference in grass competition between the two areas. However, plants on the pitted area had more available moisture due both to the pits and the reduced stand of shrubs. The soil and vegetation at this site are very similar to that of the main Tombstone study area. Forty plants were sprayed for each treatment in 1958 and 20 plants per treatment in 1959.

In both studies each plant was carefully sprayed with propylene-glycolbutylether esters of 2,4,5-T to insure uniform applications of spray proportional to plant size.
Previous Studies on Susceptibility of Different-aged Plants and Sprouts

Most studies reporting differences in susceptibility of herbicides of different-aged plants of woody species indicate that seedlings and sprouts are more susceptible to herbicides than mature plants. Hull and Vaughn (1951) observed that there was not much difference in the susceptibility to herbicides of different ages and sizes of big sagebrush. Hyder (1953) reported that young mature stands of sagebrush were generally more resistant to 2,4-D herbicides than old mature stands during the peak period of susceptibility but the reverse was true later in the season. Pechanec et al. (1954) observed that the young sagebrush plants in stands of mixed ages were least injured by 2,4-D and 2,4,5-T. Robertson and Cords (1956) noted that young plants in young stands of sagebrush were most susceptible to 2,4-D and 2,4,5-T herbicides; that suppressed juveniles in an old stand were intermediate in susceptibility; and that the old plants were most resistant. These studies indicate that insofar as big sagebrush is concerned the relative susceptibility of different-aged plants to herbicides varies with unknown seasonal changes.

In a study on shrubs in the California chaparral, both young seedlings and fire sprouts of shrubs were found to be more susceptible to phenoxy herbicides than the mature plants (Leonard and Crafts, 1956). This was attributed to the shorter distance of translocation necessary before the herbicide reached the roots and to the greater susceptibility
of the meristematic tissue in the root crowns of the young plants to herbicide injury. Loomis (1955) also noted that young plants were more subject to herbicide injury than old plants due to differences in the reactions and activity of young versus old tissues. This latter conclusion was substantiated by Leonard and Carlson (1958) who found that seedlings of some California brush species were slightly more susceptible than their fire sprouts.

**Results of Age-of-plant Studies on Creosotebush**

Young creosotebush plants were slightly more susceptible than old plants to 2,4,5-T at the 1/2- and 1-pound rates of application (Figure 18). Although these differences were significant only at the 10-percent level of probability, they do tend to confirm the conclusion of Leonard and Crafts (1956) that the greater susceptibility of the young plants was due to the shorter distance that the herbicide must translocate in order to reach the root crown. This conclusion was also substantiated by the radioisotope studies which showed that relatively little translocation of herbicide occurred in creosotebush (Figures 7, 8, 11). These studies also substantiate the conclusion of Loomis (1955) and Leonard and Crafts (1956) that differences in susceptibility between young and old plants was due in part to differences in age and activity of tissues in the root crown. Physiological differences also may have played a part since the younger plants were not involved in flower and
Figure 18. Susceptibility of sprouts and young plants versus mature plants of creosotebush to various applications of 2,4,5-T.
fruit development and therefore more food may have been translocated toward the roots during the susceptible period.

**Results of Regrowth Studies on Creosotebush**

In general, the same relationships existed between the susceptibility of sprouts and mature plants to herbicides as were found between young and mature plants (Figure 18). Again the same reasoning applies that the herbicide was more effective on the sprouts due to the shorter translocation distance, but differences due to meristematic age would not apply. A further contributing factor might have been the better moisture relations found in the pitted, regrowth area. However, good moisture conditions existed on both areas at and subsequent to the time of spraying.

Although not measured directly, results of this study indicate that there was no significant difference in susceptibility between the sprouts and young plants of creosotebush (Figure 18).
CHAPTER XII

SOIL-SITE STUDIES

The purpose of this phase of the study was to determine if fac­tors related to the soil-site complex, such as soil texture, fertility, and moisture, have a combined or interacting influence on plant suscep­tibility to herbicides.

Method of Study

Two adjacent areas on the side of a small intermittent stream near the Tombstone study site were treated in 1958 and 1959. Paired treatment plots were laid out end-to-end at right angles to the stream, one in the deep soil of the bottomland and the other immediately adjoin­ing on the upland. In 1958 two replicates of 10 plants each were treated on each site with 8 pounds per acre of propyleneglycolbutylether esters of 2, 4, 5-T. In 1959 one replicate of 10 plants was treated at both 1/2- and 2-pound rates on each site.

The upland site is similar in soil and vegetation to the main Tombstone study area. The bottomland site has a deep sandy-loam alluvial soil, 4 feet or more in depth. The dominant vegetation consists
of tarbush, mesquite, littleleaf sumac (*Rhus microphylla*), sacaton (*Sporobolus wrightii*), and tobosa grass. Creosotebush occurs as a sub-dominant.

Review of Soil Effects on Plant Susceptibility to Herbicides

Several studies have been made on the relationships of different soil characteristics to the effectiveness of soil-applied herbicides but soil effects on foliar-applied herbicides have been studied only indirectly and with varied results.

Some of the major characteristics which result in marked differences in plant growth include soil depth, soil texture, moisture availability, and soil fertility. These soil characteristics affect photosynthesis and photosynthate translocation, moisture stress and transpiration, cuticular development, and stomatal opening, all of which may affect absorption, translocation, and toxicity of herbicides in plants.

Desai (1937) noted that certain nutrient deficiencies resulted in less active response by stomata to light, water, and other environmental factors. Skoss (1955) noted that absorption of water, oil, or salts of 2,4-D was greatest when stomata were open and plants were under a slight water deficit. However, Weintraub et al. (1954b) and Hauser (1955) found that absorption of 2,4-D was decreased by moisture stress. Skoss also noted that plants under water stress and high temperatures produced cuticle higher in wax and that at moderate temperatures
leaves directly exposed to the sun produced the heaviest cuticle with a corresponding lower rate of absorption. Pallas and Williams (1962) found that soil-moisture stress had no effect on absorption of 2,4-D but translocation was markedly reduced. They attributed reduced translocation, in part, to a decrease in the effective area (vacuole and protoplasm) of the phloem due to cell contraction. Young (1954) reported that moisture stress did not significantly reduce translocation of 2,4-D but did slow absorption. Ashton (1956) noted that soil-moisture stress decreased photosynthesis which in effect might reduce translocation. Hyder (1953) found that sagebrush plants on a south exposed (upland) site were more susceptible to herbicides during the peak period of toxicity than on a bottomland site, but when the upland soil became dry and growth dropped off the plants on the upland soil became less susceptible than those on the bottomland. These studies suggest that the plants growing on deep soils with more favorable moisture and nutrient relations would be more susceptible to herbicides than those on relatively shallow soils.

Results of Soil-site Studies on Creosotebush Susceptibility

Soil-site studies on creosotebush produced variable results (Figure 19). Although results were not statistically significant at the 5-percent level, significance at the 10- and 20-percent levels of probability showed that the trend of total plant kill was higher on deep soils than on
Figure 19. Effect of soil-site factors on creosotebush susceptibility to 2,4,5-T.
shallow soils at the 1/2-pound-per-acre rate of application and lower at the 2- and 8-pound rates.

These differences may be explained in part on the basis of previous studies and observations on conditions at the time of treatment. Soil moisture at the time of spraying was high in both soils so moisture tension should have had no direct, differential effect on absorption. Because of the high moisture condition photosynthesis and translocation were probably rapid in plants on both soils. However, as noted by previous investigators, prior moisture-stress conditions probably resulted in leaves with thicker cuticle and higher wax content on the shallow-soil plants, a factor that would decrease absorption. This difference in absorption may account for the variable results obtained. At the 2- and 8-pound rates of herbicidal treatment, rapid absorption in the thinner-cuticled, deep-soil plants may have caused high phloem injury and blocked translocation, thus resulting in a lower plant kill. In contrast, on the shallow soil, plants with a heavier cuticle would absorb the herbicide applied at high concentrations at a reduced and regulated rate with the result that phloem injury would be less and translocation of herbicides and plant kill would be higher. At the 1/2-pound rate, the herbicide concentration would probably not be sufficient to cause serious phloem injury. Therefore, greater absorption by the thinner-cuticled plants on the deep soil would result in higher plant kill.
CHAPTER XIII

GENERAL DISCUSSION AND CONCLUSIONS

This study confirmed earlier findings that greatest susceptibility of creosotebush to phenoxy herbicides occurred approximately 30 days after initiation of effective summer rains (Figure 7). These findings indicate that the maximum susceptibility of creosotebush is primarily associated with certain physiomorphological growth stages in the plant and secondarily with direct influences of environmental factors such as relative humidity, temperature, and soil moisture. This conclusion is supported by the following:

1. Maximum susceptibility of creosotebush to phenoxy herbicides occurred during the late-flower, early-seed stage rather than in the earlier flush season of growth, indicating an association with physiological development. However, it also may be associated with morphological factors. An association with both physiological and morphological factors was further evidenced by the generally greater translocation of radioactive 2,4,5-T* from old leaves, flowers, seeds, and young bark than from young leaves (Table 4). The relatively high toxicity at this stage of growth may also be due in part to the greater
resistance of older leaves to contact injury by herbicides with a resultant reduction in disruption of the transport mechanism.

2. Young and sprouting plants were more susceptible to 2,4,5-T than mature plants (Figure 18) indicating that herbicide susceptibility was associated with relatively active young and meristematic stages of growth.

3. The effect of fertilization on the susceptibility of creosotebush to 2,4,5-T varied with the time of prior application indicating that a change in the physiology of the plant influenced herbicidal effects (Figure 16).

4. Soil moisture at the 3- to 5-inch depth reduced creosotebush susceptibility to 2,4,5-T only when at or below the wilting coefficient (Figure 7, Table 5).

5. Seasonal temperatures and relative humidity influenced herbicidal toxicity for the most part indirectly through their effects on plant growth (Figure 7). However, wide year-to-year variations in plant susceptibility indicate that climatic factors may have both direct and indirect effects.

An important conclusion from this study is that translocation of herbicides in creosotebush was a major problem in its effective treatment with herbicides. Evidence that this was so is indicated by the following observations:
1. The rate of translocation was very slow, mostly less than 2 cm and not exceeding 5 cm per hour under the most favorable conditions (Figures 7, 8, 11, Table 4).

2. Branches missed in spraying remained green several months after leaves on the rest of the branch had died (Figure 6).

3. Basal parts of the plants not thoroughly wetted with spray sprouted rapidly even after treatment with high rates of herbicide (Figure 6).

4. Absorption of 2, 4, 5-T* was relatively high at certain seasons of the year, but subsequent translocation was low or nonexistent (Figures 7, 9).

A major reason for the slow translocation of herbicides in creosotebush appears to be blockage of the phloem due to contact injury by the herbicide, thus preventing translocation of lethal amounts of 2, 4, 5-T in the assimilate stream of the phloem. Evidence that herbicidal injury blocked phloem transport in creosotebush was indicated by the following observations:

1. Downward translocation of 2, 4, 5-T* stopped after 18 to 24 hours (Figure 8).

2. The heavy esters of 2, 4, 5-T, which generally cause less contact injury, produced highest total-kill in creosotebush (Table 3).

This conclusion is also verified by numerous previous reports which have noted high contact injury from 2, 4, 5-T and related compounds.
On the other hand, evidence that translocation occurred rapidly in the phloem without blockage due to contact injury was shown by the high total-kill of creosotebush which occasionally resulted from low rates of herbicide treatment applied during favorable years and at times of the day when translocation of photosynthates was high (Figures 5, 10).

Although evidence is largely circumstantial, this study indicated that movement of 2,4,5-T in creosotebush also occurred in the intercellular spaces in the oil stream. These evidences include the following:

1. Increasing rates of oil synergistically increased total-kill of creosotebush by 2,4,5-T (Figure 13) indicating that oil may have increased movement of herbicides after the phloem was blocked.

2. The rate of 2,4,5-T* movement in creosotebush (less than 5 cm per hour) coincides with rates of movement for light oils cited by Minshall and Helson (1949) and Rice and Rohrbaugh (1953) rather than the usual rate of photosynthate movement (10 to 100 cm per hour).

These studies also indicated that absorption of herbicides may be a problem in the herbicidal treatment of creosotebush. This conclusion is substantiated by the following observations:

1. Seasonal radioisotope studies with 2,4,5-T* showed that differential absorption does occur (Figure 9).
2. The soil-site study indicated that differential cuticular development produced by deep and shallow soil factors may affect absorption.

However, evidences were also noted which indicated that absorption of herbicides may not be a serious problem, particularly during seasons of high susceptibility. These include the following:

1. The large number of stomata on both leaf surfaces of creosotebush probably increased absorption of herbicides and reduced the probability that absorption would be a limiting factor.

2. Leaf-wiping tests showed that high absorption of 2,4,5-T* occurred during favorable summer and fall growing seasons (Figure 9).

3. The use of oil and surfactant in the spray emulsion undoubtedly increased absorption and reduced the probability that absorption was a critical factor.

The findings from this study point out several avenues of investigation needed to expand our knowledge of herbicidal activity and to increase herbicidal effectiveness on desert shrubs. From the standpoint of foliar-applied herbicides, studies are needed to solve the paradoxical situation in which toxic substances must be translocated in the plant without causing injury to the translocating mechanism. Possible approaches to this problem include:
1. Development of a slow acting, stable herbicide which has low contact injury but which through prolonged activity results in eventual death of the plant.

2. Development of a herbicide that may be absorbed and translocated in a nontoxic form and which later converts into a toxic form. This approach may not only be used to increase the toxic effects of herbicides but may also result in greater herbicidal selectivity.

Still another approach to the problem is the development of more effective adjuvants or formulations to increase the absorption and/or translocation of herbicides within the assimilate stream. This may be accomplished by the development of additives and formulations which result in slow, ordered penetration of herbicides at such a rate that the herbicide tolerance of the living mesophyll and phloem is not exceeded, yet lethal doses are built up in the roots of the plants. Possible adjuvants already being studied include sulfur (Crafts and Reiber, 1948), boron (Gauch and Dugger, 1953; Mitchell et al., 1953), sucrose stearate (McCarthy, 1960), and formulations of polyethylene and propyleneglycol esters (King and Kramer, 1951; Crafts, 1960).
A comprehensive herbicidal study was initiated in 1957 to determine the problems involved in the chemical control of creosotebush, a major dominant over vast acreages of desert and semidesert rangelands in the southwestern United States and northern Mexico.

Both foliar-spray and radioisotope methods were used to determine the susceptibility of creosotebush to various phenoxy herbicides at different seasons of the year and times of the day and to determine the effects of various environmental, physiological, and chemical factors on herbicide absorption, translocation, and toxicity. Major studies were conducted at both Tombstone and Tucson, Arizona, in areas bordering between typical southern desert shrub and desert grassland with an average annual rainfall of approximately 12 inches. Effects of foliar-spray treatment were measured by evaluation of total-kill and topkill of plants. Radioisotope effects were measured by radioautographs showing relative absorption and translocation. Except for the screening tests in which several formulations were tested, the propyleneglycolbutylether
esters of 2, 4-D and/or 2, 4, 5-T were used in the foliar-spray studies. Radioisotopes used were $^{14}$C-labeled acid forms of 2, 4-D* and 2, 4, 5-T*.

Screening tests showed that the trichlorophenoxyacetic formulation was more effective on creosotebush than the trichlorophenoxypropionic and that the low-volatile propyleneglycolbutylether, isooctyl, and tetrahydrafurfuryl esters of 2, 4, -D and 2, 4, 5-T were generally more effective than the pentyl.

Susceptibility of creosotebush to 2, 4-D and 2, 4, 5-T varied widely from year to year and from season to season with highest susceptibility generally occurring 12 to 60 days after the first summer rain of at least 1/2 inch and during above-average rainfall years. Translocation of radioactive 2, 4, 5-T* coincided with high seasonal toxicity, being greatest approximately 30 days after initiation of effective summer rains. This period coincided with the full-flowering to mid-fruitering stage of phenological development and overlapped the period of old-leaf drop. High susceptibility during this period may be explained in part by the generally greater basipetal translocation of radioactive 2, 4, 5-T* from old leaves, flowers, seeds, and young bark than from young leaves. Seasonal temperatures and relative humidity appeared to influence herbicidal toxicity mainly in an indirect fashion through their effects on plant growth.

Creosotebush susceptibility to 2, 4, 5-T at different times of the day did not show a consistent pattern. Current and prior environmental
and phenological conditions conducive to high midday susceptibility of creosotebush appeared to be a high maximum-minimum night-day relative humidity of ca 90 and 30 percent, respectively; a favorable prior rainy season; 3- to 5-inch depth soil-moisture conditions at or above the wilting coefficient; moderate to heavy cloud conditions; and active leaf growth and flower production.

Diesel oil added to the spray emulsion generally resulted in significant increases in creosotebush kill even at high rates of 12.5 to 25 gallons of oil per acre, but increasing the amount of surfactant in the commercial 2,4,5-T preparation had no consistent effect.

Ammonium phosphate applied 10 to 14 days prior to 2,4,5-T treatment resulted in a slight increase in plant susceptibility to herbicides but fertilizer treatments applied 26 to 32 days prior resulted in a slight decrease.

Sucrose added to the treatment emulsions significantly increased topkill of creosotebush by 2,4,5-T, but did not increase translocation of radioactive 2,4,5-T*.

Young plants and sprouts of creosotebush were slightly more susceptible to 2,4,5-T than mature plants.

pH of the spray emulsion, gibberellic acid treatments, and soil-site factors had insignificant or inconsistent effects on creosotebush susceptibility.
Translocation of herbicides appears to be the major problem in the chemical control of creosotebush. The translocation rate of radioactive 2,4,5-T* was relatively slow, 1 to 5 cm per hour maximum. Basipetal movement ceased after 18 to 24 hours, following which the 2,4,5-T* largely disappeared from the tissues of the stem. Absorption of herbicides also appeared to be limiting during certain seasons of the year but probably had little effect during the season of high plant susceptibility.


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