Environmental and Other Factors in the Response of Plants to Herbicides

Agricultural Experiment Station
Oregon State University
Corvallis, Oregon

A WESTERN REGIONAL RESEARCH PUBLICATION

AUTHORS: Lawrence O. Baker, Plant and Soil Science Department, Montana State University, Missoula, Montana; Howard P. Cords, Division of Plant, Soil and Water Science, University of Nevada, Reno, Nevada; H. B. Currier, Department of Botany, University of California, Davis, California; Lambert C. Erickson, Department of Plant Science, University of Idaho, Moscow, Idaho; Chester L. Foy, Department of Botany, University of California, Davis, California; Virgil H. Freed, Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon; M. L. Montgomery, Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon; T. J. Muzik, Department of Agronomy, Washington State University, Pullman, Washington; D. C. Tingey, Department of Agronomy, Utah State University, Logan, Utah; J. B. Vernetti, Chipman Chemical Company, 6200 N. W. St. Helens Road, Portland, Oregon; R. F. Wagle, Department of Watershed Management, University of Arizona, Tucson, Arizona; J. Wayne Whitworth, Department of Agronomy, New Mexico State University, University Park, New Mexico.

Environmental and Other Factors in the Response of Plants to Herbicides

Edited by V. H. FREED and R. O. MORRIS

Foreword

Over the several years of the existence of the Cooperative Regional Research Project on Weeds, a number of research workers in the western states have given serious attention to those factors in the environment which alter the efficiency of herbicides. The project was initiated as W-11, but in 1958 it became W-63, with the title "The Effect of Certain Climatic and Soil Factors on the Response of Plants to Herbicides." Its objectives were summarized by three stated aims:

1. To measure the effect of atmospheric and soil factors on the response of plants to herbicides.
2. To determine the relationship between herbicidal effect and environmentally induced variation within certain plant species.
3. To study the chemical and physical properties of herbicides in relation to environment and effectiveness.

The results of studies directed by these three aims are the concern of this bulletin.

Because of the wide scope of the objectives, the spread of individual studies was correspondingly broad. Experiments were performed on the mechanisms of uptake and translocation of herbicides by plants; on the soil and chemical factors influencing behavior of herbicide when applied; and on the influence of soils, moisture, and fertilizers upon herbicide effectiveness. Further experiments dealt with the effects of enzyme cofactors and other additives, the effects of plant variation, and (not included here) the effects of light upon herbicide action.

The benefits accruing from the studies embrace not only more effective weed control but safer use of chemicals. Here we are concerned with herbicides, but it may be that the findings reported can be extrapolated to help guide the use of other agricultural chemicals in the years ahead. Much of the information is of a basic nature leading to a better understanding of the interaction of the environment, the applied toxicant, and the living organism.

1

Contents

CHAPTER ONE

The Penetration, Absorption, and Translocation of Herbicides

C. L. Foy, J. W. Whitworth, T. J. Muzik, and H. B. Currier

Higher plants, whether desirable and known as crops or undesirable in a given situation and called weeds, are living organisms; hence they are dynamic, complex in structure and function, and ever subject to change. Thus it becomes a challenge to the weed research worker and to the ultimate users of herbicides to alter, interfere with, and regulate the growth of plants—to kill some and to spare others in the same environment according to economic purpose.

The physiological approach to improve the efficiency of chemical weed control measures has been fruitful. Without doubt, future improvements in this field will come not only through the discovery or invention of new chemicals and by developing better methods of application, but through a better understanding of total plant function and of the ways in which various internal and external factors influence function.

Most of the discussion to follow refers to foliar absorption of herbicides since this aspect has been studied more thoroughly than root absorption of these substances.

Consideration of General Concepts

Three phases in the interaction of plants and herbicides can be distinguished: absorption, translocation, and toxic action.

Herbicidal selectivity and toxic action

Herbicidal sprays may be selective or nonselective depending upon many factors (Ashton, Harvey, and Foy, 1961) as follows: (a) leaf properties (size, shape, angle, waxiness, corrugations or other unevenness, and amount and nature of cuticle); (b) location of growing points; (c) growth habits; (d) differential absorption (influenced by the physical and chemical properties of the cuticle, the location, number, size and degree of opening of stomata, and the presence or absence of wetting agents); (e) differential translocation; (f) biophysical-biochemical factors (e.g., inactivation by adsorption, differential tolerances of cellular membranes to toxicants, enzymatic reactions, and *in vivo* activation or detoxification); (g) position of the

3

herbicide in the soil; and (h) selective herbicide placement. It is apparent that many of the above factors influence penetration and translocation of herbicides; these in turn influence ultimate toxicity.

Herbicidal foliar sprays may act as contact toxicants or be translocated systematically following absorption. A systemic herbicide must move from the leaf surface into the phloem and be translocated throughout the plant where it "accumulates" to toxic concentrations in certain tissues. Contact herbicides must also penetrate the leaf.

In order for a foliar-applied chemical to perform as a herbicide, whether selectively or nonselectively, several processes are involved. The herbicidal spray must (a) contact and (b) be retained by the leaf surface; it must then (c) be sorbed (adsorbed to the surface, sorbed into the external or internal cuticle or the more or less continuous outer layer of the plant body, hence flooding of substomatal chambers is termed stomatal absorption, and finally desorbed); then follows (d) penetration, including destruction or the relatively harmless crossing of the outer cell membrane (the plasmalemma); (e) translocation, from cell to cell, and sometimes for great distances in the case of systemic growth-regulator type herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D)* and 2,2-dichloropropionic acid (dalapon); and (f) toxicity at the (often nebulous) morphological and biochemical "site of action."

Factors influencing penetration and translocation

Environmental factors (both physical and chemical) clearly influence the amount of herbicidal penetration and translocation, and therefore ultimate toxicity. Not only short-term effects, but long-term environmentally induced ontogenetic changes appear to be important.

As examples, high temperature and low humidity are detrimental to absorption, since under these conditions the cuticle becomes thicker and less penetrable, spray drops dry rapidly, and stomata may close if water stress develops. As a further example, light stimulates opening of stomata and supports production of photosynthates which promote systemic movement of herbicide in the phloem; thus cuticle-to-vein diffusion gradients are maintained. Light also may influence extension of ectodesmata (protoplasmic threads in the outer epidermal wall), but this remains to be demonstrated.

By varying what might be called the "chemical environment," the experimenter can determine the best formulation of solution to be applied, i.e., the combination of ingredients most effective in killing the plant. This is, of course, an artificially imposed environment, but it is properly considered an environment nonetheless. Greater effi-

---

* See the glossary for abbreviations used.

ciency in herbicide technology already has been achieved through providing the right herbicide and the proper adjuvants and solvents, and through maintenance of pH levels optimum for penetration.

Environmental influences often are mediated by structural changes. Hence, for the greatest possible benefit, pathways and mechanics, or structure and function, should be studied concurrently.

Most of the variables, either known or suspected, influencing foliar penetration and translocation by herbicides have been broadly categorized in recent reviews.

Currier and Dybing (1959) classified the factors possibly influencing penetration and movement of herbicides in plants as follows: (a) plant factors, such as character of the external and internal cuticle, distribution, size and degree of opening of stomata, water balance, leaf morphology, stage of growth, injury, and metabolism; (b) physical environmental factors, including light, temperature, relative humidity, rain, and the effect of spray drop itself; (c) chemical environment or formulation, including structure of the toxicant, pH and buffers, surfactants, humecants, deposit builders, cosolvents, oils and emulsions, and miscellaneous additives; and (d) method of application.

Plant surfaces in relation to uptake and distribution

The importance of the chemical and physical nature of plant surfaces in relation to the use of herbicides has long been recognized. This subject has been reviewed recently (Crafts and Foy, 1962).

The literature dealing with plant surfaces and foliar penetration by chemicals is very extensive. Comprehensive review of all facets is beyond the scope of the present discussion, which is intended mainly as a summary of knowledge on which a discussion of experimental results can be based. Thus for a more critical review of any subtopic, the reader is referred to the original research references, for the most part not cited directly in this bulletin.

Various aspects of the broad subject of foliar penetration have been treated in texts, theses, and other reviews as follows: (a) waxes (Kreger, 1949; Schieferstein, 1955); (b) cuticle (Orgell, 1954; Priestley, 1943; Schieferstein, 1957); (c) retention and penetration of pesticides and growth regulators (Currier and Dybing, 1959; Mitchell, Smale, and Metcalf, 1960; van Overbeek, 1956; Woodford, Holly, and McCready, 1958); (d) physiology, chemistry, and mode of action of herbicides (Crafts, 1961a; Audus, 1964); (e) translocation (Crafts, 1961b); and (f) plasmodesmata (Meeuse ,1957).

Pathways of initial penetration. Both surfaces of leaves function in the absorption of chemicals. Usually the lower epidermis is

more penetrable than the upper. Not all areas of the leaf are equally permeable. Preferential areas of foliar absorption named in the literature for various substances (herbicides, other pesticides, nutrients, and fluorochromes) are: (a) directly over the veins, (b) over anticlinal epidermal walls, (c) glandular and nonglandular trichomes, (d) open stomata, (e) hydathodes, lenticels, and natural fissures, and (f) insect punctures and other imperfections in the cuticle.

Penetration may be classified as stomatal or cuticular, then cellular. Opinion is divided as to whether stomatal or cuticular entry is more important as a generalization. Both are known to occur under appropriate circumstances, and the processes are not mutually exclusive. Which predominates in a given situation may be determined by many interacting factors, Entry of oils (and aqueous sprays with lowered surface tensions) into stomata is apparently by mass movement; entry through the cuticle is by diffusion, at least initially.

Stomatal and guard cell entry. The physiology and biochemistry of guard cell action and stomatal opening and closure are still incompletely understood. It is generally agreed that stomata open or close in accordance with swelling or shrinking of the adjacent guard cells. The changes have been considered to depend upon the starch-sugar equilibrium and the resulting variation in osmotic pressure. Cellular studies by Pallas *et al.* (1961), show that guard cells have an extraordinary ability to remain operative under adverse conditions. A massive bibliography exists on transpiration and stomatal operation alone (see Pallas *et al.*, 1961).

Oils and aqueous solutions with lowered surface tensions due to the addition of suitable surfactants, penetrate large open stomata readily. Completely closed stomata tend to exclude all liquids.

Even substances entering through stomata, however, must next penetrate the internal cuticle. Although perhaps reduced in amount and different in composition from the external cuticle, the internal cuticle seemingly constitutes a lipoidal barrier, nonetheless.

A new view regarding foliar penetration pathways has been proposed by Sargent and Blackman (1962). 2,4-D was alleged to move principally *through* the guard cells and accessory cells, not between them, thus involving cuticular rather than (or in addition to) stomatal penetration.

Penetration of the lower epidermis of bean leaves was greater than of the upper epidermis; this was ascribed to the greater frequency of guard cells on the lower surface. Metabolic inhibitor studies indicated that active transport is involved. Although Franke (1961) reported a concentration of ectodesmata in guard cell walls, this fact was not considered by Sargent and Blackman. If one suggests that the de-

6

gree of stomatal opening is immaterial in leaf absorption of chemicals under any condition, then the results of Dybing (1958) are inexplicable. It must be kept in mind that Sargent and Blackman (1962) were dealing with relatively long-time absorption periods (usually 8 hours), Dybing with shorter times (15 to 30 minutes).

Since the cuticle appears to deserve primary consideration, a discussion of its physical and chemical properties in relation to penetration is in order.

Cuticular penetration. Both oils and aqueous solutions apparently penetrate the cuticle slowly. Judging from evidence on cuticular transpiration and herbicide penetration, the cuticle is presumed to be permeable with difficulty. On theoretical grounds, since both polar ions and nonpolar fat-soluble molecules penetrate cuticle, both polar or aqueous routes and nonpolar or lipoid routes are assumed to exist. It is not yet possible with ultramicroscopic studies to describe these routes, morphologically, within the cuticle. However, some understanding of how the two routes could conceivably exist is gained by a consideration of the chemical as well as the physical properties of the components which make up the cuticular layers. Since roots are not normally covered by a cuticle, the following remarks apply only to foliar penetration.

The cuticle is a more or less continuous but nonuniform and, in old leaves, often imperfect layer of metabolic products laid down by the cells of laminar organs.

Frey-Wyssling (1948) summarized the chemical nature of cutinized plant cell walls; they are composed of four rather distinct substances (or groups of compounds), all of which may vary in distribution within the wall. Probably still our best concept of the submicroscopic anatomical relationships of the cutinized epidermal wall is that represented diagrammatically by Orgell (1954), as modified from Frey-Wyssling (1948), Mueller *et al.* (1954), and Roelofsen (1952). (See Figure 1.) These substances are (a) cutin, (b) cutin waxes, (c) pectin, and (d) cellulose.

The term "cuticular layer" refers to the semi-lipoidal lamellae of the surface covering that have become embedded with wax and cutin. It may be referred to as cutinized cell wall and include pectin and the cellulose of epidermal walls when it has become impregnated with lipid substances.

All constituents impart their own physical and chemical properties to the surface layer of the plant. Each of the four major components will be discussed briefly.

"Wax" rodlets
"Wax" layer
"Wax" lamellae
Cutin
Cellulose lamellae
Pectic lamellae
Cellulose cell wall
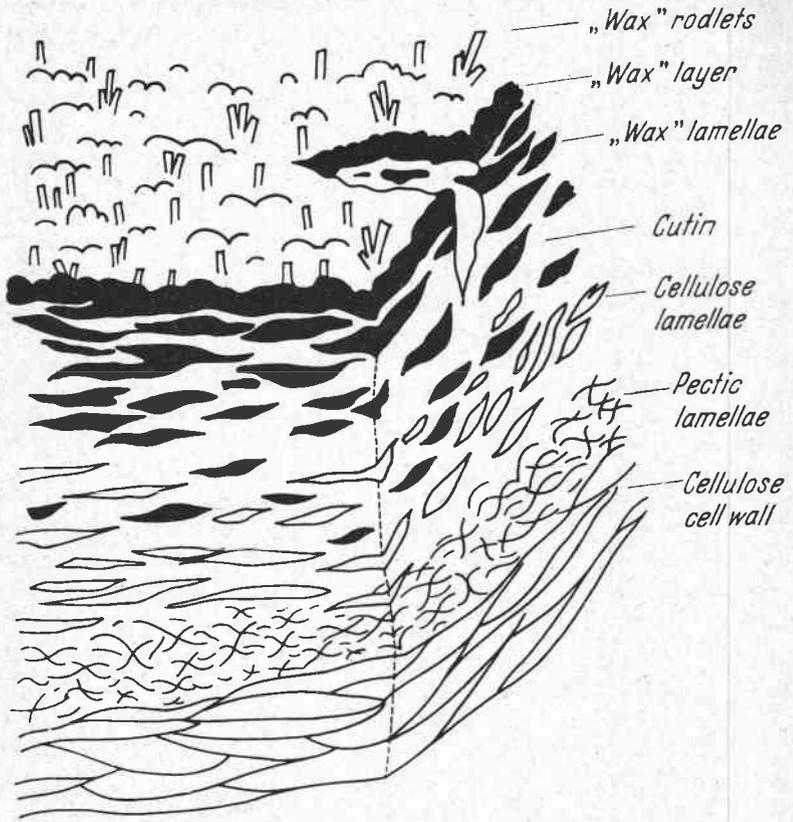
Figure 1. Submicroscopic structure of the cuticle. Lamellae are interlinked and cracks may occur in the outer surface. From Orgell (1954), as modified from Frey-Wyssling (1953), Roelofsen (1952), and Mueller et al. (1954).

Cutin is described as a semi-lipoidal oxidative polymer of long-chained fatty acids and alcohols. Such compounds, products of metabolism, migrate to the protoplast surface and then to the outer epidermal wall. Here at the surface they tend to become oriented with their polar groups in the water phase and with their hydrocarbon chains toward the outside. With age, as water is lost and reaction with oxygen proceeds, the more or less continuous "varnish-like" layer is formed. Cutin, then, results from the oxidation and polymerization of various unsaturated lipid compounds. Once formed, it is insoluble in most organic solvents and apparently constitutes the matrix of the cuticle.

Suberized and cutinized wall layers contain unsaturated high-molecular-weight ketones, alcohols, or esters of unsaturated alcohols; polymerization to form chains of complex structure is made possible by their end groups which react to form esters. Since cutin possesses a negative charge in water, has selective cation permeability, and is stainable with basic dyes, it must be only partially esterified, with some free carboxyl groups exposed at the surface. Also, since it is optically isotropic, cutin is assumed to possess a reticular linkage structure like lignin. Because of its chemistry, cutin has both polar and apolar properties; it is semipolar.

Waxes. Cuticular wax refers to the petroleum ether-soluble mixture of more-saturated lipid substances embedded in the cuticular layers. Surface wax refers to the usually irregular deposits of similar material found on the cuticle surface of some species. Part of the cuticular wax impregnating the framework of cutin is believed to be in chemical combination with the cuticle. Cutin waxes apparently consist of short rod-shaped molecules having no reactive end groups; being relatively inert, they are unable to polymerize and are of low molecular weight. Cutin waxes are optically negative, stainable in lipid dyes, and melt above 220° C.

Waxes are hydrophobic and resistant to wetting with pure aqueous sprays. The waxy rodlets of leaves forming a "bloom" may prevent contact of a spray droplet with the leaf surface (Figure 2). The selectivity of certain formulations of 4, 6-dinitro-2-s-butylphenol (DNBP) contact sprays in peas, for example, is dependent upon this lack of contact. As cited by Woodford, Holly, and McCready (1958), pretreatment with certain herbicides such as trichloroacetic acid and dalapon, which interfere with or alter wax deposition and thereby increase the wettability of peas to aqueous sprays of DNBP, may cause a loss of herbicidal selectivity based on differential wetting. More recently, ethyl-N,N-di-n-propylthiolcarbamate (EPTC) and related compounds have also been shown to interfere with the deposition of surface wax in *Brassica* spp. (Gentner, 1961).

Oils or aqueous sprays containing a suitable surfactant normally wet waxy leaf surfaces. In the case of surfactants, however, it should be pointed out that enhanced wetting is not always synonymous with enhanced penetration. Surface wax deposits may interfere appreciably with wetting but little with penetration, providing good surface contact is insured; despite adequate external wetting, however, internal cuticular wax may still constitute a serious barrier to penetration of aqueous substances. Once laid down, the waxes are rather inert.

Pectin consists of long-chain polygalacturonic acid molecules having side carboxyl groups. They are capable of forming salts; they
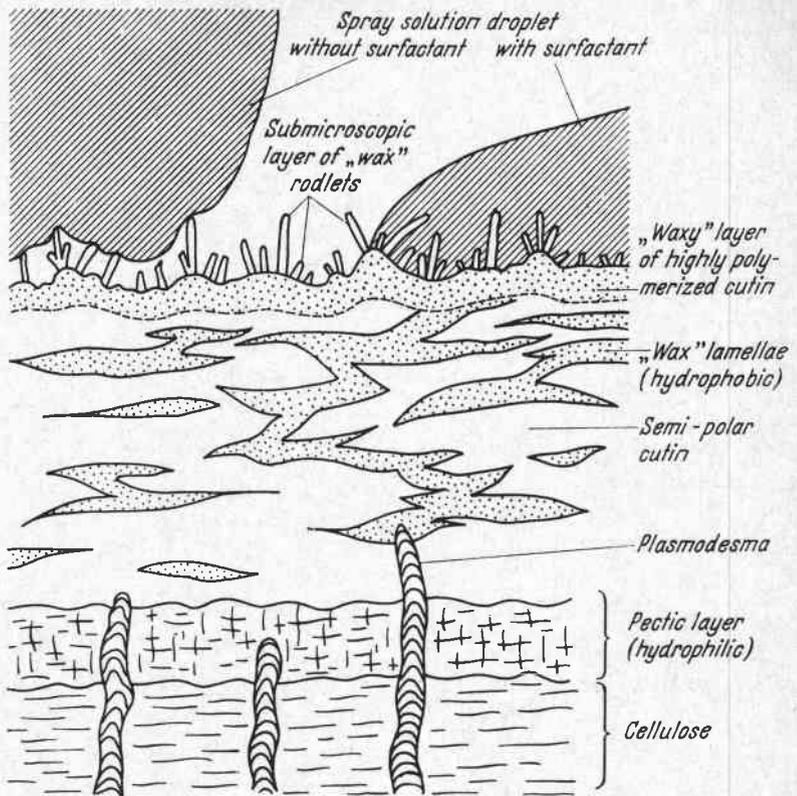
Figure 2. Hypothetical structure of the functional aspects of the plant cuticle. The waxy rodlets of leaves having "bloom" may prevent contact of a spray droplet with the leaf surface. The use of dinitro contact sprays selectively in peas depends upon this mechanism. From Orgell (1954).

impart to pectins base exchange properties. Both polygalacturonic acid and its methylated derivative are soluble in water; however, calcium pectate is insoluble. Pectic substances have little tendency to crystallize; they occur in an amorphous state and are responsible in large measure for the strong water-holding capacity of cell walls. Thus the pectic layer and the cellulose which it bounds are hydrophilic or polar.

Cellulose is composed of long-chain molecules that are relatively stable. These molecules are associated into microfibrils. Because of its microfibrillar organization, cellulose imparts tensile strength and elasticity, but it is not an important obstacle to the penetration of aqueous sprays as are cutin and cuticular waxes in the cuticle.

10

Since the hydrophobic wax molecules repel the hydrophilic cellulose, it seems probable that the polar (but semi-lipoidal) cutin molecules are interposed between them predominantly with their hydrophilic OH, COOH, and OCH_3 groups bounding the cellulose and their hydrophobic hydrocarbon chains in contact with the waxes.

Cutin may contain appreciable quantities of polymerized carboxylic acids. Having many polar groups, such cutin may absorb water and swell appreciably. This hydration could spread the wax components apart and tend to increase the permeability of the cuticle to polar molecules, such as water-soluble herbicides.

This proposed "value system" may have real significance with respect to the absorption of herbicides via an aqueous (polar) route as contrasted to a lipoidal (apolar) route. High turgidity of underlying tissue, high relative humidity (near saturation) and, locally, the spray droplet itself may influence this phenomenon.

Generally speaking, there exists a gradient from low polarity at the exterior of the cuticle to relatively high polarity in the layers bordering the epidermal cell wall. Lipophilic waxes predominate toward the outside; the outer layers contain only wax and semi-lipoidal, semipolar cutin. Hydrophilic substances, such as cellulose and pectins, predominate in the inner regions, where they lend strength and water-retaining properties.

Coefficients of asymmetry of penetration of water through cuticle have been calculated. Schieferstein (1957) found ivy cuticle to have a greater permeability to water in the inward direction than in the outward direction.

The polarity of herbicide molecules determines their solubility in the carrier solution, the cuticle, cell wall, and membrane. The less polar the molecules, the more lipid soluble they are. Undissociated solutes are relatively nonpolar; therefore, they are oil-like and penetrate lipoid barriers more readily. Thus, one may speak of the apolar or oil-like properties of even a polar compound like dalapon if it is at a low pH in water (i.e., relatively undissociated) or modified by the addition of surfactants which combine both polar and apolar properties.

The possibilities for cuticular penetration, then, may be somewhat as follows:

For aqueous solutions of inorganic salts, acids, and bases and polar organic compounds, substances may enter through cracks, punctures, or areas of leaves not completely covered by waxy lamellae and then follow a polar (aqueous) route, presumably by the hydrated cutin or the hydrophilic pectic and cellulose portions of the wall. Of course, even polar molecules may diffuse slowly across hydrophobic

11

layers. The thickness of such layers can be as little as a fraction of a micron.

For oils or apolar solutes, absorption directly through the waxy portions of the cuticle via an apolar (lipoid) route must occur, at least initially.

For substances exhibiting both polar and apolar properties (e.g., many formulated organic herbicides and most surfactants), entry and transport likely occurs via a combined aqueous and lipoid route through the cuticle proper as well as through imperfections. Later, ectodesmata (Franke, 1961) may play an important role in further penetration, but this needs confirmation.

Hydrocarbons and surfactants may "solubilize" into the cuticle or the plasma membrane, displacing the lipoid molecules and increasing permeability. Oils penetrate lipid surfaces readily; aqueous sprays are given some of the properties of oils by addition of suitable surfactants.

Since penetration of herbicides is ultimately controlled by adhesion of molecules, the composition and surface chemistry of the cuticle are important. Both chemical and physical properties are involved and the two are not easily separable.

Movement of substances through cuticle involves diffusion which is conditioned by particle size, pH, molecular structure of the penetrant, hydration, and possibly other factors. Interactions between cuticle and applied substances may be (a) mechanical (size of penetrant particle relative to pore size), (b) physicochemical (competition for adsorption sites), and (c) chemical (chemical or electrical reactions). The nature of the cuticle and penetrating substances and their physical and chemical environments determine the extent of these interactions and whether they will help or hinder penetration. The steric, polar, electrical (ion charge), and chemical properties of the penetrant molecule will influence its reaction with cuticle (Mitchell, Smale, and Metcalf, 1960; Orgell, 1954).

Finally, it has long been recognized that surfactants may facilitate and accentuate the emulsifying, dispersing, spreading, wetting, solubilizing, or other surface-modifying properties of herbicidal formulations to bring about enhancement of penetration and herbicidal action. Surfactants act primarily by virtue of their combined polar and apolar properties, rendering compatible two phases (e.g., lipoidal and nonlipoidal substances) which were otherwise incompatible. Some surfactants whose properties are known probably orient polarly and become solubilized in the cuticle, thus causing a loosening or swelling of the cuticular architecture and thereby enhancing penetration. The most effective spray additive to enhance penetration may depend on several

12

factors of the plant, environment, and spray formulations, either related to or possibly independent of wettability properties.

Despite the foregoing attempts to generalize, it must be borne in mind that the cuticle of plants is highly variable both in composition and in physical structure. Like other plant structure, it undergoes ontogenetic changes. Hydration, weathering and degradation, insect punctures, abrasion, and other physical stresses may also render the cuticle more permeable. Much of the above is subject to and deserving of further experimentation.

Ectodesmata. The occurrence and possible importance of cuticular pores and ectodesmata (protoplasmic strands protruding for various distances into the exterior walls of epidermal cells) have been of recent interest (Franke, 1962a,b; Schnepf, 1959; Crafts and Foy, 1962). Currently these structures are the subject of active research and controversy.

Most ideas center around the epidermis which contains pores in the outer wall through which lipids or cutin are secreted. Still nebulous are (a) the distance outward to which such pores normally extend; (b) whether "cuticular pores" correspond generally to ectodesmata; (c) their alleged diurnal rhythm as to stainability; (d) the prevalence of these structures in various parts of a given leaf, among species, and under different environmental conditions; and (e) their role and importance in the absorption of pesticides.

Franke (1961), for example, believes that these structures (ectodesmata) constitute the principal pathways followed by foliar-applied chemicals into the leaf. A strict proof of this hypothesis, however, has not been presented. Structures or pores observed in the outer epidermal walls may have quite different functions, one possibility being the transport of cuticular precursors to the leaf surface.

The plasma membrane. Once cuticular penetration has taken place, the plasma membrane may be affected in various ways by different toxic molecules (Figure 3). The normal unaltered membrane is believed to consist of a double layer of fatty molecules stabilized by protein layers. Materials essential to life are kept inside the cell by the structural integrity of, particularly, the central fatty portion of the membrane. This and other plant membranes control the flow of materials (foods, gases, water, organic substances, salts, and so forth) in and out of the cell and between the major cell components. Increasing the spacing between the individual units of the lipid layer leads to increased permeability, which when severe enough (as with carrot oils or aromatic weed oils) causes vital materials to leak out, causing death. Organic solvents, polycyclic hydrocarbons, and surfactants

protein layer ⟶

hydrophilic groups { 10A

lipophilic double layer { 45A

hydrophilic groups { 10A

protein layer ⟶

N C S N

D F E N
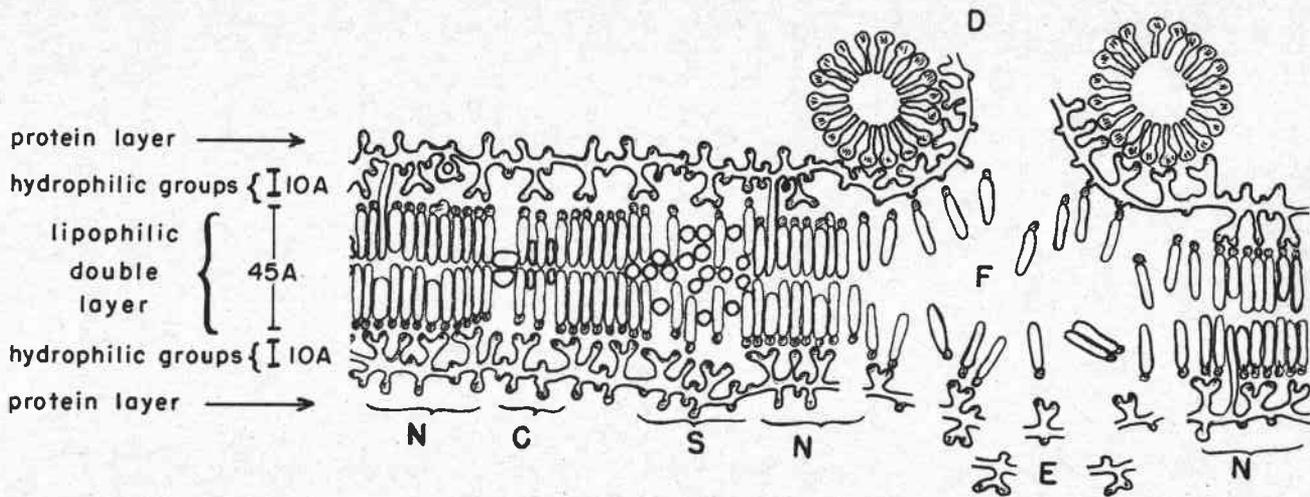
Figure 3. Schematic representation of the plasma membrane as affected by various toxic molecules. The normal unaltered membrane (N) consists of a double layer of fatty molecules stabilized by protein layers. Materials essential to life are kept inside the cell by the central fatty acid portion of the plasma membrane. Increasing the spacing between the individual units of this fatty layer leads to increased permeability, which, when severe enough, causes vital materials to leak out of the cell, causing death. At S, molecules such as xylene are seen solubilized into the fatty layer and at C the natural units are being pushed apart by polycyclic hydrocarbons. Detergent micelles at D are shown pulling away the protein layer, thereby rendering the fatty layer (F) unstable. At E, a similar disruption of the protein layer is brought about by agents which liquefy the protein. Although this drawing might give the impression that the molecular arrangement in the plasma membrane is static, it must be remembered that in reality, all molecules are in a state of vigorous thermal agitation. From van Overbeek and Blondeau (1954).

may be visualized to increase the permeability and/or cause the death of cells in this manner. Also, conceivably, some surfactants and organic compounds that tend to liquefy or denature proteins may similarly disrupt the protein layers of the membrane directly.

In some cases of phytotoxicity, there is a rapid and obvious destruction of cell membranes, which produces a leakage of cell contents and a "water-soaked" appearance preceding death. In other instances, permeability changes are subtler and penetration occurs without dramatic effect.

Movement within the leaf and export

Transport routes from leaf surface to phloem or xylem. The following possibilities exist for the movement of spray substances from an intact leaf surface to the phloem or xylem.

Across cuticle, then (1) anticlinal walls of epidermis and mesophyll, especially those of the bundle sheath extensions, or (2) via plasmodesmata in the outer periclinal epidermal wall and symplast (interconnected protoplasts) to the phloem, or (3) a combination of (1) and (2).

Through stomata, then (1) across internal cuticle, then wall channels, (2) across internal cuticle, then into plasmodesmata and symplast, (3) in intercellular spaces, spreading along wall surfaces, or in bulk, to vascular bundles, or (4) combinations of these.

The tissues involved in the movement of herbicides from cuticle to phloem and xylem are not well known. Various pathways by which pesticides may enter plants have been reviewed (Crafts, 1961c; Crafts and Foy, 1962; Currier and Dybing, 1959; Mitchell and Linder, 1957; Mitchell, Smale, and Metcalf, 1960; van Overbeek, 1956).

Some herbicides such as monuron and simazine are believed to move primarily apoplastically (i.e., via the interconnected nonliving wall phase), whereas others (e.g., dalapon, 2,4-D, and amitrole) are thought to be transported symplastically in the interconnected protoplast system or by a combination of symplast and apoplast. Direct and conclusive evidence is lacking, however.

Vascular translocation. Once inside the xylem, most if not all chemicals appear to be swept passively toward transpiring surfaces. This is true for both leaf and root absorption. Important basic processes that require study are those that determine to what extent substances enter xylem, phloem, or both. These processes are inherent in parenchyma cells contiguous to vessel, tracheid, and sieve tube.

Once inside the phloem, growth regulators and toxicants (in sublethal concentrations) appear to move passively in association with

15

food materials. A pressure mass-flow system, modified by many active ancillary processes, is the most plausible mechanism for phloem transport of solutes. However, herbicide molecules differ greatly in their relative mobilities in plants, following both root and foliar uptake (Crafts, 1959; Crafts and Yamaguchi, 1958, 1960).

Only rarely are many of the major factors affecting penetration and performance of herbicides evaluated in a given practical study on intact plants. One pertinent example is cited. For the translocatable growth regulator, maleic hydrazide, Smith *et al.* (1959) found that absorption, rather than translocation, chemical instability, or volatility, was the important factor limiting its effectiveness. Light, temperature, and application rate were not critical in influencing absorption rate within the normal range. Plant species and plant conditions had significant effects, however. Relative humidity and formulation were also very important. All formulations were absorbed poorly at low relative humidity. At moderate and high relative humidity, formulation differences were evident.

Outlook and Statement of Objectives

The foregoing has dealt primarily not with details but rather with general concepts developed over a long period of time by many researchers.

For example, much new information, both fundamental and practical in nature, was brought forth by the previous Western Regional Research Project W-11, concerning various "Physiological and Ecological Factors Related to Weed Control." With further study, however, the complexity and interactions of factors governing the effectiveness of foliar-applied herbicides have also become even more apparent.

Thus research along the same line of interest, and specifically on the penetration of herbicides as influenced by factors of the physical and chemical environment, was continued from 1959 to 1964 under Western Regional Research Project W-63. The remainder of this report will constitute a summary of the results of these studies.

The primary objective of the research was to obtain information that could be used to enhance the absorption (and sometimes translocation) of herbicides by plants, and consequently to provide improvements in herbicidal efficiency. At the same time, theoretical knowledge of basic plant physiology should be advanced.

More specifically, the project aims were as follows:

1. To determine morphological pathways and physiological mechanisms attending the absorption of herbicides applied (primarily) to leaves.

2. To evaluate the effects of several varying factors of the physical environment (light intensity, temperature, and relative humidity) and of the chemical environment (solvent, surfactant, and pH) on these pathways and mechanisms.

3. To distinguish and compare cuticular and stomatal components of foliar penetration, and to observe relative and changing magnitudes of chemicals absorbed via the two pathways under varying external conditions.

4. To study cellular transport of herbicides in plant tissues with attention to the participation of the cell walls (apoplast) and the protoplasm, and to determine the effect of callose formation on such transport.

5. To study the processes involved in the absorption of herbicides by conducting elements of the phloem and xylem, respectively.

6. To constantly attempt to improve formulation of applied solutions through addition of adjuvants in order to increase penetration. To seek relationships between surfactant structure and properties, and additive effectiveness, is a continuing objective.

General Experimental Procedures

Numerous experimental plants, both cultivated crops and weed species, were selected according to suitability for specific purposes. The various desired environmental conditions were obtained by growing or transferring plants into constant environment chambers or by bagging; other plants were grown in the open greenhouse.

Methods employed for the study of morphological pathways, descriptive for the most part, included fluorescent tracers used in connection with ultraviolet illumination; histochemical methods, by which precipitates are caused to form in location where movement occurs; and radioactive tracer macroautoradiography, histoautoradiography, and counting. Inhibitors (HCN, azide, and DNP) were employed to help determine to what extent metabolic processes control or affect movement from cuticular surface to vascular elements.

Stomatal opening was determined by direct microscopic examination. The amount of stomatal penetration of bulk solution was assessed by the use of tracers, either the fluorescence method (e.g., sodium pyrenetrisulfonate) or radioisotopically-labeled herbicides and surfactants. Cuticular penetration was also followed by fluorochromes and radioactive tracers. Hypostomatous species were especially useful in comparing cuticular penetration alone with somatal plus cuticular absorption.

Cell wall symplast movement was followed by one or more of the tracer techniques noted earlier. Callose is easily detected in both living and dead cells by the aniline blue fluorescence method (Currier, 1957). Satisfactory methods for studying transport into and out of sieve tubes and tracheary elements have not yet been devised.

Various additives were tested in spray solutions. Most attention was given to surfactants of accurately known structure which have markedly improved penetration of herbicides. Physical properties such as surface tension, contact angle, and spreading coefficient were compared with observed wettability and herbicide enhancement as an aid in understanding surfactant action. Limited studies with three radioactive surfactants obtained recently also were conducted.

Toxicity of solutions was determined by spraying whole plants. On a cellular basis, plasmolysis was the preferred criterion of vitality.

Results and Discussion

Morphological pathways of initial penetration

Histochemical precipitation, fluorescent dye, and radioactive tracer techniques were employed, the last two sometimes in combination.

Histochemical precipitation (Dybing, 1958). A Prussian-blue precipitation method (Rudolph, 1925) was used to detect the penetration and internal movement of ferric iron from $Fe_2(SO_4)_3$ solutions. *Zebrina* leaves were immersed for 5, 15, 30, or 60 minutes in 10% ferric sulfate with or without surfactant (Vatsol OT). After washing, the leaves were infiltrated under vacuum with 10% potassium ferrocyanide, producing the insoluble (Prussian blue) precipitate which is clearly visible microscopically and remains at the locus of reaction.

The distribution patterns were similar to those obtained with fluorescent and radioactive tracers. Iron penetrated readily via open stomata when a surfactant was employed. However, no surfactant assistance in cuticular penetration was evident in this particular study.

When leaves were sectioned with a freezing microtome, the precipitate was found in substomatal chambers, mesophyll, and epidermis. It was located mainly in the cell walls, but the chloroplasts were often stained (indicating intracellular penetration) and the intracellular spaces of the mesophyll were sometimes filled with precipitate. Cuticular penetration over veins, via hairs, and at the anticlinal walls was also confirmed. When applied without surfactant, the iron was

sometimes found in the guard cells and accessory cells, but it was not known whether entry was through these cells or through stomatal pores.

Fluorescent tracer experiments (Dybing, 1958). In studies of penetration by the fluorescent dye method (Dybing and Currier, 1959; Dybing and Currier, 1961), 0.1% solutions of the fluorochrome sodium 3-hydroxy-5,8,10-pyrenetrisulfonate were applied as droplets or by immersion of the test leaves in the dye solution. Of the 14 dyes tested, this compound exhibited the most suitable properties for use as a fluorescent tracer. Penetration of surfactant-free solutions was compared with solutions containing surfactants. At the end of the test period, nonabsorbed dye was removed by washing the leaves in running water; then the leaves were examined and photographed in ultra-

Table 1. EFFECT OF VATSOL OT CONCENTRATION ON STOMATAL PENETRATION BY FLUORESCENT DYE IN A FIVE-MINUTE TREATMENT PERIOD

Plant	Stomatal aperture	Fluorescence intensity (Photometer reading, avg. of 5 leaves)				
		Surfactant conc., percent				
		0	0.01	0.05	0.1	0.5
Phaseolus vulgaris L. (trifoliate leaf)	Open	....	3	1	4	49
	Closed	....	3	7	3	6
Vicia faba L.	Open	2	0	0	80	443
	Closed	0	1	8	6	13
Prunus armeniaca L.	Open	8	12	146	171	203
	Closed	0	0	2	3	2
Pyrus communis L.	Open	0	4	110	218	267
	Closed	0	1	3	11	32
Citrus sinensis Osbeck	Open	0	0	9	22	70
	Closed	3	3	1	1	1
Sorghum halepense (L.) Pers.	Open	0	8	8	5	132
	Closed	0	0	0	0	0
Lactuca scariola L.	Open	1	0	13	124	136
	Closed	4	7	1	4	15
Vinca major L.	Open	4	2	5	19	118
	Closed	4	4	3	8	9
Chenopodium album L.	Open	32	24	25	36	77
	Closed	1	0	8	1	0
Convolvulus arvensis L.	Open	4	3	0	2	129
	Closed	0	0	4	3	2

SOURCE: Dybing and Currier, 1961.

Table 2. EFFICIENCY OF DIFFERENT SURFACTANTS IN PROMOTING STOMATAL PENETRATION OF FLUORESCENT DYE INTO APRICOT LEAVES[1]

Surfactant, 0.1 percent	Fluorescence intensity (Photometer reading, avg. of 5 reps.)	
	Stomata open	Stomata closed
Vatsol OT (Na dioctylsulfosuccinate)	468	9
Tween 20 (Polyoxyethylene sorbitan monolaurate)	235	19
X-77 (Alkylarylpolyethylene glycols plus free fatty acids and isopropanol)	423	9
Brij 30 (Polyoxyethylene lauryl ether)	375	5
Nonic 218 (Polyethylene glycol tertdodecyl thioether)	305	6
Tergitol NPX (2-ethylhexylphenyl ether of polyethylene glycol)	317	6

[1] Treatment period: 5 minutes.
SOURCE: Dybing and Currier, 1961.

violet light. A relative measure of dye penetration was obtained by measuring the net increase in fluorescence intensity of the treated leaves with a photometer.

Stomatal penetration by aqueous solutions containing wetting agents was demonstrated with 15 species (see partial list in Table 1) and 6 surfactants (Table 2).

When a suitable surfactant was employed, entry via open stomata was very rapid and the leaves became intensely stained in 30 seconds. The surface tension of the applied solutions determined stomatal uptake for a given species and surfactant. When many plants and additives were studied, however, surface-tension reduction was not a limit-

Table 3. ABSORPTION OF SURFACTANT-FREE FLUORESCENT DYE SOLUTION IN A THIRTY-MINUTE TREATMENT PERIOD

Plant	Stomatal aperture	Fluorescence intensity (Photometer reading, avg. of 10 reps.)
Zebrina pendula Schnizl	Closed	6
	Open	7
Pyrus communis L.	Closed	21
	Open	34
Prunus armeniaca L.	Closed	11
	Open	101

SOURCE: Dybing and Currier, 1961.
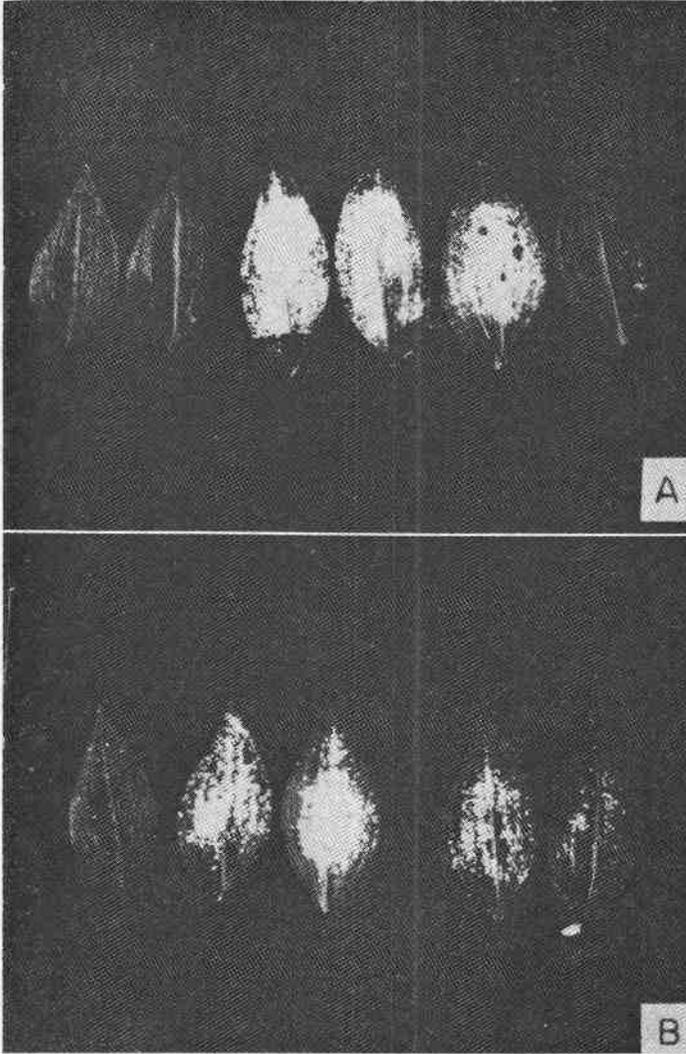
Figure 4. Penetration of fluorescent dye solutions into Zebrina leaves. A. Left to right: untreated control, dye alone, dye plus Vatsol OT at 0.5, 0.1, 0.05, and 0.01%, respectively. All treatments were for 5 minutes. Lower surface of leaves is shown. B. Left to right: untreated control, two leaves treated for 30 seconds, and two leaves treated for 10 seconds. Wetting agent included in test solution. Stomata open. From Dybing and Currier (1959).

ing factor. Promotion of stomatal penetration undoubtedly can be a very important function of a wetting agent (Figure 4).

Fluorescent dye was absorbed poorly from surfactant-free solutions, by leaves having either closed or open stomata (Table 3). Clear evidence for stomatal entry of surfactant-free dye solutions was not obtained. However, some observations indicated that this type of penetration may take place slowly with some chemicals and in certain readily penetrated species. Stomatal penetration by small droplets under high pressure was confirmed.

Penetration of the cuticle was confirmed. Leaf hairs, the epidermal cells over veins, and the cuticle over anticlinal walls of the epidermal cells appeared preferentially penetrated.

The species variable proved to be very important. Apricot, pear, and *Zebrina* leaves were readily penetrated through the stomata by surfactant-containing solutions, but cuticular penetration by the fluorescent dye was relatively slow. The fluorochrome rapidly entered bean leaves by cuticular penetration of the epidermal cells (including hairs) over the veins. Stomatal penetration was also demonstrated with this species, but less dye was present in the leaf after a five-minute treatment than with any other species studied.

PTS, when applied with a surfactant, accumulated first in the substomatal chambers of *Zebrina* leaves (Figure 5). It then moved via the intracellular spaces and walls into the mesophyll, lower epidermis, and upper epidermis (Figure 5). The fluorochrome also accumulated in epidermal cells. Since the dye reportedly does not enter living cells, this result was presumably due to the toxic action of the surfactant.

Combination fluorescent and radioactive tracer experiments (Dybing, 1958). Radioactive substances employed in the initial studies included the sodium and triethanolamine salts of 2,4-D, the triethanolamine salts of 2,4,5-T and benzoic acid, urea, amitrole, maleic hydrazide (all labeled with C^{14}), and phosphoric acid labeled with P^{32}. With regard to stomatal flooding, the results complemented the fluorescent dye studies. This was especially true when dye and radioisotope were applied in the same droplet. The same penetration patterns were revealed in autoradiographs of frozen-dried plants as produced by penetration of the dye recorded photographically (Figure 6). This result indicated that both tracers penetrated via the same route, the stomata.

Cuticular penetration of radioactive tracers was not correlated with that of the fluorochrome, however. This leads to the conclusion that different penetrants follow varying paths across the cuticle. Also, since PTS does not enter living cells, its internal movement would be expected to differ from that of most herbicides.
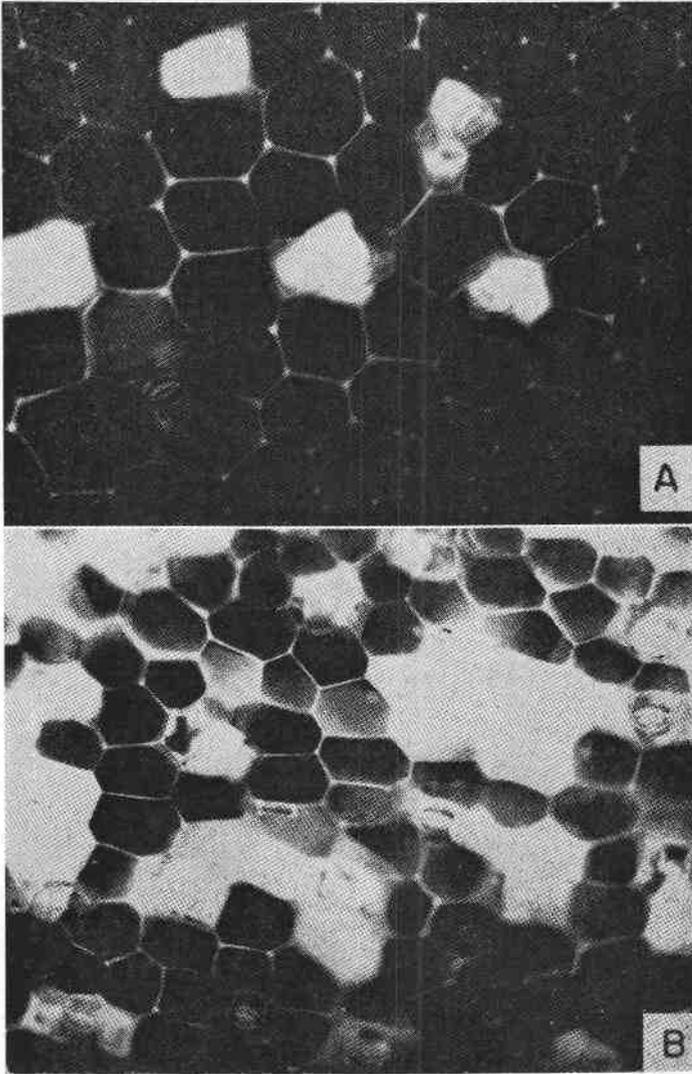
Figure 5. Photomicrographs showing dye in Zebrina leaves. In A, leaves were immersed for 5 minutes; only substomatal chambers contain dye. In B, the treatment was 30 minutes, and dye is present in substomatal chambers, anticlinal walls, and some epidermal cells. Surfactant was included in both tests. From Dybing and Currier (1959).
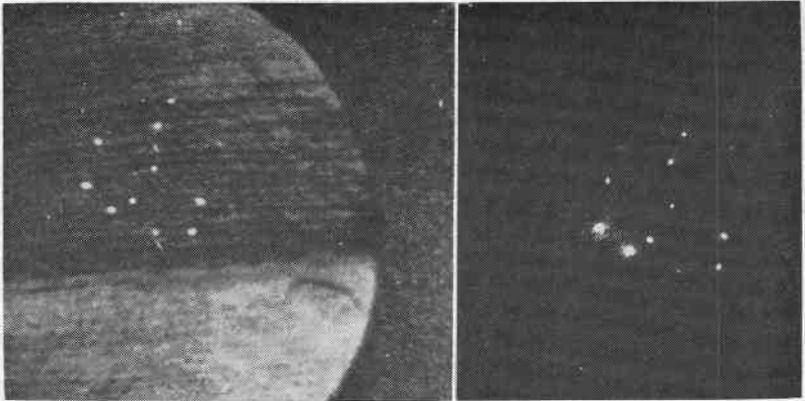
Figure 6. Penetration of fluorescent dye compared with that of C^{14}-labeled maleic hydrazide. Test solution contained 0.5% dye, 0.1% Vatsol OT, and 0.05 μc maleic hydrazide per μl drop. Treatment was for five minutes. On the left is a view of a leaf showing distribution of dye, and on the right is an autoradiogram of C^{14} distribution (ca 5X). From Dybing and Currier (1959).

The tracers were rapidly absorbed only when the stomata were open and when the solution contained a surfactant (Vatsol OT). Stomatal penetration without surfactant was not clearly demonstrated. The surfactant Vatsol OT increased both the cuticular and stomatal uptake of P^{32}. This finding is contrary to several previous reports.

Histoautoradiography. Methods of histoautoradiography were developed for the study of foliar pathways involved in the movement of herbicides at the tissue level. Apparatus and methods were modified after those of other workers (cited in Jensen, 1962). Lyophilization of quick-frozen tissue, paraffin embedding, and coating of sections with liquid nuclear emulsions were useful techniques for localizing and studying *in situ*, several water-soluble compounds. Chief difficulties have been in obtaining sufficient levels of tracer in the tissue and achieving good tissue preservation in the case of grass leaves. Not rinsing the leaves prior to freeze-drying, embedding, and sectioning results in heavier deposits on the leaf surface but often higher "backgrounds" also.

Labeled herbicides employed in various studies were tritiated monuron, 2,4-D, 2,3,6-TBA, amiben, and silvex, as well as several C^{14} ring-labeled alkylamino triazines.

Bean, *Zebrina*, morning glory, lambsquarters, and cotton served as test species in one or more experiments. Tissue preservation was never really satisfactory with corn or johnsongrass leaves.

In studies thus far, NH_4TBA-H^3 and Na 2,4-D-H^3 have been found mainly on the outer leaf surface, apparently in the cuticle, and occasionally in outer periclinal walls. Monuron-H^3, on the other hand, was almost completely removed from the surface of the leaf by rinsing, but it was found in small quantities in the outer epidermal walls, in the walls of parenchyma tissue, and in a few instances seemingly in the cytoplasm of palisade parenchyma cells. Activity also was found in and over the secondary bundle sheath and in substomatal chambers nearby. Although interpretation must be made cautiously, monuron seemingly follows an apoplastic route in transport but eventually does enter living cells in small amounts. There are other indirect evidences that this is the case (Crafts and Yamaguchi, 1958, 1960). In studies with amiben-H^3 and silvex-H^3 using bean, *Zebrina,* and cotton, only amiben at the highest concentrations (10 $\mu c/ml$) gave satisfactory autographs. Apparently the maximum specific activity used with silvex (1.3 $\mu c/ml$) was insufficient. As with 2,4-D and TBA, there was little appreciable penetration of the tissue. Apparently the levels of herbicide employed for these exposure periods were not high enough to exceed the threshold level after satisfying all adsorption sites of the cuticle. Amiben was found on and in the cuticle of the upper or lower surface of the leaf. Although none of the herbicide could be said to be within the tissue, activity often appeared most concentrated over the anticlinal walls of the epidermis. Some amiben was found on the epi-
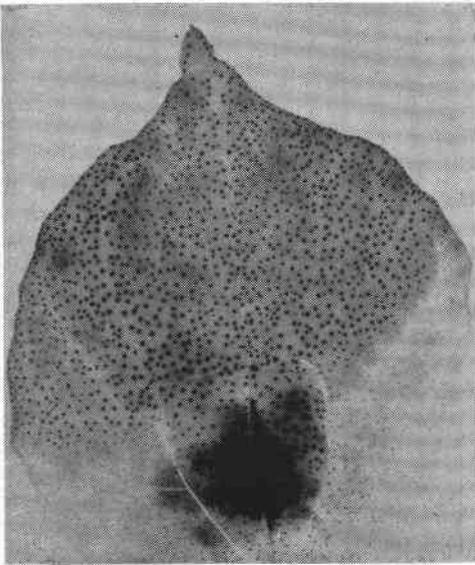
Figure 7. Autoradiogram of a cotton leaf showing pronounced accumulation of C^{14} in the lysigenous glands of cotton 72 hours after drop treatment with 10 μl (23.4 μg, 0.05 μc) of ipazine-2,4,6-C^{14}. Also note the wedge-shaped pattern of radioactivity distal to the point of application resulting from apoplastic movement during transpiration. From Foy (unpublished).

25

dermis of the secondary vein bundle sheath. Some actual penetration of the tissue as viewed in cross section was suggested in the case of lower leaf surface treatments in *Zebrina* at 70°C, 60% relative humidity (RH), and 800 to 900 foot-candles.

Efforts with the triazine compounds in cotton were much more successful (Foy, 1962c). Eight C^{14} ring-labeled alkylamino s-triazines were employed earlier in tracer studies on several species under another project. Although the degree of penetration was variable with many factors and among compounds, all 2-chloro and 2-methoxy herbicides apparently moved apoplastically and were translocated negligibly out of the treated leaf. C^{14} from two innocuous 2-hydroxy analogs was more widely distributed, both diffusely throughout the treated leaf and concentrated in vascular channels. The important difference observed among species was the marked accumulation of certain compounds, but not of others, in the lysigenous glands of cotton (Figure 7).
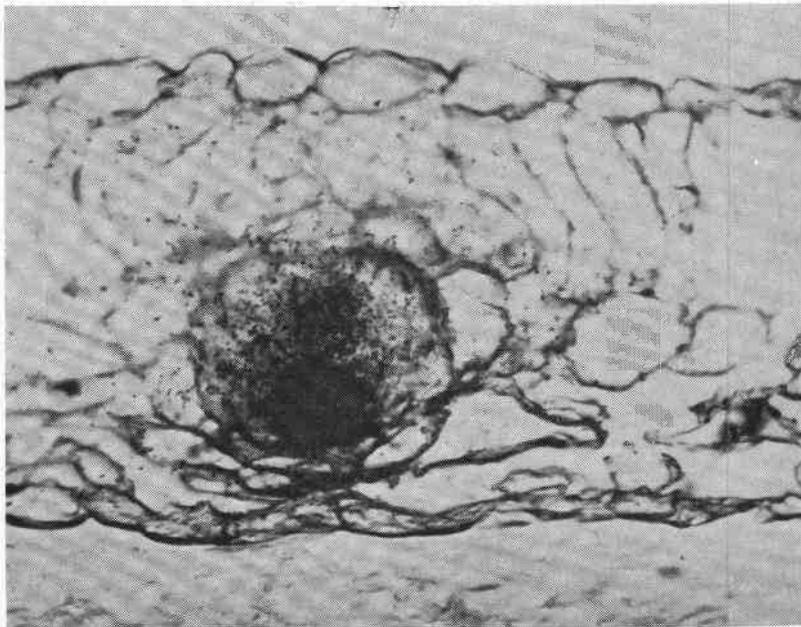
Figure 8. Histoautoradiogram of a transverse section of a cotton leaf, approximately 2 cm distal from the region of drop treatment with ipazine-C^{14}. Note the marked accumulation of C^{14} in the lysigenous gland as suggested in Figure 7. Photograph taken at "high emulsion" level to emphasize silver grains, therefore tissue is slightly out of focus. Magnification approximately 25 X. From Foy (unpublished).

Further studies showed the pronounced accumulation in lysigenous glands to be correlated with lipid solubility of the compounds. Thus the phenomenon was observed with simazine and four other 2-chloro substituted triazine herbicides; it was most pronounced with prometone, the only 2-methoxy analog included, and it was not observed at all for the 2-hydroxy analogs.

Histoautoradiographic procedures described earlier were used to confirm the cellular localization of C^{14} in cotton (Figure 8). In addition to the pronounced accumulation in lysigenous glands, activity was also found in the cuticle, epidermal cell walls, distributed throughout the mesophyll cell walls and cytoplasm, in the phloem of midveins and in veinlets, and in bundle sheath cells above and beow the midveins. Guard cells commonly contained activity, especially in the lower surfaces of leaves that had been treated on the upper surfaces. The presence of activity in epidermal walls and cuticle (especially the lower surface) is of particular interest, since the upper surface was treated and sections were taken a few centimeters distal to the (confined) treated spot in each case.

Although the techniques and interpretation may be questioned, absorption and movement of these compounds tentatively are visualized as follows: Herbicides containing surfactant enter stomata in bulk solution and also (more slowly) penetrate the cuticle by diffusion. Once inside, these compounds of low solubility move primarily in the walls, initially. However, they eventually enter living cells, at least in small quantities, and (in the case of cotton) are known to accumulate in lysigenous glands. Such behavior may be explained on the basis of (a) solvent partitioning or phase distribution between aqueous and lipoid media and (b) asymmetrical permeability of cell or gland membranes to these substances, or (c) an active accumulation requiring metabolic energy. The matter requires further study.

Activity in the lower cuticle and epidermis was observed only where the tracer had penetrated the entire thickness of the leaf blade. Movement outward in the leaf also apparently occurs in or surrounding the xylem portion of the vascular channels. Although activity was found in or associated with phloem tissue also, these substances (the 2-chloro or 2-methoxy compounds) were not translocated downward with photosynthates in a manner comparable to dalapon, amitrol, and 2,4-D. The exact reason for this apparent anomaly has not been explained. In the case of the 2-hydroxy analogs, and possibly the other compounds also, some of the observed C^{14} is likely to be no longer in the starting compounds but in degradation products thereof. It is hoped that related physiological studies under another project will provide answers to this problem.

Studies on ectodesmata. Attempts to demonstrate "ectodesmata" (Schumacher, 1942 *et seq.*) in the outer epidermal wall of several kinds of leaves were made, but the results were mostly negative (unpublished). Materials studied included *Primula, Liriodendron,* and *Pelargonium* leaves; potato tubers; and onion bulb epidermis. Only in *Eucalyptus* leaves were structures visible with sufficient clarity to say confidently that they resembled the ectodesmata in published photographs. Leaves collected under conditions of light and dark showed no differences. Histidine in the low concentrations suggested by Schumacher and Lambertz (1956) failed to promote the appearance of the structures, nor did leaves treated with this stimulant change the pattern of cuticular absorption of either PTS or K-fluorescein dye.

Franke (1961), using the berberin thiocyanate precipitation, found that entry of KSCN into leaves was localized in regions where ectodesmata were abundant, and he apparently discounts stomatal entry as being an important factor in foliar penetration. He finds that ectodesmata are particularly numerous in guard cells. However, evi-
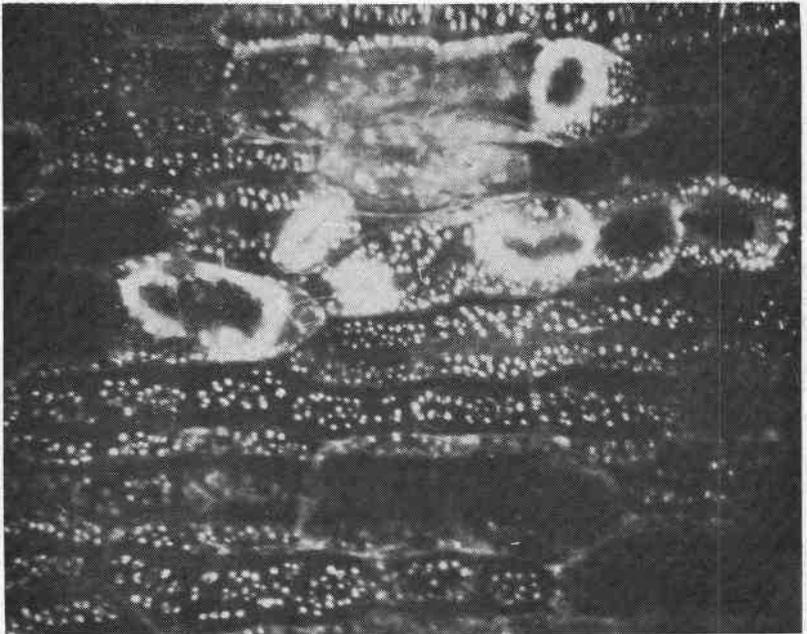
Figure 9. Wound callose reaction in pith region of epicotyl of young Pisum sativum seedling. Longitudinal section, with callose-staining pits and circular patterns of small dots on exposed walls of intact wound border cells. Living, stained 15 minutes, 220 X. From Currier (1957).

dence under this project shows that the presence of a surfactant in the spray solution still can effect a mass movement of solution into the intracellular spaces where it is protected from weather and can be absorbed over a period of time. Pathways across the cell wall adjacent to intracellular spaces into the protoplast were not studied, but this is no reason to minimize the importance of stomatal entry. Plasmodesmata of some sort may be involved in movement across external cuticle or internal cuticle, but this is uncertain.

Callose studies

Several lines of evidence suggest that the formation of callose in the pit areas and other regions of cell walls (Figure 9) impedes cell-to-cell movement of substances in plant tissues. In many instances callose is an indication of injury. This appears to be especially true of the leaf blade. Toxic chemicals, among other kinds of stimuli inducing injury, can promote callose development, and so the matter is of interest in foliar penetration. Also, since there is increasing evidence that the callose plugging of sieve elements occurs rapidly upon injury to the phloem, translocation of substances out of the leaf comes into view in an important way. Maestri and Currier (1958) showed that endothal inhibited foliar export of radioactive maleic hydrazide and amitrole. Endothal injures all living cells, including phloem elements, and thus prevents or restricts its own movement, as well as all movement within the phloem.

Chemical stimulation of callose formation in sieve elements and parenchyma cells does not appear to be very specific with respect to the nature of the chemicals that have been applied externally. Dunning (1958) concluded that callose develops in injured cells, injured by any of a number of ways, as long as the cells remained capable of carrying on the synthesis. However, of a number of poisons tested, endothal proved to be the most efficient. This is in line with its relatively slow action and its stimulating nature.

Two phases in the time course of the pit callose reactions can be distinguished: First, a rapid initial synthesis that occurs in a fraction of a second under optimum conditions, or at least a few seconds generally; and second, a prolonged synthesis that results within hours or days in the accumulation of the substance in the form of cellular pads, pegs, and solid inner-wall lamella. Endothal seems to stimulate both phases, but especially the second.

The physiological meaning of callose formation is uncertain at present. While the substance would appear, on the basis of location, to act as a sealing and plugging substance, clear proof is lacking. In sieve tubes and laticifers, such a function seems a certainty, but in

Table 4. Absorption and Initial Translocation of Dalapon in Corn Leaves Following Drop Applications[1]

Exposure time before washing	Leaf sections[2]	Total net radioactivity (counts per minute)[3]			
		Vatsol OT		No surfactant	
		Midrib	Off midrib	Midrib	Off midrib
	No.				
15 seconds	3	9	0	0	0
30 seconds	3	1	14	0	0
1 minute	2	0	0	0	0
	3	5	20	0	0
	4	0	10	0	0
5 minutes	2	1	94	0	0
	3	46	179	28	6
	4	1	42	0	0
30 minutes	1	16	13	3	0
	2	11	25	4	0
	3	1,493	2,779	15	44
	4	10	20	0	2
	5	10	8	0	0
3 hours	1	229	2,657	521	618
	2	291	239	483	405
	3	5,664	3,766	19,599	12,792
	4	176	124	677	274
	5	184	70	314	105

[1] Applications were either directly on, or well off, the midvein. The 5-μl droplet of dalapon-2-C[14], applied either with or without 0.1% Vatsol OT, represents approximately 21 μg dalapon (0.05 μc) and is equivalent to 31,366 cpm (gas flow counter).

[2] In all cases, leaf section (1) is the basal section and (3) is the central treated section.

[3] Figures represent total net radioactivity (counts per minute) in each section and are averages of four replications.

SOURCE: Foy, 1962a.

parenchyma there is but little basis for such a postulation. Work is continuing on the problem. Recent investigations by Eschrich (1961) reveal reversible callose syntheses, depending upon physiological conditions in the plant, that are of much interest to physiologists. Such disclosures suggest that callose substance is not merely a terminal secondary substance produced by dying tissues, but rather a truly regulatory substance important in cell-to-cell interchanges. It is as yet premature to predict how important callose may be in regard to uptake and movement of herbicides.

Absorption and translocation of radioactive dalapon

Corn (Foy, 1958, 1962a). Dalapon-2-C[14] applied as droplets to an individual leaf was employed in tracer studies by autoradiography and counting. That dalapon remained nonmetabolized throughout the
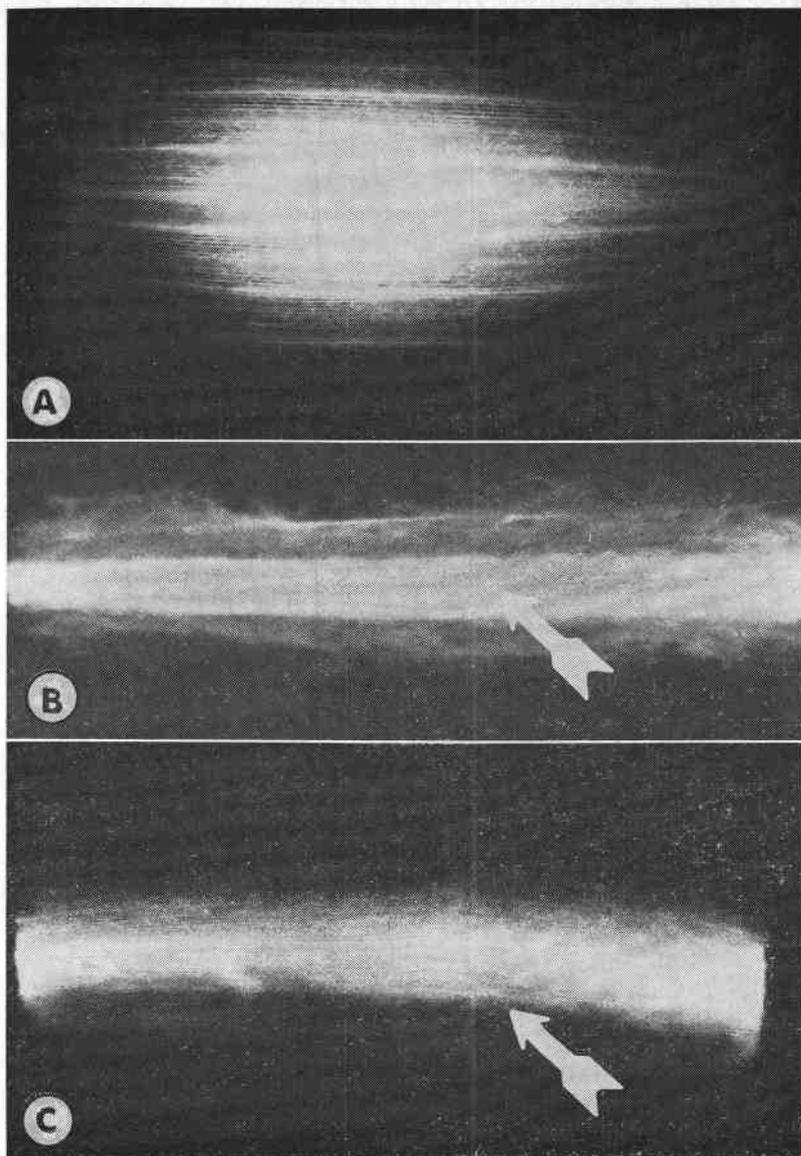
Figure 10. Distribution of dalapon-2-C^{14} in a corn leaf 3 hours after application of a 5 μl droplet (21 μg, 0.05 μc). Radioactivity appears light against a dark background in all photographs. Arrow points to midvein. A. Enlargement of treated area (over the main vein) and surrounding tissue. Initial spread around the applied drop appeared as a diffusional pattern; however, movement soon became channelized in the small veinlets and larger vascular bundles. Activity in the smallest cross-connecting veinlets is probably due to distribution in the xylem as a result of transpiration and/or drying of the section. Movement occurred in both directions from the spot of application. B. Enlargement of basal section of corn leaf (A) treated on the midvein a few cm distally. Note that the greatest concentration of C^{14} is associated with the main vein. C. Similar to B except that application of dalapon-C^{14} was off the midvein. From Foy (1962a).

experiment was confirmed by chromatography. Surfactants greatly enhanced cuticular penetration. As shown in Table 4, small amounts of herbicide were sorbed almost instantaneously (15 to 30 seconds) when a surfactant was used.

Movement away from the treated area appeared first as a diffusional pattern, but soon showed channelization in veinlets and larger vascular bundles (Figure 10). Dalapon applied off the midvein did not enter the midvein in appreciable quantities during basipetal transport. Dalapon which entered the midrib originally remained highly concentrated in this channel.

During transport, some dalapon was retained or retarded by tissues through which it passed. Retention was greatest in the basal sections (intercalary meristem regions) of young grass blades. A tenfold build-up of dalapon was demonstrated following drop application off the main vein, but not after treatment on the midrib. This work indicates that anatomical differences existing among plant species are of primary importance in determining distributional patterns following absorption. Such tracer patterns may or may not bear any clear relationship to herbicidal selectivity of the compound among species, however. For example, 2,4-D and dalapon, compounds having generally opposite herbicidal specificities, are "restricted" alike in the basal portions of grass leaves (Weintraub, Reinhart, and Scherff, 1956; Foy, 1958). Also, both dalapon (Foy, 1958) and 2,3,6-TBA (Mason, 1959) were more readily accumulated in the terminal buds of dicotyledons than in corn, yet they exhibit opposite herbicidal specificities. Thus the foregoing should serve as a word of caution when interpreting mechanisms of selective toxicity based on tracer distribution patterns alone. Results with dalapon are explicable on the basis of the known anatomy of *Zea* and the translocation of dalapon with assimilates.

In related studies (Foy, 1958, 1963), nonlabeled dalapon was found to penetrate corn leaves most readily in the nondissociated form, or at a low pH in aqueous solutions. However, when growth inhibition (which is dependent upon translocation) was used as the criterion, acute toxicity at low pH's created an opposing trend and optimum herbicidal results were thus obtained at about pH 6, fortuitiously near that of a solution of commercial dalapon sodium salt in tap water (Figure 11).

A comparison of several formulations of dalapon showed the acid and the Na salt to be about equal in effectiveness, both being more inhibitory than the NH_4, K, and Ca salts (listed in decreasing order of activity). Some interesting interactions between formulations and surfactants also were disclosed.

32
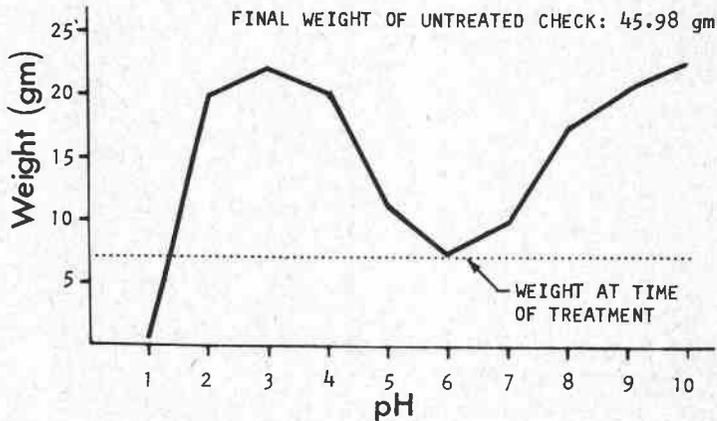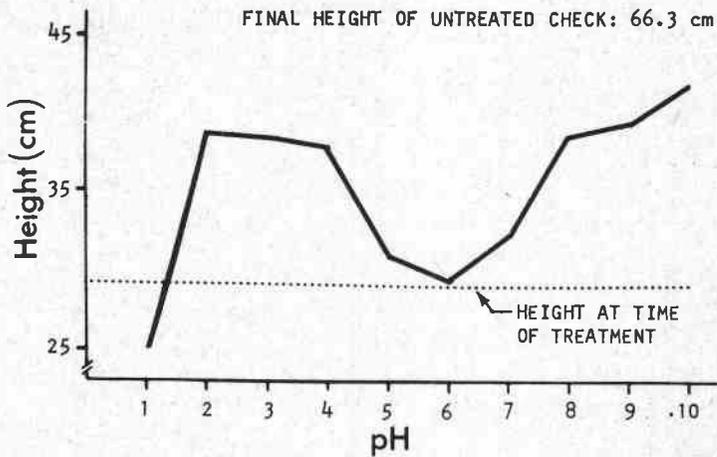
Figure 11. Measurements of corn plants two weeks after spraying to wet with buffered solutions of commercial dalapon plus 0.1% Vatsol OT, using 4 pounds of active dalapon in 40 gallons of water. Each value is the average of 16 plants (4 pots). From Foy (1963).

Tradescantia (Foy, 1958, 1962b). Patterns of absorption and distribution of dalapon-2-C^{14} and -Cl^{36} in *Tradescantia fluminensis* were determined by autoradiography and counting. Dalapon was absorbed through the cuticle and through the large open pores of hypostomatous leaves. The penetration of dalapon-2-C^{14} after two hours is shown in Table 5. Stomata were clearly the most expeditious routes of entry, but results were erratic when no surfactant was included. An

Table 5. Effect of Sodium Dioctylsulfosuccinate on the Penetration of Dalapon-2-C^{14} into *Tradescantia* Leaves Within Two Hours[1]

Total net radioactivity (counts per minute)			
Upper surface		Lower surface	
Surfactant	No surfactant	Surfactant	No surfactant
13	26	445	9
13	12	510	102
24	12	470	6
54	9	630	141
83	8	393	801
90	13	635	2
Avg. 46	13	514	177

[1] Droplet applied either to the upper or lower leaf surface represents approximately 21 μg dalapon (0.05 μc) and is equivalent to 10,185 cpm (thin end window G-M tube).
SOURCE: Foy, 1962b.

Table 6. Foliar Absorption and Translocation of Labeled Dalapon by Cotton and Sorghum as Function of Time[1]

Treatment period	Percent of dalapon-Cl^{36} applied[2]		Percent of dalapon-2-Cl^{14} applied[2]	
	Cotton	Sorghum	Cotton	Sorghum
	%	%	%	%
1 hour	1.2	0.7	0.4	0.7
8 hours	1.4	1.7	1.5	2.2
3 days	18.5	11.7	23.1	19.3
2 weeks	20.5	26.6	32.0	29.8

[1] Counts are total net radioactivity in dalapon translocated out of a section of leaf, either 20 mm in diameter (cotton) or 2 inches in length (sorghum), centered over the treated spot. (Figures are averages of four replications.)
[2] Dosages applied as 20 μl drops are equivalent to the following (counts by thin end window G-M tube): dalapon-Cl^{36} (102 μg or 0.0091 μc), 1,131 cpm; dalapon-2-C^{14} (106 μg or 0.200 μc), 8,280 cpm.
SOURCE: Foy, 1961a.

anionic surfactant, sodium dioctylsulfosuccinate, enhanced both (a) cuticular and (b) stomatal plus cuticular penetration threefold to fourfold during a two-hour absorption period. Studies confirmed the view that dalapon is translocated to regions of high metabolic activity or storage in association with and dependent upon the movement of food materials. Dalapon did not move appreciably out of albino leaves or green leaves previously depleted of food reserves, but was transported readily out of normal leaves in the light when a surfactant was used to enhance cuticular penetration.

Cotton and sorghum (Foy, 1958, 1961a,b). Dalapon-2-C^{14} and -Cl^{36} were employed in two series of tracer and metabolic experiments

Table 7. Root Absorption and Translocation of Labeled Dalapon by Cotton and Sorghum as Function of Time[1]

Treatment period	Percent of dalapon-Cl[36] applied[2]		Percent of dalapon-2-C[14] applied[2]	
	Cotton	Sorghum	Cotton	Sorghum
	%	%	%	%
1 hour	1.0	1.6	1.2	1.1
8 hours	12.2	8.9	6.9	8.0
3 days	20.6	37.7	10.6	38.9
10 days	42.5	78.4	28.6	54.0

[1] Counts are total net radioactivity in a single intact plant.
[2] Dosages applied as 10 ppm in the nutrient solution are equivalent to the following (counts by thin end window G-M tube): dalapon-2-C[14], 47.17 μl (250 μg or 0.4717 μc), 19,528 cpm; dalapon-Cl[36], 48.90 μl (250 μg or 0.0223 μc), 2,765 cpm.
SOURCE: Foy, 1961a.

Table 8. Effect of Age and Assumed Photosynthate-exporting Ability of Sorghum Leaves on Foliar Absorption and Translocation of Dalapon-Cl[36]

Leaf treated	Net activity[1]	
	Counts per minute[2]	Percent of applied dose transported out
		%
1st	104	3.7
2nd	616	21.8
3rd	649	23.0
4th	406	14.4

[1] Counts are total net radioactivity in dalapon translocated out of a 2-inch leaf section centered on the treated spot within six days.
[2] Dosage applied as a 50 μl drop (225 μg dalapon or 0.0223 μc) is equivalent to 2,825 cpm (thin end window G-M tube).
SOURCE: Foy, 1961a.

on cotton (a tolerant species) and sorghum (a susceptible species). Autoradiography and counting yielded both qualitative and quantitative data. Extraction, fractionation, and paper chromatographic procedures were used to detect dalapon and metabolic degradation products.

Dalapon entered and moved readily through leaves and roots of cotton and sorghum (Tables 6 and 7).

Some foliar sorption (primarily cuticular) occurred almost immediately in both species. Transport out of treated leaves was directly proportional to the assumed photosynthate-exporting ability of leaves of various ages (Table 8).

Table 9. EFFECT OF DOSAGE RATE AND ACUTE TOXICITY ON FOLIAR ABSORPTION AND TRANSLOCATION OF LABELED DALAPON IN SORGHUM

Treatment volume (μl)	Net activity[1]	
	Counts per minute[2]	Percent of applied dose
Dalapon-Cl[36]—acute toxicity (total dosages applied in 10 or 20 μl increments)		
10	183	32.4
20	167	14.8
40	105	4.6
80	136	3.0
160	125	1.4
Dalapon-2-C[14]—little acute toxicity (total dosages each represent a single application)		
2.5	218	21.1
5.0	492	22.7
10.0	963	23.3
20.0	1,726	20.8

[1] Counts are total net radioactivity (thin end window G-M tube) in dalapon translocated out of a 2-inch leaf section centered on the treated spot within six days.

[2] Data may be expressed in other forms by using the following interconversions: Dalapon-Cl[36]: 10 μl = 51 μg dalapon, 0.0045 μc, = 565 cpm; Dalapon-2-C[14]: 10 μl = 53 μg dalapon, 0.100 μc, = 4,140 cpm.

SOURCE: Foy, 1961a.

In the first series, acute toxicity at the point of foliar application, whether due to excess H^+ ions, herbicide, or toxic surfactant, reduced or prevented systemic distribution (Table 9). In the absence of acute toxicity, absorption and movement out of the region of application were protracted (two weeks or longer).

Transport occurred readily via the transpiration stream (following uptake by roots, severed vein, or severed petiole), in the phloem (associated with and apparently dependent upon the movement of photosynthates), and laterally in phloem-xylem interchange. In both tolerant and susceptible species, retranslocation and accumulation occurred in response to shifts in loci of high metabolic activity.

Some leakage of foliar-applied dalapon occurred, especially from roots of cotton and also from sorghum under certain conditions, and slight radioactivity was detected in the guttate from hydathodes of sorghum.

Despite its ready mobility, some dalapon was retained in transport. Restriction of dalapon movement was especially noticeable in the basal sections (intercalary meristem regions) of young sorghum leaves. Probable factors are accumulation by living cells and absorption on colloidal surfaces.

Neither penetrability nor metabolic inactivation appears to play

a major role in determining species specificity in the action of dalapon to sorghum and cotton. Furthermore, differences in translocation observed among these species would tend to minimize or refute the importance of such differences in determining herbicidal selectivity. Penetration and translocation are quantitatively influenced by numerous factors.

In the second series, ready absorption and translocation of dalapon, including redistribution and accumulation (e.g., in vegetative meristems, flowers, fruits, and seeds) in response to shifts in loci of high metabolic activity were confirmed.

Dalapon was absorbed, translocated, redistributed, and accumulated in higher plants, principally as the intact molecule or dissociable salt thereof, and remained essentially nonmetabolized for long periods, especially in dormant or quiescent tissues.

Seven days after foliar treatment of cotton and sorghum, for example, dalapon, and no other prominent radioactive substance, was recoverable with water or ethanol from all plant parts and from the nutrient solutions in which the plants were grown. This is in contrast to several other herbicides that are readily metabolized in plants.

Ten weeks after treatment (applied through severed petioles) approximately 85 to 90% of the dalapon was recoverable from cotton fruits of various ages. The remainder of the radioactivity was associated with the ether-soluble portion, the neutral and cationic fractions of an ethanol extract, and the insoluble plant residue.

Some slow metabolic decomposition resulted in the release of Cl^{36} (from dalapon-Cl^{36}) or the incorporation of C^{14} (from dalapon-2-C^{14}) into other compounds. Eventual breakdown possibly involves an initial dehalogenation followed by normal or modified propionate oxidation.

Dalapon stimulus was traced to the third generation in wheat; it was transmitted in the seeds after exposure of first-generation seedlings to preplant applications of dalapon (4 lbs./acre) in the field.

Two physiologically distinct types of action—acute toxicity and delayed growth regulatory responses—were confirmed. The former is believed to be due to the action of the herbicide as a fairly strong acid and protein precipitant, which nonselectively caused disruption of the plasma membranes and destruction of cellular constituents. The second response, resulting from lower concentrations in meristematic tissues, exemplifies true biochemical selectivity among species.

Experiments indicated that neither penetrability, translocatability, nor metabolic inactivation of dalapon is pre-eminently responsible for herbicidal specificity. The key to the moderately selective growth-regulating activity of dalapon still seems unquestionably to reside in the protoplasm. Results are not at variance with hypotheses that dala-

pon inhibits pantothenic acid synthesis and disturbs coenzyme A and pyruvate metabolism.

Few histoautoradiographic studies have been conducted on herbicide translocation. In work in California related to this project, Radwan, Stocking, and Currier (1960) described methods for determining the cellular distribution of two C^{14} -labeled herbicides—3-amino-1,2,4-triazole (amitrole) and 2,4-D. The herbicides were applied to roots and leaves of *Vicia faba*. Histoautoradiographs of freeze-dried petioles and stems of leaf-treated plants always showed activity in the phloem. In addition, the xylem frequently showed some activity. As the distance from the point of application (blade) increased, the activity in the phloem decreased and that in the xylem increased.

Root and hypocotyl sections of root-treated plants showed most activity in the xylem, but there was always a certain amount in the phloem.

The results suggest lateral transfer between xylem and phloem for both leaf- and root-absorbed tracers. The extent of metabolic degradation of the herbicides was not determined. Additional studies of this kind are needed with other herbicides that show differing mobilities.

Controlled environment studies

Stomatal movement. For the most part, plants were collected from the field or greenhouse when their stomata were either closed or naturally open to various degrees, according to the experimental need.

Stomata could also be opened experimentally by a combination of moderate to high light intensity, high humidity, and CO_2-free air. The stomata of *Zebrina* and *Tradescantia* were often found open when grown in moderate or even reduced light, in a misty or humid environment under greenhouse benches.

The effect of light in promoting foliar penetration in short-term experiments is believed to be more a matter of light-controlled stomatal movement than an influence on the cuticular component of absorption.

Exclusive of effects on stomatal movements, temperature effects on stomatal penetration of aqueous (dip) solutions were slight within the range of $11°$ to $40°C$.

Exposure of detached leaves of *Zebrina pendula* L. to ultrasonic vibrations was found to effect closure within a minute. The apparatus used was a low-energy type (35 watts) and cells did not appear injured as a result of exposure. The method promises to be of value in the study of guard-cell control of foliar penetration.

Stomatal opening and uptake of radioactive chemicals (Dybing and Currier, 1961). Drops of seven radio-labeled compounds

Table 10. Penetration of Radioactive Chemicals Into *Zebrina* Leaves in a Five-minute Treatment Period

| | Net counts per minute (avg. of 5 reps.) | | | |
| | 0.1 percent Vatsol | | No Vatsol | |
Chemical	Stomata open	Stomata closed	Stomata open	Stomata closed
C^{14}-*labeled*				
Urea	234	2	6	4
3-amino-1,2,4-triazole	203	38	42	8
2,4,5-T[1]	115	8	8	12
Benzoate[1]	89	3	4	3
2,4-D[1]	127	13	18	6
2,4-D[2]	117	8	4	3
$H_2P^{32}O_4$	787	321	155	175[3]

[1] Triethanolamine salt.
[2] Sodium salt.
[3] Four replications.
SOURCE: Dybing and Currier, 1961.

with and without surfactant were applied to the lower surface of *Zebrina* leaves, and penetration was measured by counting after a five-minute treatment period. In one set of plants stomata were open; in the other, stomata were closed.

By far the most absorption occurred in the open stomata-surfactant treatment (Table 10). Leaves with closed stomata absorbed only small amounts of radioactive tracer within five minutes (with the exception of P^{32}). Leaves with closed stomata were penetrated by P^{32}, but uptake by those with open stomata from surfactant-containing solution was markedly greater.

Since appropriate surfactants (a part of the physicochemical environment) have been shown repeatedly to enhance both stomatal and cuticular penetration, most studies on the influence of changes in the natural environment also include surfactants as a variable.

Correlation of stomatal penetration with injury response. From earlier studies it was concluded that aqueous herbicidal solutions penetrate stomata of leaves quickly when the stomata are open and when an efficient surfactant is present at a proper concentration in the spray. The criterion of entry was the detection of either chromophoric precipitates, fluorescent dyes, or radioactive tracers in the solution. The question is properly asked whether the tracers, the first two kinds at least, accurately reflect the movement of the herbicide, and if so, in the final analysis, whether herbicidal effectiveness is related to the extent of stomatal penetration.

Additional experiments (unpublished) were conducted in an attempt to correlate (a) degree of stomatal opening, (b) amount of herbicide (2,4-D or dalapon) entering the substomatal cavities (using fluorescent dye tracers), and (c) the degree of injury subsequently shown by the plants. Quantitative assaying was done in separate experiments using radioactive herbicides.

In the first series, tests were performed on kidney bean (first true leaf), johnsongrass (from rhizome transplants), and *Zebrina* (rooted cuttings) in a controlled-growth chamber, 22°C, 60% RH, and about 800 to 900 foot-candles light (diurnal cycle: 16 hours light, 8 hours dark). Three procedures served to provide plants with open stomata and plants with closed stomata. In the first procedure (Experiments 1 to 4), some plants were placed in the dark for 24 hours, others remained under the 16 to 8 hour light-dark cycle. Spraying was performed in late morning after the light plants had been exposed to several hours of light. In the second (and better) method (Experiments 5 to 9), all plants were placed in the dark for approximately 48 hours; to open stomata, some of the plants received light for 3 to 5 hours before spraying. In the third method (Experiments 10 and 11), plastic bags were placed over the shoots during the light exposure.

The spray solution contained Na 2,4-D at 0.05 or 0.1%, dalapon at 0.1 or 2%, and PTS at 0.1%. Spray was applied to run off. Both upper and lower surfaces were wetted. Dark plants were sprayed in a darkened room, light plants in a lighted room. The spray was prevented from reaching the soil in those cultures that were to remain growing.

After spraying, pots were turned on their sides to drip. After five minutes, the shoots were washed 30 seconds in a flat pan into which tap water ran vigorously. Leaves were detached from one set of plants, placed between blotters, and examined immediately with ultraviolet illumination, using the naked eye for estimation of gross fluorescence and microscopes for cellular distribution. The fluorescence intensity was rated visually.

Treated plants and untreated controls not used in fluorescence analysis were removed to the greenhouse or growth chamber, and injury responses were observed over a period of two weeks. Data recorded included height, fresh weight at harvest, and abnormal growth.

General results. In the majority of cases stomatal penetration occurred in the light plants, not in dark plants.

The degree of injury was positively correlated with degree of stomatal opening in most cases, but the differences were small.

Other pathways of penetration did not seem to be influenced by light. Margins of leaves of all three species were indicated as regions

of enhanced absorption. In beans, cuticular absorption (lower surface) was greatest over the veins. Occasional cracks and punctures revealed the dye. In johnsongrass, in addition to stomata, margins and leaf bases were preferential areas of uptake.

Stomatal penetration on the lower surface exceeded that on the upper. This result is accountable partly to the lower density of stomata on the upper surface. However, the lower stomata also seemed to be more penetrable.

Within the penetration time of five minutes, enough 2,4-D and dalapon were absorbed to produce typical symptoms of injury subsequently.

Since the degree of stomatal opening was never marked, and since dark plants often showed no stomatal opening but a definite injury response, a significant portion of the absorption must have occurred through the cuticle.

The strikingly great absorption via open stomata demonstrated by Dybing (1958) in detached leaves could not be duplicated in intact leaves, with the possible exception of results obtained by the plastic bag technique. Bagging the shoots for six hours was effective in opening the stomata. A limiting factor in field application of herbicides may be a small degree of stomatal opening.

Continued studies showed, in confirmation of earlier results, that (a) degree of stomatal opening, (b) entrance of herbicide into the substomatal cavities (using fluorescent dye tracer), and (c) final herbicidal response were positively correlated.

Additional tests with *Chenopodium album* provided a clearer demonstration of a positive correlation among the three factors. The plastic bag technique for obtaining widely open stomata again gave the greatest difference between light- and dark-treated plants. From this, it can be concluded that the greater the stomatal opening (under humid, warm conditions in the light), the greater the herbicide absorption by leaves. Tentatively it is proposed that (in short-term experiments) temperature, light, and relative humidity all exert their most pronounced effect on the stomatal component of absorption. This does not rule out their possible effects (long term, principally) on cuticular penetration. A suitable surfactant enhanced both stomatal and cuticular penetration.

Although the trends were the same as in earlier studies with detached leaves, differences due to environment were less distinct. One explanation is that the stomata are not as widely open nor as completely closed in attached leaves as they are in detached leaves. It may also be, as Dybing noted, that stomata of bean leaves are very small, and the surfactant effect is less striking than on *Zebrina*, which pos-

sesses large stomata. A third explanation is that the dye tracer cannot give a complete picture of the absorption pathway followed by the herbicide. Especially is this true when we consider cuticular absorption and the fact that PTS does not enter living cells. The dye can move slowly through the cuticle, appearing in the anticlinal walls of the epidermis. It can move more rapidly through cracks and perforations, but we have little basis for saying that the herbicide moves in the same exact pathways and at the same rate.

Effects of environment and solution additives on foliar penetration of dalapon (Foy 1962d). *Tradescantia*—Earlier studies (Foy, 1962b) showed that dalapon was absorbed through the cuticle and large open stomata of hypostomatous leaves of *Tradescantia fluminensis* Vell, var. *variegata*. Both cuticular and stomatal penetration (the most expeditious route of entry) were enhanced by the addition of a surfactant. Studies were continued with the same species to determine the influence of temperature, relative humidity, and spray additives on foliar penetration.

Rooted cuttings growing in nutrient cultures in the greenhouse were transferred into constant-environment chambers. After a preconditioning period of 8 to 12 days, plants were treated with dalapon-Cl^{36} by drop placement ($10\mu l$) on either the upper or lower surface of one or more leaves. Experimental variables were as follows: treatment time—2 hours, 16 hours; treatment solutions—(a) dalapon only, (b) dalapon plus 0.1% X-77 (surfactant), (c) dalapon plus 0.1% R-163X (humectant), (d) dalapon plus surfactant and humectant; environment— (1) 37.5°C, 25 to 40% RH; (2) 26°C, 25 to 40% RH; (3) 26°C, 80% RH. All applications were made early during the light period of an 18-hour light, 6-hour dark regime. At the end of the experiments, leaves were washed free of nonabsorbed dalapon, and autoradiographed to determine gross distribution. The leaves were later quantitatively assayed for absorbed dalapon by counting.

After 2 hours almost all of the radioactivity was still in the treated leaf; after 16 hours, approximately 6 to 29% of that absorbed had translocated out of the treated leaf. Quantitative data were quite variable among replications under most experimental conditions. Penetration was generally greater through the upper surface (except at 37.5°C, 25 to 40% and 80% RH); the surfactant seemingly enhanced both stomatal and cuticular penetration (the latter a slower process), whereas the humectant seemed less advantageous, either with no effect, or was even detrimental on occasion. At 37.5°C, however, the humectant also apparently enhanced penetration slightly. Differences in results among treatments were not as great as anticipated.

42

Bean, barley, and miscellaneous (Prasad, Foy, and Crafts, 1962). From several studies related to those reported for *Tradescantia*, the following conclusions seem justified:

1. Experimental condition: (bean leaf, upper surface, 15-hour treatment, 26°C, 60% RH, 800 foot-candles.) *Results:* Relative amount of dalapon-C^{14} absorbed and translocated out of the treated area was approximately doubled due to the addition of a surfactant (X-77, 0.1%). The addition of a humectant (glycerol, 5%) did not enhance penetration and translocation of dalapon when used as the sole solution additive, and it completely nullified the enhancing effect of the surfactant when the two spray additives were used in combination.

2. Experimental condition: (bean, upper surface, 30-hour treatment, greenhouse). In this series, one-half of the plants were treated and left in the open greenhouse (possibly 26°C, 50% RH), whereas the remainder of the plants were covered with polyethylene bags throughout the treatment period. In the latter, the RH was soon at or near saturation and the temperature also was elevated somewhat above that of the open greenhouse. As before, the treated area was punched out and discarded; then the relative amounts of dalapon absorbed and translocated out of the treated area and into various plant parts was determined.

Once again, X-77 enhanced penetration and translocation; glycerol did not increase penetration and translocation when used alone and, in fact, nullified the enhancing effect of X-77 when the two were used in combination. This trend was true under both environmental conditions. After three hours, dalapon was found in all plant parts. The treated leaf (excluding area treated) still contained more radioactivity than the shoot, bud, and roots combined. Of the dalapon transported out of the treated leaf, greater quantities were found in the bud than in other parts, indicating an accumulation in the buds.

There was little to indicate that the humectant properties of glycerol were of any benefit in the uptake of dalapon under these environmental conditions. Even under higher temperature and lower relative humidity, a humectant might be expected to attract moisture unto itself but not necessarily to release it readily, along with dissolved herbicide, to plant tissues.

3. In later experiments under conditions of low (25 to 40%) relative humidity, the addition of humectant (glycerol, 0.5%) caused only a slight enhancement of dalapon penetration. A surfactant (X-77, 0.1%) almost doubled the uptake. Other solution additives (hexadecanol, Plyac, and castor oil) tested under the same conditions were

Table 11. Effects of Solution Additives on Foliar Absorption and Translocation of Dalapon-2-C^{14} After Six Hours Under Low Relative Humidity Conditions[1]

Additives	Counts per minute/gram dry weight
Dalapon alone	36.2
Dalapon plus X-77 (0.1%)	64.3
Dalapon plus glycerol (0.5%)	42.4
Dalapon plus hexadecanol	40.8
Dalapon plus Plyac (0.3%)	27.7
Dalapon plus castor oil	41.2

[1] Concentration of dalapon: 1,500 mg/l; humidity conditions: 25 to 40%.

either detrimental or showed only slight effect. Typical count data are shown in Table 11.

4. Based on all preceding observations, it was tentatively proposed that relative humidity, temperature, and light, just prior to and during treatment, exert their greatest influence on the stomatal component of absorption. Of these factors, relative humidity exerts perhaps the most pronounced effect.

In later experiments, after a suitable acclimatization period, leaves were treated with droplets of (a) dalapon, (b) dalapon plus 0.1% X-77, (c) dalapon plus 0.1 to 5% glycerol, and (d) dalapon plus surfactant and humectant. After 15 hours of absorption, the treated area was punched out; radioactivity remaining in various plant parts was determined. Again, whereas the surfactant enhanced penetration and translocation, glycerol was without effect.

For determining the role of humidity, two procedures were adopted: Plants were grown at 27°C, 800 foot-candles, and 60% relative humidity. After treatment, batches of plants were moved into cabinets maintained at 95% and 30% RH and allowed to absorb for 2 and 15 hours. Counting data and autoradiographs showed that greater amounts of dalapon were absorbed and translocated under high relative humidity conditions.

Bean plants were grown in the greenhouse in the second procedure. After treatment, plants of one series were covered with polyethylene bags to ensure a saturated atmosphere; plants of another series were left uncovered. The bagged plants contained far greater radioactivity; C^{14} or Cl^{36} was chiefly concentrated in leaves and growing apices.

Penetration and translocation were obviously greater in the covered (high humidity) plants than in uncovered plants, when promoted by the addition of a surfactant.

Table 12. EFFECTS OF REWETTING THE DROPS ON UPTAKE OF DALAPON[1] BY LEAF DISKS OF *Zebrina* WITHIN SIX HOURS

Condition	Counts per minute/disk	
	Adaxial	Abaxial
Low humidity (drop dried)	82	230
Low humidity (drop rewetted)	107	328
High humidity	121	411

[1] Concentration: 1,500 mg/l.

Table 13. UPTAKE OF DALAPON BY LEAF DISKS AFTER SIX HOURS UNDER MID-HUMIDITY CONDITIONS[1]

Species	Counts per minute/disk	
	Adaxial	Abaxial
Coleus blumei	150	251
Tropaeolum majus	175	204
Zebrina pendula	86	430

[1] Concentration of dalapon: 1,500 mg/l; humidity conditions: approximately 50 to 60%.

Initially, five possibilities were considered as contributing to or accounting for the enhanced penetration and translocation of labeled dalapon under high relative humidity conditions, as follows:

1. Slower rate of droplet drying; therefore, better opportunity for prolonged uptake (stomatal and cuticular) from solution;

2. Greater stomatal opening because of increased turgor (of guard cells in particular);

3. Increased hydration of the cuticle, permitting continuous uptake via an aqueous continuum;

4. Reversal of the transpiration stream; consequently, herbicide drawn in passively;

5. Involvement of ectodesmata in the uptake of herbicides.

At least two factors, (1) rate of droplet drying and (2) stomatal location and behavior, were shown experimentally to contribute to the humidity effect. Droplets dried rapidly under conditions of low humidity. Periodic addition of water to the dried drops increased absorption and translocation but they were not equal to that under sustained high-ambient humidity (Table 12).

Stomata were more widely open under high than low humidity. Also, uptake of dalapon was greater through the abaxial than the adaxial surfaces of leaf disks of three hypostomatous species—*Coleus, Zebrina,* and *Tropaelum* (Table 13).

Environmental effects are usually much less apparent when penetration is not enhanced by the use of a surfactant. The large effect of high relative humidity (also with somewhat higher temperature) in increasing penetration, especially in short-term experiments, is thought to be largely attributable to increased stomatal penetration.

However, experimental evidence shows that cuticular penetration may also be higher under conditions of high relative humidity or when the applied drops remain moist longer than normal. Possibly cuticle hydration (item 3), and in some instances reversal of the transpiration stream (item 4), may then be involved. Earlier studies (Dybing, 1958) showed that penetration of cuticle does not occur uniformly over the leaf surface, but occurs intensely at points where insect punctures, thin areas, cracks, or other modifications exist.

Cuticular penetration was greater in leaves possessing a water deficit than in fully turgid leaves. The cause is interpreted as a strong DPD (diffusion pressure deficit) gradient across the cuticle. Rapid drying of spray droplets may be more a cause of herbicidal ineffectiveness under hot and dry conditions than a poorly hydrated cuticle. Thick and relatively impermeable cuticles developed in drought-hardened conditions are not considered here.

Little progress has been made on this project to date as to either the occurrence or possible role of ectodesmata (item 5) in foliar penetration, as German workers have suggested.

Johnsongrass and Bermudagrass (Foy and Sukartaatmadja, 1963). Interpretation of results from radioisotope tracer studies alone is sometimes limited because nonherbicidal dosages are most commonly used. To supplement such studies, the influence of relative humidity on growth regulating and/or herbicidal response of two perennial grass weeds to dalapon was also investigated.

Rhizome cuttings of uniform length and containing three nodes were grown in greenhouse flats for two weeks, then transferred to one-gallon cans (two plants each) for an additional period of four weeks before treatment. Dalapon (10 lbs./100 gal.) containing formulated anionic surfactant was sprayed on the foliage by use of a hand sprayer attached to a compressed air outlet.

1. The first experiment involved bagging to achieve high humidity, and a sublethal dosage (4 lbs./A in 40 gal.) was used to permit the observation of subtle differences in growth. The treatments were as follows:

a. Treated, unbagged for 24 hours, bagged for 24 hours;
b. Treated, left unbagged;
c. Treated, bagged immediately for 24 hours;

Table 14. EMERGENCE OF INFLORESCENCES, TILLERING, AND HEIGHT OF JOHN-SONGRASS AT INDICATED INTERVALS AFTER SPRAYING WITH DALAPON UNDER SEVERAL ENVIRONMENTAL CONDITIONS[1]

A. *Inflorescence Emergence*

Treatment	Number (one week after treatment)	Number (two weeks after treatment)
a) Treated, unbagged 24 hours, bagged 24 hours	0	0
b) Treated, left unbagged	1 (abnormal)	1 (poorly developed)
c) Treated, bagged immediately for 24 hours	0	0
d) Untreated check	4	8
e) Bagged 24 hours before treatment only	1	1 (abnormal)

B. *Tillering*

| | Number of new sprouts after transfer to cans | | |
Treatment	At treatment	Two weeks after treatment	Four weeks after treatment
a) Treated, unbagged 24 hours, bagged 24 hours	23	23	42
b) Treated, left unbagged	19	19	23
c) Treated, bagged immediately for 24 hours	18	20	37
d) Untreated check	19	33	33
e) Bagged 24 hours before treatment only	17	18	33

C. *Height*

Treatment	Average height at treatment	Percent increase (2 weeks after treatment)	Percent reduction of ht. increase (4 weeks after treatment)[2]
	cm	%	%
a) Treated, unbagged 24 hours, bagged 24 hours	62.0	20.0	74.2
b) Treated, left unbagged	69.9	31.6	50.1
c) Treated, bagged immediately for 24 hours	78.9	22.3	60.3
d) Untreated check	84.1	50.3	......
e) Bagged 24 hours before treatment only	78.2	17.5	70.2

[1] Dalapon was used at a rate of four pounds per acre in 40 gallons. Each figure in sections A, B, and C represents eight plants originally (four replications x two plants each).

[2] Treated plants increased less than 2% in height between two weeks and four weeks after treatment. Comparable untreated plants increased another 6% during the same period.

d. Untreated check; and

e. Bagged 24 hours before treatment only.

Conditions in the open greenhouse were 22 to 29°C and 68 to 78% RH. During bagging, the RH approached saturation and the temperature was also slightly elevated. Responses were noted periodically, and the plants were harvested four months after treatment.

Dalapon symptoms, wilting and browning of foliage, were observed after 24 hours; treatment c gave the most rapid response. This likely is related to slower droplet drying and possibly to an influence on stomatal opening. After 48 hours and two weeks, however, treatments a, b, c, and e were indistinguishable from each other; all showed about 25 to 30% necrosis of the sprayed foliage.

Inflorescence emergence, tillering, height, and mortality ratings (both shoots and rhizomes) were recorded. The data in sections A, B, and C of Table 14 are largely self-explanatory. The experiment was terminated four months after treatment. All treatments (bagged and unbagged) caused 62.5 to 75.0% kill of shoots but only 12.5 to 25.0% kill of rhizomes. Thus, there were only minor and temporary differences between environmental regimes using sublethal rates of dalapon.

2. A second replicated experiment involved the use of lethal dosages of dalapon (10 lbs./100 gal./A) in the greenhouse and in walk-in Minneapolis-Honeywell constant environment chambers. The treatments were as follows:

a. Untreated check (greenhouse);

b. Treated and left (greenhouse);

c. Untreated check (chamber);

d. 48 hours in chamber, treated, left in chamber;

e. Treated, transferred to chamber immediately;

f. Treated, transferred to chamber after 24 hours; and

g. Treated, transferred to chamber after 48 hours.

Conditions in the open greenhouse were 22 to 29°C and 60 to 78% RH; in the chamber, 28 to 29°C (daytime temperature in the greenhouse) and 90 to 95% RH. Light intensity was slightly lower in the chamber (800 foot-candles) than in the open greenhouse (1000+ foot-candles), but day lengths were identical (15-hour light, 9-hour dark). Two weeks after treatment all plants in the chamber were returned to the greenhouse. Other procedural details were similar to those used in the preceding experiment.

Spray droplets dried in the greenhouse within 30 minutes, in the chamber after about two days. The data are given in Sections A, B, and C of Table 15. It is evident that injury, height-reduction, and percent kill were greater in the growth chambers than in the greenhouse.

Table 15. VISUAL INJURY RATINGS, HEIGHT, AND KILL OF SHOOTS AND RHIZOMES OF JOHNSONGRASS AT INDICATED INTERVALS AFTER SPRAYING WITH DALAPON UNDER SEVERAL ENVIRONMENTAL CONDITIONS[1]

A. *Injury Ratings*

Treatment	One week after treatment	Four weeks after treatment
a) Untreated (greenhouse)	0[2]	0
b) Treated, left in greenhouse	2	2
c) Untreated (chamber)	0	0
d) 48 hours in chamber, treated, left in chamber	4	8
e) Treated, transferred to chamber immediately	6	8
f) Treated, transferred to chamber after 24 hours	4	7
g) Treated, transferred to chamber after 48 hours	3	6

B. *Height*

Treatment	Average height at treatment	Percent height increase (2 weeks after treatment)[3]	Percent reduction in height increase (2 weeks after treatment)
	cm	%	%
a) Untreated (greenhouse)	62	81	----
b) Treated, left in greenhouse	55	7	92
c) Untreated (chamber)	61	52	18
d) 48 hours in chamber, treated, left in chamber	59	12[4]	86
e) Treated, transferred to chamber immediately	62	8	91
f) Treated, transferred to chamber after 24 hours	66	3	96
g) Treated, transferred to chamber after 48 hours	65	3	96

C. *Kill of Shoots and Rhizomes*

Treatment	Percent of plants with complete shoot kill After 1 month	Percent of plants with complete shoot kill After 4 months	Total fresh weight of shoots	Percent kill of rhizomes
	%	%	gm	%
a) Untreated (greenhouse)	0	0	172	0
b) Treated, left in greenhouse	0	63	36	50
c) Untreated (chamber)	0	0	150	0
d) 48 hours in chamber, treated, left in chamber	50	100	------	100
e) Treated, transferred to chamber immediately	38	100	------	100
f) Treated, transferred to chamber after 24 hours	0	75	14	63
g) Treated, transferred to chamber after 48 hours	0	75	23	75

[1] Dalapon was applied at a rate of 10 pounds in 100 gallons per acre. Each figure in sections A, B, and C represents eight plants originally (four replications x two plants each).
[2] 0 = normal, unaffected; 10 = all foliage affected (browned or necrotic).
[3] Measured only once; almost no further increase in height after two weeks.
[4] Sprayed two days after all other treatments.

Similar studies on Bermudagrass showed less distinct differences among treatments, and the results must be considered inconclusive.

Effect of temperature on the uptake of simazine by wheat roots (Montgomery and Freed, 1963). Absorption and translocation studies conducted under this project have dealt with foliar absorption for the most part. The following example is included, however, to illustrate the marked influence of one factor of the physical environment (temperature) on the uptake of a soil-applied herbicide. Numerous other examples could be cited from outside this project.

Residue studies in the greenhouse showed that wheat is not as tolerant of simazine under these conditions as field studies indicate. Even at low rates of application, injury to the wheat was encountered. In attempting to determine the reason for this behavior, uptake studies from soil were carried out at two different temperatures, 16° and 26°C, under the same light intensity. Two different treatments were made at each temperature; one involved applying 0.5 pound per acre of simazine to the soil surface, while the other consisted of blending soil with sufficient simazine to give a concentration of 0.5 parts per million.

Radioanalysis of the plants after four weeks indicated large differences in the uptake at the different temperatures. In the 0.5 pound per acre treatment, 84% more radioactivity was found in the plants growing at 29°C than in those at 16°; with the 0.5 parts per million concentration, six times as much radioactivity was taken up at the higher temperature. The smaller temperature differential with the 0.5 pound per acre treatment was probably due to leaching of the chemical from the soil surface to the root zone, since considerable moisture is required in greenhouse culture. This would make a much higher concentration of simazine in the root zone of the plants growing at 26°C.

These results would tend to explain why applications of simazine to fall wheat do not result in as serious injury of the crop as applications in the spring. Shortly after seedling development the plants are not very active due to the low winter temperatures, so little simazine would be taken up during this time. It is likely that by the time plants resume growth in the spring, a good deal of the chemical has been dissipated in the soil.

Surfactant studies

It has long been known that surfactants increase herbicidal effectiveness (Currier, 1954; Currier and Dybing, 1959; Foy, 1958, 1961a, 1962a,b,d, 1963; Freed and Montgomery, 1958; Hughes and Freed, 1961; Jansen, Gentner, and Shaw, 1961).

Currier and Dybing (1959) summarized nine factors to which the response of surfactants may be due: (a) improving coverage; (b)

removing air film between spray and leaf surface; (c) reducing interfacial tension between relatively polar and apolar submicroscopic regions of the cuticle; (d) inducing stomatal entry; (e) increasing the permeability of the plasma membrane, through stimulation or incipient toxicity; (f) facilitating cell wall conduction in the region of the wall-cytoplasm interface; (g) acting as cosolvents; (h) interacting directly with the herbicide in some manner; and (i) acting as humectants secondarily.

A surfactant may exhibit one of three characteristic types of action on herbicidal activity: progressive enhancement with increasing surfactant concentrations, progressive suppression, or no effect (Currier, 1954; Jansen, Gentner, and Shaw, 1961). Certain surfactants are more effective than others. Orgell and Weintraub (1957) found that the pH and type of surfactant were the outstanding factors modifying sorption of acidic and basic substances by intact cuticle. Darlington and Cirulis (1963) reported a mathematical relationship describing the penetration of various chemicals into apricot leaf cuticle. They concluded that cuticular penetration of chloroacetamide derivatives is a diffusion process influenced by temperature, concentration, and relative solubility in organic solvents. Although penetration could be increased markedly by lipophilic substituents on the molecule, there were secondary effects not correlated with chloroform-water partitioning. In the studies of Darlington and Cirulis, substitution itself, the presence of polar heteroatoms in the substituent (chlorine, oxygen), and cyclization of the side chain did not increase (and maybe decreased) the rate of cuticle penetration to the degree suggested by the increased chloroform partitioning of the derivative. Unsaturation, olefinic or aromatic, did not affect the relationship greatly.

Surfactant effects are not necessarily the same on different species, with all herbicides, or at all initial levels of surfactant or herbicidal activity. Webster (1962) found the entry of radioactive 2,4-D into leaves of *Kalmia angustifolia* L. was variably affected by the addition of a surfactant, depending upon the age of the leaves. Entry rate of 2,4-D into recently formed leaves decreased with the increase in Tween 20 within the range of 2,4-D/Tween 20 ratios used (roughly 5/1 to 0.5/1). Entry rate of 2,4-D into older leaves increased, however, with increase in Tween 20.

Currier's observations (1954) that root absorption of dalapon is enhanced by the addition of sodium dioctylsulfosuccinate (Vatsol OT) is of interest, since roots are readily wetted by water; in all probability the action must be primarily on root-cell protoplasm. This is in contrast to foliar absorption, where the plasma membrane is only one of the lipid surfaces where surfactants can exert their effect. These re-

sults possibly may be explainable on the basis that Vatsol OT caused an imperceptible subacute toxicity at the protoplast surface, thus increasing permeability.

Surfactants may represent anionic, cationic, nonionic, ampholytic, and blended classes of compounds. As yet, no clear correlation has been found between any of the effects of a surfactant and the ionic class to which it belongs. The precise mechanisms of herbicide enhancement by surfactants still require clarification. Much additional study is needed.

Amitrole (Freed and Montgomery, 1958). Solutions of amitrole with and without surfactants were applied to bean leaves. Absorption was followed by removal of the residue on the leaf (by washing with water) and analysis of this solution. Addition of a surfactant (0.1% X-77) had a marked influence on the rate and amount of chemical absorbed. Among three surfactants studied—Mult. C., Mult. L., and X-77—at the same concentration (0.05%), greatest absorption generally was correlated with greatest surface-tension lowering. However, specific herbicide-surfactant interrelationships were suggested. Quantitative results are shown in Tables 16 through 18.

Table 16. THE TIME-COURSE OF ABSORPTION OF AMITROLE AS INFLUENCED BY A SURFACTANT

Solution	Surface tension (dynes/cm)	pH	Percent applied amitrole absorbed		
			21 hrs.	93 hrs.	117 hrs.
			%	%	%
Amitrole alone	69.6	5.87	11	13	55
Amitrole plus 0.1 percent X-77	33.3	5.82	28	44	87

SOURCE: Freed and Montgomery, 1958.

Table 17. THE EFFECT OF DIFFERENT SURFACTANTS ON THE FOLIAR ABSORPTION OF AMITROLE

Solution	Surface tension (dynes/cm)	pH	Percent applied amitrole absorbed	
			24 hrs.	48 hrs.
			%	%
Amitrole	69.6	5.87	13	....
Amitrole plus Mult. C (0.05%)	50.3	5.90	19	42
Amitrole plus Mult. L (0.05%)	59.1	6.40	26	....
Amitrole plus X-77 (0.05%)..	33.3	5.82	78	82

SOURCE: Freed and Montgomery, 1958.

Table 18. THE EFFECT OF DIFFERENT SURFACTANTS AT THE SAME SURFACE TENSION ON FOLIAR ABSORPTION OF AMITROLE

Solution	Surface tension (dynes/cm)	Percent amitrole absorbed in 24 hours
		%
Amitrole	69.6	12
Amitrole plus Mult. C (0.1%)	49.8	17
Amitrole plus Mult. L (0.5%)......	47.5	37
Amitrole plus X-77 (0.013%)	50.9	51

SOURCE: Freed and Montgomery, 1958.

The data suggest that: (a) reduction in surface tension is an important factor in absorption and translocation; (b) the relationship of molecular interaction between the surfactant and the herbicide is perhaps of equal or more importance than that of lowering of surface tension; and (c) highly specific requirements for surfactant formulation to fit the herbicide in order to achieve maximum effectiveness are indicated.

Indole-3-acetic acid (Hughes and Freed, 1961). Studies similar to those above were later conducted with IAA. The chemical was removed from the bean leaf surface with a dilute basic wash and quantitatively determined with a spectrophotofluorometer. Quantitative data are shown in Tables 19 and 20.

Table 19. AVERAGE PERCENTAGE OF IAA ABSORBED BY BEAN LEAVES

Treatment	Surface tension (dynes/cm)	Percent IAA absorbed in:		
		2 days	4 days	6 days
		%	%	%
IAA	69.2	27	36	40
IAA plus 0.1% polyoxethylene sorbitan monolaurate	41.8	56	80	88
IAA plus 0.1% isooctyl phenoxy polyethoxy ethanol	34.8	63	76	80
IAA plus 0.1% alkyl aryl polyoxyethylene glycol	41.8	37	47	----
IAA plus 0.1% sodium di-octylsulfosuccinate	33.5	45	66	77
IAA plus 0.1% lauryl sarcosine	26.4	55	76	85
IAA as calcium salt	....	....	9	17

SOURCE: Hughes and Freed, 1961.

53

Table 20. The Influence of Surfactant on Absorption of IAA

	IAA plus surfactant[1]			IAA alone		
	Percent IAA absorbed in:					
	2 days	4 days	6 days	2 days	4 days	6 days
Six separate runs						
of each period	80	92	96	48	55	48
	55	83	92	31	40	42
	46	74	81	10	30	48
	55	87	96	16	29	40
	43	60	73	33	35	33
	58	84	88	22	26	31
Average	56.2	79.5	87.7	26.7	35.8	40.3
Std. dev.	13.1	11.1	9.2	13.6	10.6	7.2
Coef. of variation	22.3	14.0	10.5	51.0	29.6	17.8

LSD .05 = 6.0
[1] 0.1% polyoxyethylene sorbitan monolaurate.
SOURCE: Hughes and Freed, 1961.

The following inferences may be drawn from the data: (a) The foliar absorption pattern of IAA follows an initial phase of rapid absorption, declining markedly in rate from the fourth through the sixth day. (b) All five surfactants at 0.1% concentration increased both rate of absorption and total amount of chemical absorbed. (c) There is an interaction between the surfactant and the species of chemical being absorbed which involves not only the ability of the surfactant to reduce surface tension, but also the chemical nature of this agent. (d) The presence of a surfactant markedly reduces the variation in absorption induced by climatic conditions. It is felt that this factor may be important in field applications of herbicides in that the use of surfactants may reduce the variation in results under adverse conditions. (e) It has been shown in this study that foliar absorption of IAA follows a first-order rate law.

Dalapon (Foy and Smith, 1964a). The following surfactants were prepared at various concentrations in distilled water, with and without 3% (w/v) technical or commercial 2,2-dichloropropionic acid (dalapon): *anionic*—Vatsol OT (sodium dioctylsulfosuccinate) and Dupanol WA (sodium lauryl sulfate); *nonionic*—Dynawet (chemistry undisclosed), X-77 (alkylarylpolyoxyethylene glycols, free fatty acids, and isopropanol), Tween 20 (polyoxyethylene sorbitan monolaurate), Tween 80 (polyoxyethylene sorbitan monooleate), and T-1947 (polyoxyethylene polyols).

Surface tensions were measured with a CENCO Du Noüy tensiometer; contact angles, by means of a micro- or macroprojector.

54

Height and fresh weight of corn plants two weeks after spraying served as measures of herbicidal activity. Usual greenhouse culture and spraying procedures were employed. Of the seven surfactants, Vatsol OT gave the greatest reduction in surface tension when present at optimum concentration. However, other surfactants such as X-77 and Tween 20 gave better surface-tension reduction at lower concentrations.

All surfactants influenced surface tensions of commercial dalapon solutions only slightly; technical dalapon solutions were affected to a somewhat higher degree. Curves depicting surface-tension lowering in distilled water were most dramatic and varied greatly among surfactants. Contact angles on paraffin-coated plexiglass and corn leaf surfaces correlated well with surface-tension measurements. With solutions having surface tensions of 31 dynes per centimeter or less, leaf wetting was complete and contact angles could not be measured.

Although surfactants differed considerably, all markedly enhanced herbicidal activity.

Solutions containing either technical or commercial dalapon wet the foliage rather poorly but better than those without dalapon. Vatsol OT gave the best visible wetting of the leaves. A concentration of 0.05% Vatsol OT gave wetting equivalent to 5% T-1947 and Tween 20. Vatsol OT at 0.1% caused thorough drenching, i.e., much better wetting than 5% Tween 20 or T-1947. Separate experiments on (1) extent of droplet spreading, (2) gross observations of contact angle between droplet and leaf surface, and (3) rate of sinking of weighted corn leaf sections in various solutions, again confirmed the superiority of Vatsol OT as a surface-tension reducer and wetting agent.

Vatsol OT alone was toxic to corn, especially at high concentrations; T-1947 and Dupanol WA produced early symptoms of toxicity, but caused no lasting injury; all other surfactants were nontoxic at all concentrations.

Minimum surface tensions and contact angles occurred at 0.1 to 0.5% concentration for all surfactants; however, maximum herbicidal activity was observed at 10 times these levels or greater. Thus, herbicidal enhancement was not correlated with surface-tension lowering, contact angle, observed wettability, or initial toxicity of the surfactants above 0.1 to 0.5% concentration. This confirms the view that herbicide-surfactant-plant surface interactions, subtler and more specific than mere increased wetting, are undoubtedly a part of total surfactant action. Essentially the same trends were apparent whether conclusions were based on height or fresh weight determinations.

Dimethyl sulfoxide as an absorption and translocation aid (Norris and Freed, 1963a). DMSO was tested as an absorption and

translocation aid for 2,4,5-T in bigleaf maple (*Acer macrophyllum*) seedlings three to five years old. The triethanolamine salt of 2,4,5-T was applied in (a) basal ,(b) injection, and (c) foliar type treatments at concentrations normally used in the field. Following exposure, all trees were divided into treated section, new growth, stem and roots. The sections were then dried, ground, extracted, and counted.

DMSO was found undesirable as a solvent for injector-type treatments. The results of the basal treatment, however, seem to indicate that DMSO may have some value as a basipetal translocation aid. In field tests of basal and injection treatments with 2,4,5-T and 2,4-TP in DMSO, however, poor kills of bigleaf maple resulted. The foliar application in 100% DMSO solutions exhibited a certain degree of phytotoxicity to maple leaves.

Tracer studies with radio-labeled surfactants. *Absorption and metabolism* of C^{14} surfactant and 2,4,5-T in bean plants (Norris and Freed, 1962, 1963b). Black Valentine beans (*Phaseolus vulgaris*) were grown in sand in the greenhouse. Four groups were treated as follows: (1) 0.25 ml 1.0% C^{14} Pluronic L-62; (2) 0.25 ml 220 ppm C^{14} triethanol amine 2,4,5-T; (3) 0.125 ml 440 ppm C^{14} triethanol amine 2,4,5-T and 0.125 ml 2.0% unlabeled Pluronic L-62; and (4) 0.125 ml 2.0% C^{14} Pluronic L-62 and 0.125 ml 440 ppm unlabeled triethanol amine 2,4,5-T.

The treatments were applied in droplets to a single leaflet of the first trifoliate leaf when fully expanded. After 72 hours, the plants were harvested and sectioned. The treated leaflet was washed with 80% ethanol to remove nonabsorbed material. The tissues were then dried, ground, and counted in a gas-flow G-M counter.

The preliminary data showed the usual enhancement of herbicide uptake and translocation with the use of a surfactant. But more significant in this study is the definite increase in the movement of the surfactant when it is applied along with a herbicide. This indicates once again that some sort of interaction between herbicide and surfactant does exist.

Whether or not the surfactant actually moves in direct association with the herbicide is not yet evident. The fact that upward translocation of the herbicide was enhanced while that of the surfactant was not, indicates that they do not necessarily move together. However, this point is not yet fully evident from the data available.

Later studies confirmed the marked effect of the herbicide on the uptake of the surfactant. The mode of action is not known. Furthermore, the exact form in which the surfactant exists once inside the plant has not been determined. Preliminary chromatogram scans and the evolution of $C^{14}O_2$ from treated plants indicate some metabolism

of the labeled surfactant. The results also seem to indicate that while the surfactant with herbicide is metabolized to CO_2 at a slightly faster rate, the surfactant when applied alone is actually being degraded or metabolized to other products at a much faster rate. The significance of this information is not fully known. Further studies are planned to investigate more intensively this herbicide-surfactant interaction.

Tracer studies with labeled surfactants and dalapon (Foy and Smith, 1964b). Dalapon-Cl^{36}, T-1947-C^{14} (polyoxyethylene polyols), and SLS (sodium lauryl sulfate)-S^{35} were employed in tracer studies on cotton and barley. Labeled and unlabeled herbicides and surfactants were applied to leaves by drop treatment in the following combinations: (1) dalapon-Cl^{36} alone; (2) dalapon-Cl^{36} and T-1947; (3) dalapon-Cl^{36} and sodium lauryl sulfate; (4) T-1947-C^{14} alone; (5) sodium lauryl sulfate-S^{35} alone; (6) T-1947-C^{14} and dalapon; (7) sodium lauryl sulfate-S^{35} and dalapon; and (8) untreated. Because of low specific activity, the rather large droplets required (100 μl approximately) were confined within lanolin rings on the leaves. Wetting patterns and acute toxicity were observed, and plants were harvested for autoradiography and counting 1 hour, 24 hours, and 7 days after treatment.

No radioactivity was detected outside treated leaves within one hour. Dalapon-Cl^{36} alone entered all plant parts within 24 hours. T-1947-C^{14} alone penetrated poorly, tended to remain liquid, and had moved only distally within the treated leaf after seven days. Sodium lauryl sulfate-S^{35} alone penetrated rapidly. Although both T-1947-C^{14} and SLS-S^{35} moved out toward the tips of leaves (e.g. in cotton, forming a wedge pattern, characteristic of apoplastic movement with the transpiration stream), they apparently followed different systems. SLS-S^{35} (nonmetabolized after 24 hours) moved predominantly in the veins, whereas T-1947-C^{14} (also apparently nonmetabolized) occurred mostly in interveinal areas. Moreover, T-1947-C^{14} (but not SLS-S^{35}) tended to accumulate in the lysigenous glands of cotton. After seven days, SLS-S^{35} had become metabolized and S^{35} was distributed widely throughout the plants.

Nonlabeled dalapon increased T-1947-C^{14} uptake slightly by producing acute toxicity in the region of absorption; with SLS-S^{35}, which penetrated readily alone, similar dalapon toxicity impaired the transport mechanism and reduced translocation of S^{35} out of the treated leaf. Nonlabeled SLS enhanced, whereas T-1947 apparently interfered with, absorption and translocation of dalapon-Cl^{36}. The latter result was unexpected since SLS and T-1947 both markedly enhanced the herbicidal activity of dalapon in spray tests on corn. Differences in drop size, degree of leaf coverage, and concentration of chemicals in

solution between the two treatment methods probably account for the anomalous results with T-1947-C^{14} in the tracer experiments.

Studies along these lines will be continued and expanded. Additional labeled surfactants representing other ionogenic classes will be included.

Summary

1. The effectiveness of herbicide application depends on three factors: penetration of the chemical, movement within the plant, and biochemical (toxic) action.

2. Insufficient penetration can be limiting, both as to gross toxicity and to selective action of herbicides. Studies of penetration pathways and mechanisms have led to improved methods of spray application.

3. The major experimental techniques used in penetration studies have included radioactive and fluorescent tracers, in connection with autoradiography, counting, and ultraviolet microscopy. Herbicidal or growth-regulatory responses on plants also served as indirect measures of absorption and translocation.

4. Environmental factors enhancing penetration include: moderate temperature, high humidity, and moderately high light intensity.

5. The chemical environment, i.e., the formulation of the spray solution, exerts important influences. Dalapon, for example, displayed a definite pH optimum for absorption and translocation; rather specific interactions between formulations and surfactants were also suggested. Surfactants, penetrants, deposit builders, and cofactors, enhance both stomatal and cuticular penetration under many conditions. Surfactants, especially, are effective if selected according to use and employed at proper concentrations.

6. Herbicidal enhancement by surfactants is only partially explained by improved wetting. Enhancement was not closely correlated with surface-tension lowering, contact angle, observed wettability, or initial toxicity of surfactants above 0.1 to 0.5% concentration in solution. Neither is the ionogenic class of a surfactant a satisfactory criterion for judging its herbicidal enhancement effects. Some radiolabeled surfactants were themselves absorbed and translocated, perhaps in association with herbicide uptake and transport. The precise mechanism(s) of herbicide enhancement by surfactants require much further study. Rather specific herbicide-surfactant-plant surface interrelationships are indicated.

7. Foliar penetration pathways can be described as cuticular and/or stomatal. Penetration of intact cuticle is slow and long con-

tinuing, provided the spray deposit remains in an absorbable condition. Cracks and perforations enhance the process. High humidity reduces the rate of droplet drying, promotes stomatal opening, and doubtlessly increases the hydration and thus permeability to polar substances. Stomatal penetration of aqueous solutions occurs when stomata are open sufficiently and when an efficient surfactant is present in the solution. Once in the intracellular space system, the herbicide must move across the so-called "internal cuticle" to enter the mesophyll cell walls or protoplasm.

8. Cellular pathways followed by chemicals from the cuticle to vascular tissue are imperfectly known. However, some herbicides such as monuron appear to move in the cell walls primarily; others, such as 2,4-D, appear to move in the protoplasm (symplast).

9. Why some substances enter the phloem, others the xylem, is not clearly known. Also, why herbicides differ markedly in their tendency to "accumulate" in living cells (e.g., in leaf vascular parenchyma, young roots, and along the stem transport pathways) and in the lysigenous glands of cotton is not well understood. Attention must be given to xylem- and phloem-parenchyma cells; such studies may help to divulge these secrets. Once a substance enters the xylem, movement is usually in the transpiration stream towards leaf margins. Once in the phloem, movement is usually with the assimilate produced in photosynthesis or in digestion of food reserves. Movement across the cambium from phloem to xylem and vice versa can occur readily.

10. Callose development in phloem appears to block export of applied herbicides from leaves. However, the effect of callose formation in leaf parenchyma on translocation remains to be proved.

References

Ashton, F. M., W. A. Harvey, and C. L. Foy. 1961. Principles of selective weed control. Calif. Agric. Expt. Sta. and Ext. Serv. Circ. 505.

Audus, L. J. 1964. *The Physiology and Biochemistry of Herbicides.* New York: Academic Press.

Crafts, A. S. 1959. Further studies on comparative mobility of labeled herbicides. Plant Physiol., *34*:613-620.

Crafts, A. S. 1961a. *The Chemistry and Mode of Action of Herbicides.* New York: Interscience Publishers.

Crafts, A. S. 1961b. *Translocation in Plants.* New York: Holt, Rinehart and Winston.

Crafts, A. S. 1961c. Absorption and migration of synthetic auxins and homologous compounds. In: *Encyclopedia of Plant Physiology 14.* Growth and Growth Substances. Berlin-Göttingen-Heidelberg: Springer, pp. 1044-1054.

Crafts, A. S., and C. L. Foy. 1962. The chemical and physical nature of plant surfaces in relation to the use of pesticides and to their residues. Residue Reviews, *1*:112-139.

Crafts, A. S., and S. Yamaguchi. 1958. Comparative tests on the uptake and distribution of labeled herbicides by *Zebrina pendula* and *Tradescantia fluminensis.* Hilgardia, *27:*421-454.

Crafts, A. S., and S. Yamaguchi. 1960. Absorption of herbicides by roots. Amer. Jour. Botany, *47:*248-255.

Currier, H. B. 1954. Wetting agents and other additives. Calif. Weed Conf. Proc., *6:*10-15.

Currier, H. B. 1957. Callose substance in plant cells. Amer. Jour. Botany, *44:*478-488.

Currier, H. B., and C. D. Dybing. 1959. Foliar penetration of herbicides—review and present status. Weeds, *7:*195-213.

Darlington, W. A., and N. Cirulis. 1963. Permeability of apricot leaf cuticle. Plant Physiol., *38:*462-467.

Dunning, J. J. 1958. Factors influencing the formation of callose in plant cells. Ph.D. dissertation, Univ. of Calif., Davis.

Dybing, C. D. 1958. Foliar penetration by chemicals. Ph.D. dissertation, Univ. of Calif., Davis.

Dybing, C. D., and H. B. Currier. 1959. A fluorescent dye method for foliar penetration studies. Weeds, *7:*214-222.

Dybing, C. D., and H. B. Currier. 1961. Foliar penetration in chemicals. Plant Physiol., *36:*169-174.

Eschrich, W. 1961. Untersuchungen über der Ab- und Aufbau der Callose. Z. Botan., *49:*153-218.

Foy, C. L. 1958. Studies on the absorption, distribution and metabolism of 2,2-dichloropropionic acid in relation to phytotoxicity. Ph.D. dissertation, Univ. of Calif., Davis.

Foy, C. L. 1961a. Absorption, distribution and metabolism of 2,2-dichloropropionic acid in relation to phytotoxicity. I. Penetration and translocation of Cl^{36} and C^{14}-labeled dalapon. Plant Physiol., *36:*688-697.

Foy, C. L. 1961b. Absorption, distribution and metabolism of 2,2-dichloropropionic acid in relation to phytotoxicity. II. Distribution and metabolic fate of dalapon in plants. Plant Physiol., *36:*698-709.

Foy, C. L. 1962a. Penetration and initial translocation of 2,2-dichloropropionic acid (dalapon) in individual leaves of *Zea mays* L. Weeds, *10:*35-39.

Foy, C. L. 1962b. Absorption and translocation of dalapon-2-C^{14} and -Cl^{36} in *Tradescantia fluminensis.* Weeds, *10:*97-100.

Foy, C. L. 1962c. Accumulation of *s*-triazine derivatives in the lysigenous glands of *Gossypium hirsutum* L. Plant Physiol. (Suppl.), *37:*XXV.

Foy, C. L. 1962d. Influence of spray additives and environment on foliar penetration of dalapon-Cl^{36} in *Tradescantia.* West. Weed Control Conf. Res. Prog. Report, pp. 82-83.

Foy, C. L. 1963. The influence of formulation, exposure time, and pH on the herbicidal action of dalapon foliar sprays tested on corn. Hilgardia, *35:*125-144.

Foy, C. L., and L. W. Smith. 1964a. Comparison of surface tension lowering, wettability of paraffin and corn leaf surfaces, and herbicidal enhancement of dalapon by seven surfactants. Weed Society of America (Abstracts), pp. 77-78.

Foy, C. L., and L. W. Smith 1964b. Tracer studies with radiolabeled surfactants and dalapon. Weed Society of America (Abstracts), p. 79.

Foy, C. L., and K. Sukartaatmadja. 1963. Influence of relative humidity on the herbicidal response of Johnsongrass to dalapon. West Weed Control Conf. Res. Prog. Report, pp. 92-93.

Franke, W. 1961. Ectodesmata and foliar absorption. Amer. Jour. Botany, 48:683-691.

Franke, W. 1962a. Ektodesmenstudien. I. Über pilzforming erscheinende Ektodesmen. Kritische Abhandlung über das Wesen der Ektodesmen. Planta, 59:222-238.

Franke, W. 1962b. Weitere Untersuchungen zur Stoffaufnahme durch Blätter und deren Beziehung zu Ektodesmen. Ber. Deut. Botan. Ges., 75:295-307.

Freed, V. H., and M. Montgomery. 1958. The effect of surfactants on foliar absorption of 3-amino-1,2,4-triazole. Weeds, 6:386-389.

Frey-Wyssling, A. 1948. *Submicroscopic Morphology of Protoplasm and Its Derivatives.* New York: Elsevier.

Frey-Wyssling, A. 1953. *Submicroscopic Morphology of Protoplasm,* 2nd ed. New York: Elsevier.

Gentner, W. A. 1961. Influence of EPTC on the deposition of surface wax of cabbage leaves. Weed Society of America (Abstracts), pp. 53-54.

Hughes, R. E., and V. H. Freed. 1961. The role of surfactants in the foliar absorption of indole-3-acetic acid (IAA). Weeds, 9:54-59.

Jansen, L. L., W. A. Gentner, and W. C. Shaw. 1961. Effects of surfactants on the herbicidal activity of several herbicides in aqueous spray systems. Weeds, 9:381-405.

Jensen, W. A. 1962. *Botanical Histochemistry.* San Francisco: W. H. Freeman and Co.

Kreger, D. R. 1949. An X-ray study of waxy coatings from plants. Rec. Trav. Bot. Neerl., 41:603-736.

Maestri, M., and H. B. Currier. 1958. Interactions of maleic hydrazide and endothal. Weeds, 6:315-326.

Mason, G. W. 1959. The absorption, translocation, and metabolism of 2,3,6-trichlorobenzoic acid in plants. Ph.D. dissertation, Univ. of Calif., Davis.

Meeuse, A. D. J. 1957. Plasmodesmata (Vegetable Kingdom). Protoplasmatologia, 2A (1c):1-43.

Mitchell, J. W., and P. J. Linder. 1957. Absorption and translocation of plant regulating compounds. Amer. Assoc. for Advancement of Science Symposium: Atomic Energy and Agriculture, 49:165-182.

Mitchell, J. W., B. C. Smale, and R. L. Metcalf. 1960. Absorption and translocation of regulators and compounds used to control plant diseases and insects. Advances in Pest Control Research, 3:359-436.

Montgomery, M., and V. H. Freed. 1963. The effect of temperature on the uptake of simazine by wheat. West. Weed Control Conf. Res. Prog. Report, p. 90.

Mueller, L. E., P. H. Carr, and W. E. Loomis. 1954. The submicroscopic structure of plant surfaces. Amer. Jour. Botany, 41:593-600.

Norris, L. A., and V. H. Freed. 1962. Movement of C^{14} surfactant and 2,4,5-T in bean plants. West. Weed Control Conf. Res. Prog. Report, pp. 92-93.

Norris, L. A., and V. H. Freed. 1963a. Dimethyl sulfoxide as an absorption and translocation aid. West. Weed Control Conf. Res. Prog. Report, pp. 85-86.

Norris, L. A., and V. H. Freed. 1963b. Absorption and metabolism of C^{14} surfactant as influenced by 2,4,5-T in bean leaves. West. Weed Control Conf. Res. Prog. Report, pp. 86-87.

61

Orgell, W. H. 1954. The isolation and permeability of plant cuticle. Ph.D. dissertation, Univ. of Calif., Davis.

Orgell, W. H., and R. L. Weintraub. 1957. Influence of some ions on foliar absorption of 2,4-D. Botan. Gaz., *119*:88-93.

Pallas, J. E., Jr., D. G. Harris, C. B. Elkins, Jr., and A. R. Bertrand. 1961. Research in plant transpiration. ARS, USDA Production Research Report No. 70.

Prasad, R., C. L. Foy, and A. S. Crafts. 1962. Role of relative humidity and solution additives on the foliar absorption and translocation of radiolabeled 2,2-dichloropropionic acid (dalapon). Plant. Physiol. (Suppl.), *37*:xiii.

Priestley, J. H. 1943. The cuticle in angiosperms. Botan. Rev., *9*:593-661.

Radwan, M. A., C. R. Stocking, and H. B. Currier. 1960. Histoautoradiographic studies of herbicidal translocation. Weeds, *8*:657-665.

Roelofsen, P. A. 1952. On the submicroscopic structure of cuticular cell walls. Acta Botan. Neerl., *1*:99-114.

Rudolph, K. 1925. Epidermis and epidermal transpiration. Botan. Arch., *9*:49-94.

Sargent, J. A., and G. E. Blackman. 1962. Studies on foliar penetration. I. Factors controlling the entry of 2,4-dichlorophenoxyacetic acid. Jour. Exper. Botany, *13*:348-368.

Schieferstein, R. H. 1955. Formation of wax deposits on leaves. M.S. thesis, Iowa State College, Ames.

Schieferstein, R. H. 1957. Development of protective structures of the plant cuticle. Ph.D. dissertation, Iowa State College, Ames.

Schnepf, E. 1959. Untersuchungen über Darstellung und Bau der Ektodesmen und ihre Beeinflussbarkeit durch stoffliche Factoren. Planta, *52*:644-708.

Schumacher, W. 1942. Über plasmodesmenartige Strukturen in den Epidermisaussenwanden. J. wiss. Bot., *90*:530-545.

Schumacher, W., and P. Lambertz. 1956. Über die Beziehungen zwischen den Stoffaufnahme durch Blattepidermen und die Zahl der Plasmodesmen in den Aussenwänden. Planta, *4*:47-52.

Smith, A. E., J. W. Zukel, G. M. Stone, and J. A. Riddell. 1959. Factors affecting the performance of maleic hydrazide. Jour. Agric. Food Chem., *7*:341-344.

van Overbeek, J. 1956. Absorption and translocation of plant regulators. Ann. Rev. Plant. Physiol., *7*:355-372.

van Overbeek, J., and R. Blondeau. 1954. Mode of action of phytotoxic oils. Weeds, *3*:55-65.

Webster, D. H. 1962. Entry of 2,4-dichlorophenoxyacetic acid into lambkill leaves at varied 2,4-D/Tween 20 ratios. Weeds, *10*:250-251.

Weintraub, R. L., J. H. Reinhart, and R. A. Scherff. 1956. Role of entry, translocation, and metabolism in specificity of 2,4-D and related compounds. U. S. Atomic Energy Commission, TID 7512. Proc. Conf. on Radioactive Isotopes in Agric., pp. 203-208.

Woodford, E. K., K. Holly, and C. C. McCready. 1958. Herbicides. Ann. Rev. Plant. Physiol., *9*:311-358.

CHAPTER TWO

The Influence of Environment on the Response of Plants to Certain Herbicides

T. J. MUZIK

In the lifetime of every plant, there are periods when it is especially sensitive to the vicissitudes of the environment, as well as to the application of herbicides.

It is obvious that plants differ in their responses to environmental conditions. Why they respond the way they do is less obvious. The composition of the plant community is determined by factors such as climate, soil, soil and atmospheric moisture pattern, light, temperature, fertility, and past history. Any one of these factors may be critical in determining the effectiveness of a chemical. A systemic herbicide must, to be effective, do three things: it must enter the plant, it must be translocated through the plant, and it must exert a phytotoxic effect after it is translocated.

Temperature-Dependent Changes in Plant Growth

The optimum temperature conditions for the growth of one species are not the same as for another, nor are the minimum and maximum temperatures for growth. Temperature has complex effects on plants. Relatively slight differences in temperature at critical periods may cause large differences in final growth responses, including flowering. The onset of flowering appears to be especially critical to a plant's response to chemicals.

Light and temperature bear on the nutritional status of the plant; they not only affect absorption and translocation but simultaneously alter the sensitivity of the plant to external stimuli.

Both high and low temperatures adversely affect plant growth. Plants resistant to high temperatures have been shown to produce organic acids which alleviate the toxic effects of ammonia (Petinov and Molotkovsky, 1957). Thiamin has been shown to be closely related to carbohydrate metabolism (Bonner and Bonner, 1948). Spraying certain plants with nicotinic acid, B vitamins, or ribosides markedly improved their growth at low temperatures (Ketellapper, 1963).

Temperature may affect the level of endogenous auxin. Rice seedlings at $26°C$ had a lower content of auxin than seedlings at $10°C$ (Ketellapper, 1963).

63

Currier and Dybing (1959) suggest that some of the effects of temperature on penetration are exerted through changes in physico-chemical processes, rates of diffusion, vicosity, and so forth, and physiological factors—acceleration of photosynthesis, phloem translocation, protoplasmic streaming, and growth. Drying of spray droplets and closure of stomata under a water stress may also be important. In general, warm temperatures promoted penetration; but high temperatures together with low humidity were detrimental to absorption, possibly due to increased evaporation of spray droplets.

Both translocation and absorption have been reported as being speeded up at high temperatures. Most workers have failed to separate absorption from translocation or toxic effect, and it is difficult to draw conclusions from much of the literature. The use of different species without adequate control of all environmental factors also serves to cloud the picture.

Kelly (1949) reported that bean plants grown at 15°C showed little effect from 2,4-D until transferred to 25°C, but plants grown at 5°C exhibited a marked effect after transfer to the 15°C temperature. From these results, it would appear that a change in temperature following treatment may be as critical as the temperature at the time of treatment.

Variations in temperature prior to or after spraying may affect the sensitivity of certain crops to some chemicals but not to others. Flax sprayed with 2,4-D or MCPA was injured more when sprayed at 29.5°C than at 10°C, but the greatest injury occurred when plants were sprayed at 29.5°C and subsequently placed at 10°C. Application of 2,4-D to oats before or after frost caused yield reduction, whereas MCPA caused no such reduction (Audus, 1959).

Many examples can be given of seasonal responses to various herbicides under field conditions. Unfortunately, one can never be quite sure which environmental factor is critical. Temperature, rainfall, light, and stage of growth have all been shown to affect the response of plants to chemicals. Therefore, it is necessary to control these factors within very narrow limits in order to investigate their relative roles.

For example, stage of growth of wheat is critical in its response to 2,4-D. Spraying in the seedling and early flowering stages may drastically reduce yields. Field practices, however, must be balanced between temperature and stage of growth. Thus, an early spring spray when the annual weeds are in the most sensitive stage and the wheat most resistant is not always practicable because of low temperatures. The applicator must balance a number of factors and select the best alternative.

64

Stage of growth of wheat appears to be less critical in terms of its response to bromoxynil and to the triazine herbicides. Simazine has given excellent control of downy bromegrass in the autumn without serious damage to winter wheat. Spring applications have had no apparent effect on either weed or crop. Atrazine, a closely related chemical, kills both the crop and the weed when applied in the fall or early winter (before December), but it gives excellent selective control when applied in early spring, except in light soils where it may cause serious damage to the wheat. Sprinkler irrigation caused increased damage to wheat in the spring even in heavy soils, demonstrating the importance of rainfall patterns.

IPC, on the other hand, while consistent on any one date of application, may cause diametrically opposite responses to crop and weed in applications made less than a week apart to adjacent plots. Seasonal effects with this chemical thus appear to be overshadowed by local environmental conditions at the time of or prior to application. Temperature is probably the determining factor.

IPC is a useful chemical in removing downy bromegrass from Merion bluegrass, particularly if applications are made in late fall. In this crop, it is possible to balance the sensitivity of the weed and the crop; in wheat, the differences in sensitivity between crop and weed are not great enough to permit use of this chemical under the environmental variations of the western region.

Other factors, such as soil moisture following herbicidal application, may be critical. Yield reductions and malformations frequently arise from applications in the seedling stage. In some of our studies, it appeared that a dry period of four to eight days following 2,4-D application to wheat could induce serious damage, whereas under good moisture conditions the plants recovered almost completely. This is probably related to the root damage which has been shown to occur from foliar applications of 2,4-D to wheat in the seedling stage (Johanson and Muzik, 1961).

Objectives

We have investigated the effect of temperature on growth and susceptibility to 2,4-D at different stages of growth and the effect of temperature on translocation of 2,4-D. Attempts were made to modify the effects of temperature by the addition of certain metabolites.

This work has been concerned mainly with two temperature-sensitive crops, peas and tomatoes; a temperature-resistant crop, winter wheat; and a temperature-resistant weed, fiddleneck (*Amsinc-*

kia intermedia). This weed exhibits resistance to 2,4-D in the bolting stage, but is very sensitive in the rosette stage.

Plants were germinated in the greenhouse and transferred to growth chambers under controlled conditions at the proper stage of growth and left in the chambers for at least a week before treatment. Root cultures were made in nutrient solutions under sterile conditions. The use of cultures enabled the intrinsic plant response to the herbicide to be separated from any effects due to translocation.

Illumination was kept constant within an experiment. Attempts to control flowering in *Amsinckia* were only partially successful. It was possible to select plants of the same age, but in different stages of growth, by growing them under long-day conditions.

Results: Effects of 2,4-D and Temperature

Pea and fiddleneck root cultures

Roots were grown under sterile conditions by sterilizing seeds and germinating them before excising the roots. The addition of 0.1 ppm pyridoxin to the standard pea medium was necessary to permit growth of *Amsinckia* roots.

Pea roots placed in sterile nutrient media grew well at 25°C and 40°C, elongating rapidly and producing many laterals, but roots grew very slowly at 10°C. When transferred from 10°C to a warmer temperature after one week, they elongated rapidly and appeared to grow normally.

The incorporation of 2,4-D at 0.01 and 0.1 ppm severely inhibited root growth at 25°C and 40°C. No laterals were produced and the roots elongated only slightly. Bulbous tips sometimes developed, and the roots increased twofold in thickness. No morphological effect of 2,4-D was visible on the roots placed at 10°C.

After one week the roots were rinsed in sterile distilled water and transferred to solutions without 2,4-D at 25°C. Those previously grown at 25°C and 40°C did not recover. However, those treated previously at 10°C with 0.01 or 0.1 ppm 2,4-D grew as well as or better than the controls.

Increased numbers of protoxylem elements were found in roots grown in the 2,4-D solution at 40°C. More phloem fibers formed adaxially, giving the roots a more compact appearance, and the central interstitial tissue was not visible in cross section. This tissue exhibited a disorganized appearance in roots grown at 25°C.

Isolated roots of fiddleneck were immersed in nutrient solutions containing 2,4-D and were transferred to solutions without 2,4-D after periods of 30 minutes, 1 hour, 7 hours, 24 hours, 4 days, and 7

days. Those grown at 10°C did not absorb sufficient 2,4-D in 30 minutes to cause malformations or growth inhibition when transferred to warmer temperatures and solutions without 2,4-D. A 30% inhibition of elongation occurred in roots exposed for 1 to 24 hours, a 50% inhibition in roots exposed for 3 to 4 days, and almost complete inhibition in roots exposed for 7 days. These results suggest that the entry of 2,4-D into roots requires metabolic energy and is limited under low-temperature conditions.

Tomato plants

Tomato plants (Washington Forcing) were grown in a growth-control chamber at 10°, 20°, or 30°C. One leaf on each plant was dipped in 250 ppm 2,4-D solution and removed 1, 4, 8, or 12 days later. Control plants were treated the same way except for the 2,4-D treatment. Root and stem growth was much more severely inhibited at 20° or 30°C than at 10°C when the treated leaf was removed 4 days or less after treatment. This effect was much less clearly shown in plants left for longer periods.

Fiddleneck

Toxicity of 2,4-D. Fiddleneck (*Amsinckia intermedia*) in the rosette stage showed only a slight response to 2,4-D treatment (300 μg per plant) when grown at 5°C. At higher temperatures (10° and 20°C) this amount of 2,4-D caused severe malformations and, in some instances, death.

Tests were made to ascertain the minimum amount of 2,4-D to give a response at 5°C, 10°C, and 26°C when applied to fiddleneck at the rosette or bolted stages. Curling of the apical leaves was used as a criterion for response. The plants were grown in the greenhouse at 21°C, then transferred to growth rooms adjusted to the above temperatures. After allowing a week for the plants to become adjusted to the new environment, a droplet containing 2,4-D was placed on the growing point of each plant; concentrations ranged from 1 μg to 600 μg per droplet. The chemical was dissolved in 15% acetone solution plus 0.1% Tergitol. It took approximately 5 to 10 times as much chemical to obtain curling of the leaves at the preflowering stage as it did in the rosette stage of this plant at any temperature. At 5°C, 10 μg caused curling of the leaves in the rosette. At this temperature it took 50 μg for similar curling at the preflowering stage. At 10°C, 2 μg and 20 μg, respectively, and at 26°C less than 1 μg caused curling of the young leaves whereas it took at least 5 μg for the preflowering stage.

Translocation of 2,4-D. Radioactive carboxyl-labeled 2,4-D was applied to the midrib of the tenth youngest leaf of fiddleneck plants

growing at 5°, 15°, and 25°C. In all cases the treated leaf appeared to be mature. Rosette and prebolting stages were used. All plants were of the same age.

In the rosette stage, the radioactivity moved downwards into the roots of all the plants regardless of the temperatures in which they were growing. For the first two hours, there was a definite temperature influence on the amount of radioactivity (2,4-D) translocated; that is, the plants growing at 25°C had the greatest concentration of radioactivity in the apex of the treated leaf as well as in the roots. Autoradiographs of plants treated for seven hours showed an appreciable movement of labeled material into the roots with the same well-marked temperature differences observed in the two-hour treatment. Movement into some of the young apical leaves also occurred in the seven-hour treatment. The effect of temperature on movement in the 24-hour period was not so well marked, and the radioactivity had moved throughout the plant stems and roots. Movement appeared to take place initially into the roots, but later movement was both acropetal and basipetal.

Movement of the labeled 2,4-D to the roots was considerably less in the bolting or preflowering stage at all temperatures.

Effects of metabolites. Other workers have shown that some plants growing at low temperatures did not synthesize certain substances in sufficient quantities for rapid growth and development (Ketellapper, 1963). If the addition of these substances to the leaves would improve the growth of the plant, the plant might be more susceptible to 2,4-D, even though the temperature remained unchanged.

Seedlings of *Amsinckia* were established in silica sand, and weekly treatments of certain metabolites were sprayed on the foliage. Nutrients were supplied to all plants every other day with Hoagland's solution. Thiamin (20 ppm), ascorbic acid (1,000 ppm), biotin (1 ppm), pyridoxin (20 ppm), nicotinic acid (40 ppm), adenine (1 ppm), 2-thiouracil (1 ppm), and a mixture of amino acids were sprayed on the leaves. The plants were sprayed with the solutions weekly for four consecutive weeks and then harvested. The root and shoot of each plant were separated and the dry weight taken. At the time of harvest the plants sprayed with the amino acids, d-biotin, or nicotinic acid were darker in color than the control plants and had made significant increases in dry weight of both tops and roots. Those metabolities which caused an improvement in growth at this temperature were then applied to similar plants in the rosette stage together with 2,4-D to determine if they would affect its activity. The plants were grown in the growth chamber under the same light regime and same conditions as in the previous experiment. In addition to the

amino acid mixture, biotin (0.1 μg and 10 μg), niacin (10 and 100 μg), or thiamin (10 μg), 275 μg of 2,4-D per plant was applied. Observations were recorded one month after treatment.

2,4-D alone caused a suppression of the apex and some distortion of the youngest leaves. Biotin (0.1 μg) plus 2,4-D caused severe swelling of the apex, suppression of new growth, and discoloration and necrotic areas in the mature leaves. Biotin (10 μg) plus 2,4-D had much less effect. Niacin at the rate of 100 μg per plant increased the response of the plant to 2,4-D but did not do so at 10 μg. Thiamin caused the greatest increase in response to 2,4-D, and the plants were severely injured.

Thiamin-2,4-D interaction. Fiddleneck plants were grown in the greenhouse for one month and then placed in a growth chamber at 10°C. Plants were treated with either 2,4-D (500 μg per plant), thiamin (0.05 μg to 50 μg per plant) or with mixtures of thiamin and 2,4-D. All treatments were made in volumes of one milliliter in eight aliquots on a single leaf.

The untreated plants and those with thiamin alone grew slowly, with no apparent morphological differences due to the thiamin. Applications of 2,4-D alone caused suppression of growth and slight distortion of the apex. 2,4-D plus 0.05 μg thiamin caused 100% mortality; 2,4-D plus 0.5 μg thiamin caused death of 40% of the plants and severe injury and distortion to 60%. The 2,4-D plus the higher rates of thiamin (2.5, 5.0 and 50 μg) severely injured but did not kill the plants.

Autoradiographs from plants receiving no thiamin prior to 2,4-D, or thiamin at the time of herbicide application, showed the labeled carbon from the herbicide to be present in a relatively low concentration in both roots and shoots. Movement in the shoot, in fact, was restricted to the treated leaf. Treatment with thiamin seven hours before 2,4-D application resulted in autoradiographs of a somewhat greater intensity, and faint traces of radioactivity could be seen in the younger leaves. Application of thiamin to plants 72 to 168 hours before labeled 2,4-D was applied resulted in autoradiographs of much higher intensity, in which the presence of C^{14} could be detected in several leaves (young as well as more mature ones) and in the roots.

The results of the second phase of this experiment, which was based on inhibition of growth responses, are in close agreement with the evidence obtained from the autoradiographs. When thiamin was supplied to the leaves exposed to a temperature of 10° C either 72 or 168 hours before 2,4-D was applied to the leaves, significantly greater inhibition of both shoot and root growth occurred than when no thiamin was supplied prior to application of 2,4-D (Table 1). The

Table 1. Effect of 2,4-D and Thiamin on Dry Weight of Fiddleneck[1]

Hours between applications of thiamin and 2,4-D		Dry weight (grams)	
		Shoots	Roots
No thiamin	....	2.57	1.78
0	....	2.63	1.71
7	....	2.62	1.73
72	....	1.91[2]	1.53[2]
168	....	1.78[2]	1.49[2]

[1] Plants were grown at 10°C and received 100 μg thiamin followed by 250 μg 2,4-D at the specified intervals. Dry weights were measured 14 days after treatment.
[2] Significantly different from control at the 1% level.

application of thiamin either just before or seven hours prior to 2,4-D application, however, did not result in significant growth reduction.

An explanation of the action of thiamin in increasing the effectiveness of 2,4-D is not readily apparent from these studies. However, based on the experimental work of Mitchell *et al.* (1953) and the fact that thiamin is translocated in a similar fashion to 2,4-D (Bonner, 1942), it is conceivable that the two substances together form a more translocatable material.

Fiddleneck plants, when maintained at 10° C, exhibited no significant response to thiamin if the vitamin was supplied to the roots via nutrient solution. The effects of applying thiamin to the foliage of plants at 10° C, however, differed strikingly from the effects of root applications. As shown in Table 2, treatment with 50 μg thiamin caused a highly significant increase in dry weights of both shoots and roots. Thiamin, however, had no marked effect when supplied at rates of 10 or 250 μg.

Table 2. Effect of Thiamin on Dry Weight of Fiddleneck Grown at Low Temperatures[1]

Thiamin μg per plant		Dry weight of plant (grams)	
		Tops	Roots
0	....	3.39	1.60
10	....	3.41	1.55
50	....	3.82[2]	1.88[2]
250	....	3.48	1.65

[1] Plants were grown at 10°C and received thiamin twice weekly by foliar application.
[2] Significant at the 1% level.

Plants grown in glass-faced boxes at 10° C showed significant differences in the rate of daily root growth (elongation) when foliage applications of thiamin were made (Table 3). The data show that all levels of thiamin were effective in this respect.

Table 3. RESPONSE OF FIDDLENECK ROOTS AT 10°C TO FOLIAR-APPLIED THIAMIN

Thiamin µg per plant	Root growth[1] cm/day
0	1.84
10	2.50[2]
50	2.61[2]
250	2.55[2]

[1] Each value is an average of six roots.
[2] Significant at the 5% level.

The evidence presented thus far seems sufficient to support the view that at least part of the low temperature response of coast fiddleneck to 2,4-D is due to the interference of temperature with thiamin relationships in the plant. However, the data cannot be held to support the hypothesis in full unless it can be shown that there is a definite deficiency of the metabolite at a suboptimal temperature compared to an optimal temperature.

Thiamin content at different temperatures. To detect possible differences in content of thiamin at various temperatures, *Amsinckia* plants were grown at 10°C (suboptimal temperature for coast fiddleneck), 18°C (optimal temperature), and 27°C (supraoptimal temperature) and subsequently analyzed for thiamin. The results are recorded in Table 4. Both shoots and roots of plants maintained at 10°C contained less thiamin than plants growing at temperatures of 18° or 27°C. There was comparatively little difference in values of thiamin at the latter two temperatures.

Table 4. THIAMIN CONTENT OF COAST FIDDLENECK PLANTS GROWN AT DIFFERENT TEMPERATURES

Temperature °C	Thiamin per 100 mg dry material		Root/shoot ratio
	Shoot µg	Root µg	
10	0.938	0.219	0.23
18	1.183	0.906	0.77
27	1.110	0.844	0.76

Discussion

It is obvious from the data assembled by numerous workers that the influence of temperature on plant growth is very complex. Small differences in either light or temperature may lead to some large differences in final growth. Many of these effects of light and temperature are exerted on absorption and translocation, yet it appears that some effects of light and temperature can be separated from these. It is also apparent that species differences must be considered when working with different plants. A tomato plant at 10°C is clearly under more stress than winter wheat or a winter annual weed at the same temperature. Absorption and translocation in leaves and roots are less at low temperatures.

Stage of growth is important in determining response to a chemical. In our experiments, most of the work was done with a plant (*Amsinckia intermedia*) which shows a marked reduction in response to 2,4-D when it begins to flower. In the rosette stage, it was found to be much more sensitive even when the plants were of the same chronological age. Thus, physiological condition is more important than age.

Plants grown at temperatures cool enough to reduce their growth rate failed to respond to 2,4-D, or responded to a much lesser degree than plants grown at higher temperatures. However, when these plants were sprayed with a solution containing a metabolite which would increase growth at this temperature, the response to 2,4-D was invariably more marked. The most active of these compounds was thiamin. It is of particular interest to note that the concentration of the material applied is apparently quite important, both in increasing the plant's rate of growth and its response to 2,4-D. The mechanism of this action is not yet clear, although it may be due to an interference with the plant's metabolism. Further experiments are under way to investigate this possibility.

Kelly's work (Kelly, 1949) left unexplained the causes for the effects of 2,4-D on bean plants transferred from 5° to 15°C, even though plants left at 15°C exhibited no symptoms. Our work suggests a possible explanation; i.e., the plant failed to grow at 5°C or 15°C due to lack of certain metabolites. Metabolites limiting at 5°C are different from those at 15°C. Whereas the plant can make few or none of these metabolites at 5°C, it can make some at 15°C. Growth at 15°C would originally be more prolonged than at 5°C, until certain metabolites, e.g., thiamin, become limiting. Plants at 5°C would have a more limited growth until a different unknown metabolite became limiting, leaving a small supply of thiamin. Transfer to 15°C may have permitted production of the unknown but not of thiamin, and growth re-

sumed until thiamin became limiting. Thus, a more marked effect of 2,4-D would be observed after transferring plants from 5°C to 15°C.

It seems unlikely that the slower entry and translocation at low temperatures alone is sufficient to account for the failure of *Amsinckia* to respond to 2,4-D treatments at the low temperatures. These results suggest that the lack of response of the plant to 2,4-D at low temperature is caused by a temperature-induced shortage of thiamin.

References

Audus, L. J. 1959. *Plant Growth Substances,* 2nd ed. London : L. Hill.

Bonner, J. 1942. Transport of thiamin in the tomato plant. Amer. Jour. Botany, *29:*136-142.

Bonner, J., and H. Bonner. 1948. The B vitamins as plant hormones. Vitamins and Hormones, *6:*225-270.

Currier, H. B., and C. D. Dybing. 1959. Foliar penetration of herbicides—review and present status. Weeds, *7:*195-213.

Johanson, N. G., and T. J. Muzik. 1961. Some effects of 2,4-D on wheat yield and root growth. Botan. Gaz., *122:*188-194.

Kelly, S. 1949. The effect of temperature on the susceptibility of plants to 2,4-D. Plant Physiol., *24:*534-536.

Ketellapper, H. J. 1963. Temperature-induced chemical defects in higher plants. Plant Physiol., *38:*175-179.

Mitchell, J. W., W. M. Duggar, Jr., and H. G. Gaunch. 1953. Increased translocation of plant-growth-modifying substances due to application of boron. Science, *118:*354-355.

Petinov, N. S., and Y. G. Molotkovsky, 1957. Protective reactions in heat-resistant plants induced by high temperatures. Fiziologiyo Rasteny (Plant Physiology), *4:*221-225.

The Influence of Soils, Moisture, and Fertility on the Response of Plants to Foliar-Applied Herbicides

D. C. TINGEY, L. C. ERICKSON, and H. P. CORDS

Following the discovery of the value of 2,4-D as a herbicide, Mitchell and Brown (1946) reported that when this herbicide was applied to leaves of bean plants the translocation of the resulting stimulus was closely associated with the translocation of organic food materials. From this, one might conclude that any environmental factors, such as temperature, light, water, and nutrients, that influence translocation of organic food materials would probably influence translocation of 2,4-D and other systemic herbicides. Thus the response of plants to a herbicide may be quite different when plants are grown under different environmental conditions. Results from the use of 2,4-D and similar herbicides applied to the foliage of creeping perennials has been highly variable both in experimental plots and in field applications. Some of this variation may be due to environmental factors. If such is the case, it would be valuable to have this knowledge so that recommendations could be made for more effective use of such herbicides.

Review of Literature

Soil type

Mitchell and Brown (1946) reported that annual morning glory plants grown under soil conditions favorable for rapid growth and others grown under soil conditions where the plants were relatively dormant gave a similar response when treated with 2,4-D. Three weeks after herbicide application, the vegetative response, the number of dead plants, and the depletion of the carbohydrates were the same in the two groups of plants.

Fisher, Meadors, and Behrens (1956) concluded from a three-year study in Texas that mesquite (*Prosopis juliflora*) was more susceptible to silvex when growing on sandy loams or deep sandy soils than when growing on clay loam soils. The root kill on sandy soil varied from 30 to 97%, whereas on heavy clay it varied from 0 to 40%. Other factors—notably soil moisture, root systems, depth of sprouting tissue, and fertility—also may have influenced the results.

Moisture

Erickson, Seely, and Whitman (1948) concluded from their work on 2,4-D that this herbicide was more toxic to Canada thistle, field bindweed, and white top when adequate soil moisture prevailed.

Anderson and Shadbolt (1949) obtained no difference in the yields of two varieties of sweet corn or in the control of erect red root (*Amaranthus retroflexus*) with 2,4-D on irrigated and nonirrigated plots. They reported more lodging in the irrigated plots, a delay in ripening of the corn of about three to five days for either 2,4-D or irrigation, and a five- to seven-day delay in maturity where the two were combined.

Cox (1952) reported the effect of soil moisture on the response of velvet mesquite (*Prosopis juliflora* var. *velutina*) in Arizona to phenoxy herbicides. Water was added to keep the soil moist to a depth of 48 inches in small plots enclosed by dikes within large sprayed areas, for periods of $3\frac{1}{2}$ to 5 weeks before applying the herbicides. The mesquite responded to the added moisture by more rapid terminal growth. Some differences were observed in the watered and unwatered plants soon after the herbicides were applied, but observations made 12 to 18 months following treatment led Cox to conclude that soil moisture did not have a significant effect on the long-term toxicity of phenoxy herbicides. Fisher, Meadors, and Behrens (1956) reported from observations on field plots in Texas that soil moisture seemed to be a factor influencing the response of mesquite (*Prosopis juliflora*) to silvex.

Hyder, Sneva, and Freed (1962) reported that when soil moisture dropped below 40% available at the 6-inch depth, control of big sage (*Artemesia tridentata*) with 2,4-D was less certain. With green rabbit brush (*Chrysothamnus viscideflorus*), the soil moisture had to be below 30% available in the upper 12 inches when spraying with 2,4-D in order to obtain effective control. They stated that soil moisture as an index of plant susceptibility was not as definite as the stage of growth of the plant.

Nitrogen

McKay *et al.* (1959) reported a five-year study on the control of Canada thistle in spring wheat, which included a combination treatment of fertilization and applications of 2,4-D. Annual additions of 80 pounds of nitrogen to spring wheat, together with spraying with 2,4-D, hastened the reduction of the thistle stand and increased the yield of wheat over that from nonfertilized plots. Nitrogen applied alone reduced the Canada thistle by an average of 18%, and 2,4-D alone reduced the thistle stand by 51%. The sum of the two individual

effects amounted to 69%. When 2,4-D and nitrogen were both applied, the thistle stand was reduced by 83%. Nitrogen may have acted by enhancing the translocation of 2,4-D in the thistles and thereby increasing the subsequent kill. However, the effect may have been due to competition by the more vigorous wheat, since wheat yields were increased by 16 bushels per acre.

Nitrogen fertilizer in the form of ammonium nitrate, applied well ahead of the application of 2,4-D for the control of pheasanteye (*Adonis annua* L.) in the winter wheat, seemed to cause the wheat to be more susceptible to 2,4-D in the preboot and early boot stages, but not in the 1 to 5 tiller stage. The ester formulation of 2,4-D at rates of 1, 2, and 4 pounds, as compared with the amine form, applied at preboot and early boot stages, resulted in substantial reductions in wheat yields. Nitrogen fertilizer did not affect the response of pheasanteye to 2,4-D (Tingey, 1963). Downs (1952) reported that nitrogen-fertilized wheat infested with pheasanteye was more susceptible to 2,4-D than was the unfertilized wheat and was injured relatively more at higher rates. The reaction of pheasanteye to 2,4-D was not affected by the level of nitrogen fertilization.

In contrast to these findings, Rasmussen (1954) found that nitrogen applied at the same time as 2,4-D did not alter the response of wheat to the herbicide.

Phosphorus

Rohrbaugh and Rice (1956) found that tomato plants deficient in phosphorus were more tolerant than control plants to 2,4-D, but that when phosphorus was applied at the time of 2,4-D treatment or eight hours or three days prior to the application of the herbicide, the plants became more susceptible. This experiment may not indicate that phosphorus has any specific role in the toxicity of 2,4-D. Perhaps a deficiency of any factor essential to photosynthesis or translocation may influence the toxic potential of 2,4-D. Linder, Brown, and Mitchell (1949) and Rhodes, Templeman, and Thruston (1950) have shown that when deficient factors are supplied, herbicide toxicity is increased.

Studies on Canada Thistle and Field Bindweed (Utah)

Experimental procedure

Canada thistle (*Cirsium arvense* (L.) Scop.) plants were propagated asexually and grown in either soil or vermiculite placed in galvanized cans 7 inches in diameter and 20 inches deep. While in the field, each can was placed inside an irrigation pipe which was embedded upright in the soil to a depth of 18 inches. This was done to

keep the plants from becoming overheated in hot weather. Field bindweed (*Convolvulus arvensis* L.) was grown only in soil.

The following environmental factors were investigated in one of the experiments on Canada thistle and in the experiment on field bindweed.

Soil types. (a) Milville loam, a highly calcareous soil; (b) Nibley silty clay loam, a noncalcareous soil; (c) Warm Springs fine sandy loam; and (d) Ackman loam.

Fertilization. The fertilized plants received farm manure at 20 tons per acre, nitrogen at 200 pounds per acre, and phosphorus (P_2O_5) at 200 pounds per acre. The fertilizers were mixed with the upper 8 inches of soil at planting. The following spring a retreatment with nitrogen and phosphorus was made at the same rate.

Irrigation. Three regimes were followed:

1. The soil was kept moist prior to and following the application of the herbicides.

2. The soil was allowed to become dry prior to the application of herbicide so that some of the plants were wilted at the time of application. The soil was kept dry during the top die-down period until new top growth was observed in plants treated as in the first regime. Irrigation was then commenced and continued until the experiment terminated.

3. Treatment was the same as in the second regime except that the soil was watered immediately after the application of the herbicides and was kept moist.

Herbicides used were 2,4-D and amitrole in the case of Canada thistle and 2,4-D and PBA in the case of field bindweed. They were applied in August. Unsprayed plants were left as controls. Green and dry weights of roots and tops were measured on both Canada thistle and field bindweed controls at the time the herbicides were applied.

Three replications of each treatment were used. In some cases, fewer than three replications were made because the plants died before herbicide application. In making the calculations for variance analysis, however, the average of the replications for each treatment was used and the error was based on the second and higher-order interactions.

In another experiment on Canada thistle, the plants were grown in vermiculite. Root rot developed and many of the plants died. The experiment was modified, and the only environmental variable employed was fertilizer treatment involving high and low nitrogen and phosphorus. In most cases there were enough good plants for three or four replications. In a few cases there were only enough for two replications.

Plants in both experiments were approximately 14 months old when the herbicides were applied. In the first experiment, results were based on data taken approximately one year after treatment; in the second experiment, results were based on data taken approximately four months after application of herbicide, since most of the plants had died during that time.

Results and discussion

Two explanations are in order.

As indicated by the title of this chapter, the principal objectives of the experiments in Utah were to determine to what extent soils, moisture, and fertility influenced the response of Canada thistle and field bindweed to certain foliar-applied herbicides. To determine if these environmental factors have any effect on the response of plants to herbicides, it was necessary to resort to a consideration of the interactions of soil type x herbicide (including a control), soil moisture x herbicide (including a control), and fertilization x herbicide (including a control). These interactions are a measure of the effect of soils, irrigation, and fertilizer on the response of the plants to herbicides. We were less concerned with the main effects, such as soil, irrigation, fertilization, and herbicides, except to know that these factors had caused differences in plant response, as we expected these factors to have an effect on plants. We also were interested to determine the consistency of the interaction as determined by the various methods used in measuring experimental results, such as: (1) percentage of plants killed; (2) percentage of plants not recovered (live roots, but no top regrowth); (3) dry weight of roots; (4) percentage dry matter in roots; and (5) dry weight of tops.

In field experiments the conventional method of expressing the effect of herbicides on the control of creeping perennials is "percentage of plants killed." This usually is arrived at in one of two ways: (1) an estimate is made of the percentage of the treated plot free of weeds, or (2) an estimate is made of the amount of weed growth, which is then expressed as a percentage of the control level and subtracted from 100. (Whenever possible, density of growth at the time the herbicides were applied should serve as the basis of the amount killed.)

Both methods are based on the amount of top growth left on the plots. Top growth can, however, be a very poor index of the amount of live roots left in the soil. We have, therefore, used the five tests listed above to measure the efficacy of herbicide action.

Response of Canada thistle to 2,4-D and amitrole (experiments in soil). *Plants killed.* Amitrole killed 22% of the plants and

Table 1. Interaction of Irrigation and Herbicide Action on Canada Thistle[1]

Herbicide	Irrigation treatments[2]		
	1	2	3
None	0	0	0
2,4-D	0	6	6
Amitrole	73	6	77
LSD at 5% level	17.2	17.2	17.2

[1] Results are expressed as percentage of plants not recovered (live roots but no top regrowth). Observations were made one year after treatment.

[2] Treatment 1: plants in moist soil throughout the experiment; treatment 2: plants in dry soil when herbicide applied and remained dry during die-down period; treatment 3: plants in dry soil when herbicide applied but wet during die-down period.

2,4-D killed 6%. Changes in soil, irrigation, or fertilization made singly or in combination did not alter these figures.

Plants not recovered (no top growth, live roots). Of the variables studied, only herbicides, irrigation, and their interaction (herbicides x irrigation) had any significant effects. As shown in Table 1, the percentage of plants failing to make any top regrowth was greater after treatment with amitrole than after 2,4-D.

In addition, plants responded to amitrole in a manner dependent upon their irrigation treatment. Plants treated with amitrole under dry conditions and subsequently kept dry recovered sooner than plants treated under more moist conditions. It appeared that the response to amitrole was dependent primarily upon the moisture content of the soil following the application of the herbicide. No comparable effect was noted for 2,4-D.

Root weight. Neither soil type, irrigation, nor fertilization had any measurable effect on the dry weight of roots harvested just prior to herbicide application. However, one year after the herbicides were

Table 2. Interaction of Irrigation and Herbicide Action on Canada Thistle[1]

Herbicide	Root dry weight (grams)			Relative root growth (percent of weight at time of application)		
	Irrigation			Irrigation		
	1	2	3	1	2	3
None	25.5	22.8	25.9	162	145	165
2,4-D	13.3	12.1	17.4	85	77	110
Amitrole	5.3	11.6	5.6	34	74	36
LSD at 5% level	5.8	5.8	5.8	------	------	------

[1] Results are expressed on the basis of root dry weight one year after treatment.

applied, significant interactions were noted with irrigation and fertilizers.

The herbicide x irrigation interaction, based on root weight, is illustrated in Table 2. It was very similar to that based on the percentage of damaged plants. Plants receiving amitrole under dry conditions (irrigation regime 2) had more roots than plants kept under more moist conditions. This effect was not seen in the control plants or in plants treated with 2,4-D.

Table 3 contains the results indicative of a herbicide x fertilizer interaction. Root weights of fertilized control plants were higher than those of nonfertilized plants, but following treatment with 2,4-D or amitrole the situation was reversed. It appeared that the fertilized plants were more susceptible to root injury than were their unfertilized counterparts.

Table 3. INTERACTION OF FERTILIZATION AND HERBICIDE ACTION ON CANADA THISTLE[1]

Herbicide	Dry weight of roots (grams)			Relative root growth (percent of weight at time of application)	
	Fertilized	Unfert.	Difference	Fertilized	Unfert.
None	27.7	21.7	6.0	176	138
2,4-D	12.0	16.5	–4.5	76	105
Amitrole	6.5	8.5	–2.0	41	54
LSD at 5% level ..	4.8	4.8	6.8	-------	-------

[1] Results are expressed on the basis of root dry weight one year after application.

The weight changes also have been expressed as the percentage change from the original weight of the root (Tables 2 and 3). These figures illustrate further the herbicide x irrigation and herbicide x fertilizer interactions.

Response of Canada thistle to 2,4-D and amitrole (experiments in vermiculite-nutrient culture). The following year a second experiment was performed with two strains of Canada thistle grown in vermiculite-nutrient culture. The influence of nitrogen and phosphorus on the response of the two strains to 2,4-D and amitrole was investigated. Previous tests had indicated that the strains differed in their resistance to amitrole under field conditions.

Plants killed. A high percentage of the plants treated either with 2,4-D or amitrole were dead in four months. High and low nitrogen and phosphorus did not appear to have any effect on the kill and, furthermore, no difference in the effectiveness of 2,4-D or amitrole

was evident. The two strains did not appear to differ in their resistance to the two herbicides under nutrient culture conditions.

Root weight. The small number of plants surviving 2,4-D or amitrole treatment had few roots. The overall effect, as measured by the root data, was similar to that observed on the basis of plants killed. Little difference was noted between the two strains or between plants fertilized with nitrogen and phosphorus and unfertilized plants. Although the nitrogen and phosphorus were at different levels, the control plants showed no differences in root weights. This and the high percentage of plants killed by the herbicides probably obscured any influence the nitrogen and phosphorus might have had on the plant response to the herbicide.

Response of field bindweed to 2,4-D and PBA. The five criteria described earlier were used to evaluate the results obtained in the experiment on field bindweed.

Plants killed. Only 6% of the plants treated with 2,4-D and 12% of those treated with PBA were killed. Soils, irrigation, and fertilization did not alter these figures significantly.

Plants not recovered (live roots but no top regrowth). Herbicides and the interaction of soil x herbicides were the only significant factors in this comparison. Twenty-two percent of the PBA-treated plants and 9% of the 2,4-D-treated plants had not recovered by the end of the year. A significant herbicide x soil interaction was observed, since the toxicities of 2,4-D and PBA varied with the soil type (Table 4).

PBA was more toxic than 2,4-D on three of the soils tested, but on the Nibley silty clay loam it was considerably less toxic.

Table 4. THE EFFECTS OF SOIL AND HERBICIDES UPON FIELD BINDWEED REGROWTH

Soil	Percent of plants killed		
	TBA	2,4-D	Control
	%	%	%
Milville loam	0	6	42
Nibley silty clay loam	0	25	0
Sandy loam	0	0	0
Ackman loam	0	6	22
AVERAGE	0	9	22

Root weight. Herbicides, soils, and fertilizers all produced effects significant at the 1% level. Irrigation produced an effect significant at the 5% level. However, no single interaction was noted between any

environmental factor and herbicides. The roots of control plants averaged 24.0 grams; of 2,4-D-treated plants, 30.4 grams; and of PBA-treated plants, 17.7 grams.

Percentage dry weight of roots. While herbicide treatment resulted in a significant difference in the percentage of root dry matter in plants, no differential effects due to the environmental factors were observed. The amount of dry matter in roots of controls was 21%; in roots of 2,4-D treated plants, 29.3%; and in roots of PBA-treated plants, 25.5%.

Top weight. Both herbicide and irrigation treatment influenced the dry weights of the tops, but again no interaction was found between the herbicides and any of the environmental factors. The average weight of tops for the controls was 14.6 grams, for the 2,4-D-treated plants 7.5 grams, and for the PBA-treated plants 5 grams. The differences between the control and treated plants were significant at the 1% level.

Studies on Perennial Groundcherry (Idaho)

Experimental procedure

The interacting effects of nitrogen, two soil-moisture regimes, and the herbicides amitrole, 2,4,5-T amine, and 2,4,5-T LVE on the control of perennial groundcherry (*Physalis longifolia* var. *subglabrate*) were investigated. A depleted, 13-year-old, Kentucky bluegrass, irrigated pasture in southwestern Idaho was selected for this study. It was severely infested with groundcherry and had been overgrazed by sheep. The area was divided into two blocks, one irrigated at 10-day (normal) intervals and the other at 20-day intervals. Nitrogen, phosphorus, and the herbicides were applied in the fall, and the data are based on percentage regrowth the following year.

Results and discussion

Effects of soil nitrogen and moisture. Nitrogen alone had no significant effect on the quantity of regrowth. However, the combined effects of nitrogen and the herbicides were significant in the suppression of regrowth in the high-moisture plots but not in the low-moisture plots. The differences in toxicity between the herbicides and the influence of the soil moisture regimes were significant at the 1% level. The data from this experiment are recorded in Table 5.

Effects of soil phosphorus and moisture. No significant interaction was observed between the soil phosphorus and the actions of the herbicides, nor were there significant increases in toxicity when the soil moisture content was increased. The data are recorded in Table 6.

Table 5. THE EFFECTS OF NITROGEN AND TWO SOIL-MOISTURE REGIMES ON THE TOXICITY OF THREE HERBICIDES TO PERENNIAL GROUNDCHERRY

Treatment (pounds per acre)	Regrowth (percentage of original stand)	
	Normal irrigation	Half-normal irrigation
	%	%
Control	387	261
N (75 lbs.)	326	180
N (150 lbs.)	443	231
N (75 lbs.) plus amitrole (2 lbs.)	46	87
N (75 lbs.) plus 2,4,5-T LVE (2 lbs.)	51	109
N (75 lbs.) plus 2,4-5-T amine (2 lbs.)	139	194
Amitrole (2 lbs.)	50	81
2,4,5-T amine (2 lbs.)	172	142
2,4,5-T LVE (2 lbs.)	66	116

Table 6. EFFECTS OF HERBICIDES, PHOSPHORUS, AND TWO SOIL-MOISTURE REGIMES ON THE REGROWTH OF PERENNIAL GROUNDCHERRY[1]

Treatment (pounds per acre)	Regrowth (percentage of original stand)	
	Normal irrigation	Half-normal irrigation
	%	%
Control	387	261
P_2O_5 (75 lbs.)	207	223
P_2O_5 (150 lbs.)	263	265
P_2O_5 (75 lbs.) plus amitrole (2 lbs.)	94	96
P_2O_5 (75 lbs.) plus 2,4,5-T amine (2 lbs.)	165	144
P_2O_5 (75 lbs.) plus 2,4,5-T LVE (2 lbs.)	74	38
Amitrole (2 lbs.)	50	81
2,4,5-T amine (2 lbs.)	174	144
2,4,5-T LVE (2 lbs.)	66	116

[1] Measurements made one year after treatment.

Neither nitrogen nor phosphorus deficiencies appeared to exist for groundcherry growth. The area was grazed almost continuously, and no above-ground competition was provided by the bluegrass since it rarely exceeded 2 inches in height. The data therefore suggest these possibilities: (1) Nitrogen was present in such ample quantities that the additions were of no significance; (2) interactions of nitrogen and herbicide combinations are not uniform in their phytotoxic effect; (3) the bluegrass, because of the excessive grazing, could not express competitive effects *or* it is a poor competitor with groundcherry; (4) the one-year duration of the experiment was too short to obtain full

expression of the treatment differences; and (5) groundcherry and other perennial weeds may feed so deeply that the effects of fertilizer applications are only slowly discernible.

Studies on Canada Thistle (Idaho)

Experimental procedure

In January 1964, root segments (3 inches long and bearing shoots) of Canada thistle [*Cirsium arvense* (L.) Scop.] propagated from a single clone were planted in 6 x 6 x 24 inch galvanized containers filled with 26.6 pounds of oven-dry, virgin, nutrient-deficient, McAvoy sandy loam soil. Water was then added (determined by the soil water-holding capacity), bringing the soil in all containers to field capacity. Thereafter the containers were weighed two or three times weekly to determine moisture utilization and to return each to field capacity. Because of poor growth rates, 50 pounds of nitrogen (as commercial NH_4NO_3) per acre were added in February.

In April all top growth was removed to establish a more uniform growth-rate base. The containers were then numbered and treated with 100 pounds nitrogen or 50 pounds phosphorus equivalent per acre. The latter was applied as commercial triple superphosphate. One month later the top growth was removed, oven dried, and weighed to determine plant-growth and moisture utilization ratios as influenced by nitrogen and phosphorus levels.

To establish two temperature regimes, one-half of the samples (four replications of each treatment) were moved to the field on July 3. The other half remained in the greenhouse. Simultaneously, two moisture regimes were established in both the greenhouse and in the field: (a) continuing at field capacity, and (b) at one-half field capacity.

On July 10, herbicides were applied at rates equivalent to $\frac{1}{2}$ and $\frac{3}{4}$ pound 2,4-D amine per acre to all the samples except the nontreated controls.

The plants remaining in the greenhouse were moved to the field on July 20 because of the uncontrolled high greenhouse temperatures. This terminated the two-temperature treatment intervals. The moisture levels were maintained until the study was terminated on August 20. To obtain root weights, the plants were removed from the cans and the roots washed free from all soil and then dried at 54°C.

The variables in this study, designed to determine the influence of environmental factors on the growth of Canada thistle and its susceptibility to 2,4-D, were then: moisture, phosphorus level, nitrogen level, temperature, and herbicide level.

Results and discussion

The mean temperatures for the two environments during the period of July 3 to July 20 were: in the greenhouse, 37.5°C; in the field, 18.5°C.

A statistical analysis made on the top-growth water utilization ratio showed no significant difference. However, an analysis of variance made on the root dry weights obtained at the termination of the study revealed some significant effects. Those results which showed differences significant at the 1% level are summarized in Tables 7 and 8.

Table 7. Root Growth of Canada Thistle Under Various Environmental Conditions

Condition	Root growth (grams)
High nitrogen	804
Low nitrogen	713
High phosphorus	720
Low phosphorus	797
High nitrogen—low phosphorus	435
High nitrogen—high phosphorus	368
Low nitrogen—low phosphorus	361
Low nitrogen—high phosphorus	352
High temperature	697
Low temperature	819

Table 8. Effects of Nitrogen, Phosphorus, and Temperature Upon Root Damage Caused by 2,4-D

Treatment	Root weights (grams)					
	High nitrogen	Low nitrogen	High phosphorus	Low phosphorus	High temperature	Low temperature
High 2,4-D	190	199	195	184	165	215
Low 2,4-D	251	214	223	243	195	270
Control	372	300	302	370	337	335

It appeared that maximum root growth occurred under conditions of high nitrogen alone, low phosphorus alone, or the combination of high nitrogen with low phosphorus. Root growth was also greater at the lower temperature.

The greatest root damage, measured by reduction in dry weight, occurred under conditions of high nitrogen, low phosphorus, or high temperature.

Summary

Although it is evident that some variables still remain to be controlled, several interactions between herbicides and environmental factors have been demonstrated. Thus, in both the Utah and Idaho studies, 2,4-D caused significantly more root damage under conditions of high nitrogen. The Idaho studies suggested that low phosphorus and high temperature will enhance root damage by this herbicide. The studies with amitrole on Canada thistle and silvex on perennial groundcherry also seem to indicate that a reasonable level of moisture must be present to ensure maximum herbicide effect.

Further studies are desirable in order to identify other variables which may interact with the herbicide to enhance or depress its toxicity.

References

Anderson, E. T., and C. A. Shadbolt. 1949. The effect of post-emergence treatments of 2,4-D on non-irrigated and irrigated sweet corn. No. Cent. Weed Control Conf. Res. Prog. Report, 6:128.

Cox, D. K. 1952. Effect of soil moisture on reaction of velvet mesquite to topical application of selective hormone type herbicides. Western Weed Control Conf. Res. Prog. Report, 13:50.

Downs, R. J. 1952. Control of pheasanteye as it affects yields and other factors in winter wheat. M.S. thesis, Utah State Univ., 37 pp.

Erickson, L. C., C. I. Seely, and E. W. Whitman. 1948. Using 2,4-D for selective and non-selective weed control. Idaho Agric. Ext. Bull. 172.

Fisher, C. E., C. H. Meadors, and R. Behrens. 1956. Some factors that influence the effectiveness of 2,4,5-trichlorophenoxyacetic acid in killing mesquite. Weeds, 4:139-147.

Hyder, D. N., F. A. Sneva, and V. H. Freed. 1962. Susceptibility of big sagebrush and green rabbitbrush to 2,4-D as related to certain environmental, phenological, and physiological conditions. Weeds, 10:288.

Linder, P. J., J. W. Brown, and J. W. Mitchell. 1949. Movement of externally applied phenoxy compounds in bean plants in relation to conditions favoring carbohydrate translocation. Botan. Gaz., 110:628-632.

McKay, C., G. Ames, J. M. Hodgson, and L. C. Erickson. 1959. Control Canada thistle for greater profits. Idaho Agric. Expt. Sta. Bull. 321.

Mitchell, J. W., and J. W. Brown. 1946. Movement of 2,4-dichlorophenoxyacetic acid stimulus and its relationship to the translocation of organic food materials in plants. Botan. Gaz., 107:393-407.

Rasmussen, L. W. 1954. The effect of 2,4-D and urea combination sprays on weed control and yield of winter wheat. Western Weed Control Conf. Res. Prog. Report, *15:*69-70.

Rhodes, A., W. G. Templeman, and M. N. Thruston. 1950. The effect of the plant growth regulator 4-chloro-2-methylphenoxyacetic acid on the mineral and nitrogen contents of plants. Ann. Botany, *14:*181-188.

Rohrbaugh, L. M., and E. L. Rice. 1956. Relation of phosphorus nutrition to the translocation of 2,4-dichlorophenoxyacetic acid in tomato plants. Plant Physiol., *31:*196-199.

Tingey, D. C. 1963. Yield of dryland winter wheat treated with 2,4-D. Utah State Univ. Agric. Expt. Sta. Bull. 441, pp. 1-12.

CHAPTER FOUR

Plant Variation and Herbicides

R. F. Wagle and L. O. Baker

The regional research thus far described in this bulletin has dealt primarily with interactions between herbicides and plant physiological-ecological systems. The results to be delineated here were obtained as a consequence of the introduction of the factor of plant variation into the overall project.

Certain plant-herbicide responses are explained on the basis of plant variation and adduced for the involvement of herbicides as factors in genetic selection.

Definition of Terms

Certain genetic terms are used in the next two sections of this chapter which may need some definition for a better understanding of the concepts involved. The following terms are therefore given and will have no meaning beyond those stated:

Phenotype. This refers to the appearance of an individual plant and is determined by the interaction of the genotype with the environment.

Genotype. This is the heredity constitution of the individual.

Ecotype. This is an adaptive race and refers to the fact that a species is made up of genetic races attuned to the habitat.

Ecocline. This refers to morphological or physiological gradients that often appear in plants which are associated with environmental gradients. Variations between plants at each end of the gradient may often result in their being classed as ecotypes or even species, when in fact a complete sampling would show the continuity in the variation.

Selection. This refers to the environmental forces responsible for the evolution and variation in living organisms.

Plant Variation

The experiments conducted under this project dealt with the following three kinds of plant variability:

Relation to age or maturity

Plants of the same or similar genetic constitution (genotypes) may exhibit structural and physiological differences at different stages of development that make them more or less susceptible to herbicides. An ecological principle that seems to best illustrate this type of variability is: There are times in the life history of an organism when it is in some critical phase of its development which has a narrow tolerance range for some particular factor of the environment. The narrower the range of tolerance, the more critical the factor becomes. The basic problem in utilizing this form of variability in plant control is to identify those critical phases of plant development having the narrowest tolerance ranges for particular herbicides in plants of similar genotype.

Relation to the environment

Plants of the same or similar genetic constituents may exhibit structural and physiological differences under different conditions of environment. These plants, too, may be more or less susceptible to different herbicides, depending on the environmental conditions prior to, during, or immediately after herbicidal treatment. An ecological principle relevant to this fact states that: In any area, a given phenotype is an adjustment to the soil conditions and to the diurnal and seasonal rhythms of climate. The phenotype is the result of environmental stimuli acting on genotype. Different stimuli acting on the same genotype may give different phenotypes. The basic problem in utilizing this form of variability for the selective control of plant species would be to relate the response of plants with similar genotypes to particular herbicides in different environments.

Relation to the genotype

Here we have variability that represents true genetic differences between individual plants. At least two ecological principles are basic to our understanding of the role played by genetic variability as it affects the response of a plant species to herbicides. The first of these principles involves genetic potential: The capacity of a plant or of a species to respond to its environment is a direct result of its genetic diversity. The second principle is concerned with evolution. Perpetuation of vegetation is dependent first on the ability of a species to migrate and second on the ability of a species to vary and to meet a diverse environment with genetic change. Many basic problems are inherent in utilizing this form of variability for the selective control of plant species. Among these are:

1. Controlling and/or identifying complexities introduced by the other two forms of variability.

2. Isolating chemical resistance or defining plant tolerances in relation to specific chemicals as a genetic characteristic.

3. Identifying specific physiological or morphological differences in plants and relating them to specific chemical or herbicidal effects.

4. Identifying species changes due to the influence of chemically changed environments.

In general, genetic variability will be emphasized in this section together with "selective herbicides." Genetic variability is stressed because the greatest differences in physiological function between plants are inherent in the plants themselves. Also, genetic variability is probably one of the most important causes for failure in the selective control of "herbicide susceptible" plant species. Selective herbicides are emphasized because they are designed to exploit morphological, physiological, or age and maturity differences in plants. Also, use of selective herbicides seems to be increasing more rapidly than use of the nonselective type.

Plant variation is the basis of all "selective" herbicidal work. Because the life constants of plants are accomplished with a remarkable degree of uniformity, we must assume that all plants exhibiting these functions have similar basic environmental requirements. Thus plant differences, or variations in plant populations, must be due to variations in needs which are attuned to variations in supply. Selection of individual plants by the environment has acted to modify or change the genetic capacity of plants to maintain these life constants under different extremes or conditions of environmental supply. Stebbins (1950) states that several systematists and biologists seem to agree that "Species must consist of systems of populations that are separated from each other by complete or at least sharp discontinuities in the variation pattern, and that these discontinuities must have a genetic basis."

Herbicides as Environmental Factors

Plant variations have originated in different habitats as the result of selection operating through different amounts, or supplies, of the factors constituting the environment. Thus we find that plants as a result of environmental selection may need less of some essential element for the life processes. Alternatively, they may develop more efficient organs and physiological processes for extracting and/or utilizing certain essential elements. They may develop modified or

different organs and physiological mechanisms for carrying on the life processes. Or, finally, they may develop different means of storing or conserving certain elements essential to life.

The variations existing between plants as the result of environmental selection, then, form the starting point for the development of selective herbicides. A herbicide to be selective must be designed or used as a factor of the environment which is specific for some form or forms of plant variation as described earlier.

Selective forces

The selective forces naturally operating on plants can usually be included under three classes:

Climatic factors which exert a primary form of control over plant form and distribution.

Soil factors which act as a secondary control on plant distribution and operate wholly within local climatic conditions.

Biotic factors which operate to control plant distribution within the conditions imposed by climate and soil.

Among the several biotic factors, man is considered to be the most powerful selective force in relation to herbicides. As the biotic agent responsible for the development and application of herbicides, man has added a new force to his already potent selective influence on plant distribution and evolution. However, this selective force is more or less local and operates as a limiting factor on plant distribution within the environmental boundaries already imposed on the vegetation by climate and soil. Several of the studies made by the W-63 regional research group also have indicated climate and soil as factors influencing or controlling the selective action of herbicides (Freed, 1960, 1961, 1962; Baker, 1960, 1961, 1962; Wagle, 1960, 1961, 1962).

Selective herbicides

Selective herbicides, as defined by Crafts (1961), Klingman (1961), and many others working in the area of weed control, seem to imply a genetic basis and utilize the principle that: Species differ in their physiological capacities to tolerate environmental extremes. Their selective action may depend upon a single small morphological or physiological difference between two species, or it may operate by affecting one or more of the life processes through a series of complex differences involving cell wall structure; cutinization of epidermal surfaces; presence, absence, or number of stomatal openings; presence or absence of leaf emergences; and internal physiology.

One aspect of plant variation that herbicidal studies have more or less neglected is the variation that exists within species. Two texts

in the field of herbicides (Crafts, 1961; and Klingman, 1961) recognize that the selectivity of a herbicide is dependent on variations between plants in their physiological processes and morphology. However, they fail to mention that physiological and morphological variations within a species may be as great as those occurring between species. Stebbins (1950) states, in answering the question as to whether the characteristics of external morphology and physiology which distinguish species are different in kind from those between different races or subspecies, or whether they only differ in degree, that: "The differences between closely related species are nearly always duplicated or paralleled by the differences betweten the races or subspecies of a single species. In many species the traits which characterize genera or even higher categories can be found to vary within a species." Studies made by Alley (1960, 1961, 1962), Baker (1960, 1961, 1962), Whitworth (1960, 1961, 1962), and Hodgson (1964) seem to confirm this statement.

To emphasize the need for studying the selective effects of herbicides within species as well as between species, it might be worthwhile to restate the genetic principle implied by Crafts and Klingman to include variation within species. Thus, we might say that the use of selective herbicides is based on the principle that: Species or different genotypes within a species differ in their physiological capacities to tolerate environmental extremes. Many studies have shown that different species or different genotypes within a species usually show growth variation under a given environment (Wagle, 1958; Turesson, 1922; Arneklev, 1963). Also, many studies have shown that different species or different genotypes within a species exhibit different physiological capacities for tolerating environmental extremes. These tolerances may further vary in the same plant with age, stage of growth, or season of growth (Turesson, 1922, 1930, 1936; Clausen, Keck, and Hiesey, 1940; Heslop-Harrison, 1953; Wagle, 1958, 1962; Baker, 1960, 1961, 1962; Muzik, 1961; Whitworth, 1960, 1961, 1962). Cain (1944) sums up the problem of variation and a basis for the selective control of species with herbicides in two statements: "(1) Species are not genetically homogenous populations and consequently they are composed of individuals which vary in morphological and physiological characteristics within definable limits. (2) All vegetation types, being products of environment, are subject to evolution, migration, or extinction under the compulsion of environmental change."

Another significant aspect of herbicidal influence that is missed by neglecting the genetic viewpoint is the effect of herbicides on plant evolution. When herbicides are introduced into an environment, they immediately become a factor of that environment capable of exerting

an extremely strong selective influence. A herbicide may remove a single or several entire species populations from the area into which it is introduced, or its effect on some species may be only partial, ranging from slight to severe. Where an entire undesirable species is removed from a given treated area, we might say that the selective herbicide has admirably fulfilled its purpose. One of the functions governing the existence and successful reproduction of this plant species has been interrupted by a chemical introduction to the environment which has limited the area the species could occupy. On the other hand, where only partial control is achieved, we first have to decide whether the results were due to some mistake in application, or were due to plant variations which could be classed as genotypic variation meeting ecologic variation (the introduction of the herbicide as a factor of environment) resulting in the selection of favorable ecotypes which may spread into future treated areas. If the latter is the case, the chemical introduced is probably exerting a very strong selective influence that would favor those plants physiologically resistant to it. Subsequent migration and density increases of these "resistant" plants by their propagules with an accompanying removal of the nonresistant plants by the herbicide could result in the development of a herbicide-resistant race or species. Fortunately, each chemically different herbicide constitutes a different environmental factor. Also, the effect of these chemical factors, when added to the environment, may be more or less critical at different seasons of the year, or during different phases of the life cycle, or in different segments of the area, depending on soil or other site differences.

Knowledge of the variations in the pattern of growth in a plant species and of the conditions of highest susceptibility to given herbicides increases opportunities for controlling the area of the species, but it does not eliminate the evolutionary significance of herbicides as agents of the environment. Continued selective control of some undesirable plant species, under many situations, may depend on the continuing development of new chemicals and our knowledge of chemical resistance and its genetic and physiological basis in the plant.

Methods and Procedures

Only those methods and procedures important to an understanding of the results will be mentioned, and they will be covered along with the studies discussed. Detailed descriptions of methods and procedures for each study are available in the study plans and annual reports. These are on file in the experiment stations concerned. In addition, there are several published articles covering much of this work.

Woody Plant Studies

Turbinella oak

The study on *Quercus turbinella* Greene was conducted at the University of Arizona. It was designed to investigate phenotypic variations in the species and relate them to herbicidal control. The studies were concentrated on defining some of the genetically controlled tolerance limits of the species and relating them to herbicidal control limitations.

Many unforseen difficulties were encountered in the attempt to develop a picture of the genetic variability in this species. On the basis of field observations, a great deal of variability appeared to exist between individual plants within small areas. This was attributed to the fact that the species could propagate both by seeds and root sprouts. In this way new variations or genotypes which became established from seeds by sexual reproduction could be propagated indefinitely by asexual sprouting. Thus, variation could be expected to be out of proportion to what might be expected from plants reproducing only sexually (Clausen, 1954).

Another difficulty which was experienced in studying variability in this species was its uncertain seeding habits and the transient viability of the seeds when collected. It was very difficult to get large seed collections representative of various or extreme habitats at any one time because of the long periods between good seed years that appeared to be characteristic of the species and because this periodicity varied with areas and environments. Also, no means were discovered for extending the viability of these seeds for over one year. Many seeds germinated in collection bags within a few hours after collection at ambient temperatures, and germination rates dropped drastically after six months for all methods of storage tested. Seed collections representative of different environments could not be accumulated over a period of more than one year.

The characteristics of the plant that have been described as barriers to the easy definition or isolation of specific genetic variability also offer some opportunity for genetic interpretation of herbicidal results. For example, a species with a high degree of local variability might be expected to be difficult to control and would show variable results with the application of selective herbicides. This was definitely the case with turbinella oak. It was the most difficult species in the chaparral complex to control. Mortality was extremely variable with the use of 2,4-D, 2,4,5-T, monuron, diuron, or silvex (Whitham, 1960; Schmutz and Whitham, 1962). Complete control with fenuron was accomplished only by using soil-sterilizing quantities of 8 to 16

pounds per acre (active ingredients) of the chemical (Wagle and Schmutz, 1963). At rates of 1 to 4 pounds per acre (a.i.) plant mortality varied from few to most plants. The few plants that survived the 4 lb./A a.i. application may very well be genetically resistant to this chemical at this rate, and if their range is extended, fenuron may offer effective control of turbinella oak only at excessive nonselective rates.

Soil studies. Part of the job of finding an effective means of controlling a species is to try and define some of the factors that appear to limit the distribution of the species. This would indicate some of the genetically fixed species tolerance limits for certain environmental factors which, in turn, may give a basis for control. It also would serve to define with area limits, those environmental conditions within which control methods would have to be used.

In the turbinella oak study, soil fertility and soil origin were investigated in relation to species distribution. Soil fertility bio-assay studies showed that areas having the highest amount of grass were low in nitrogen, while phosphates were indicated as being in adequate supply. Phosphate was indicated as being in low supply in six of nine soils tested where turbinella oak was a dominant part of the vegetation, and nitrogen was low in four of the nine soils. All of the soils studied on which *Quercus turbinella* was found in abundance were coarse textured, with a gravel and rock content of from 20 to 40% and sand contents of over 25%. The clay and silt contents were below 15 and 22%, respectively.

Studies on the distribution of turbinella oak in relation to soil origin indicated that the numbers of oak plants growing on both north and south slopes underlain by quartz diorite were significantly higher than those growing on either slopes or areas underlain with basalts or sediments. Numbers of oak plants growing on north slopes of areas underlain by both basalt and the sediments were significantly higher than the number of oak plants growing on south slopes underlain by these two strata. On many south slopes and on some north slopes, large open brush-tree areas predominantly covered with grass and small shrubs were associated with these underlying basalt and sedimentary strata.

The genetic significance of these studies appears in the tolerance limits of the species in regard to soil factors. Apparently turbinella oak is tolerant to soils of low fertility, particularly in regard to nitrogen and phosphorus, a characteristic of soils originating from quartz diorite. It reached its greatest density on the coarser granitic soils. The distribution of the plants growing on the soils originating from the basalts and sediments was influenced by water availability which,

in turn, was influenced by temperature extremes. Field examination showed that the parent material was deeply fractured and root development was more extensive and deeper in the coarse granitic soils. The roots grew poorly or not at all in the heavier soils, and the parent materials in the sedimentary soils were cemented and unfractured. One series of germination tests showed no significant differences in seed germination between soils from the three different parent materials. However, temperature was not introduced as a variable factor.

Herbicide studies. When fenuron was used on both mature and after-fire sprouting plants of turbinella oak, it gave much better control on the after-fire sprouts (Wagle and Schmutz, 1963). The physiological tolerance limits of the after-fire sprouts for fenuron were much less than those of mature plants. Fenuron gave the greatest plant mortality when applied at a time of the year to coincide with heavy precipitation so it could be leached rapidly into the ground and actively absorbed by the roots prior to the early spring flush of growth. These responses were examples of using a herbicide to define age and environmental variation, or differences in tolerances, to a particular herbicide. Different plant responses to fenuron were isolated as the result of applying gibberellic acid to some plants, which gave a physiological basis for the chemical-resistance differences observed between old and newly sprouting fire-treated plants (Wagle and Schmutz, 1963).

Herbaceous Plant Studies

Several of the states included genetic variation within species in their projects. These and other projects also included work that illustrates that species variation is a significant factor in selectively controlling a species.

Wild oats

Wild oats (*Avena fatua* L.) were studied at the Montana Agricultural Experiment Station. Seeds were collected from Montana and from adjacent states and provinces. Both the seeds and the plants they produced were observed to differ morphologically. Physiological differences were discovered in the degree of dormancy the seeds possessed (Haun, 1956). Differences also were observed in the longevity of the seed from different strains which were buried in a Bozeman silt loam soil (Baker, 1962).

Baker (1959) states in his station research outline: "One of the confusing aspects of the published results on wild oat research is the lack of agreement between various investigators. Undoubtedly this can be partly explained by the different strains of wild oats that have been

observed." Variable results also may be caused by differences in the environmental conditions of growth. Temperature seems to play an important role here (Thurston, 1953).

The species *Avena fatua* has been divided into subspecies and varieties on the basis of several morphological differences (Lindsay, 1956; Thurston, 1953; Toole and Coffman, 1940). Several physiological differences have been identified in seeds that were at first labeled strains simply because they came from different locations (Haun, 1956; Leighty, 1958). These physiological differences were indicated by seed dormancy differences. Certain of the strains were highly dormant several months after harvest, while other strains were practically nondormant at the same time. Strains with an intermediate degree of dormancy also were observed. Further physiological differences were demonstrated when growth-regulating substances that were isolated from the seed of various strains either stimulated or retarded growth of wheat coleoptile segments. These differences were reported to carry over into the progeny from one generation to another even when the parent stock was grown in the same environment (Lute, 1930; Haun, 1956).

Herbicide studies. Wild oats have been observed to respond to certain chemicals differently than small grains (Leggett, 1957; Leighty, 1958). *Avena* species are more tolerant to amitrole than barley or wheat. Differences in their reaction to MH (Carder, 1956) and dalapon (Josephs *et al.*, 1957; Andersen and Helgeson, 1958) have been reported.

Selective chemical control of wild oats in barley and wheat has become a widely used method of controlling this troublesome pest. Tests with DATC, barban, and other chemicals have shown that differences exist between closely related genera in the grass family. In fact, differences between varieties of wheat and barley in their response to these chemicals have been noted. While a clear-cut differential response of the wild oat strains to several chemicals has not been observed in Montana, it seems perfectly logical to assume that such differences exist.

Genetic significance. Strains of a species have been isolated which can avoid chemical control by variations in their seed-dormancy mechanism. Studies of the percentage of control obtained indicate that a good proportion of plants are not killed, and their continuing propagation may develop races of wild oat plants which are as resistant to barban and DATC as the wheat and barley plants which are selectively favored at the time of first treatment.

More details are needed in terms of local and regional variation

for the whole life cycle of wild oats. Some of the chemicals that have shown only limited promise for controlling wild oat distribution may show selectivity against other or new races.

These studies are a good example of selectivity utilizing the physiological, morphological, and phenological differences between closely related species for purposes of limiting the distribution of an undesirable species. They illustrate many of the early statements made in this section on the importance of understanding genetic variability for effective weed control.

The ecotypes described may have more exactly represented an ecocline if a larger sampling of variability had been made.

Canada thistle

Studies made in three states—Montana, Utah, and Wyoming—were reviewed in developing the material on Canada thistle (*Cirsium arvense* L. Scop.) contained in this study. Canada thistle is a highly variable species capable of extending its range asexually as well as by seeds. A detailed understanding of the extent of this variability in terms of morphology and genetically controlled physiological processes could be the best means to a predictable control of the species.

Hodgson (1964) of Montana, in reviewing the work done on Canada thistle, described a number of morphological variations in the species. As a result of observing differences in the herbicidal response of Canada thistle plants from diverse environments, he started a study on the seasonal development of annual shoot growth in Canada thistle plants developed from root sections obtained from 10 different areas. He found phenological variations in dates of spring emergence of shoots, period of maximum shoot growth, blooming, and seed development.

Hodgson also found variations in leaves and flowers. Most of these Canada thistle strains or ecotypes were found to be dioecious; however, two ecotypes that produced mainly pollen were found to produce an occasional seed and were thereby imperfectly dioecious. Seed weights varied widely among this group of 10 plants.

Physiological studies. In the Wyoming project some important light-influenced growth differences were noted between six ecotypes of Canada thistle collected from widely separated areas. Growth chambers with light and temperature controls were used in this experiment to ensure reproducible environments.

The study was undertaken to determine the influence of herbicidal treatments and light quality on the quantity and character of the pectic substances and alterations in pectic compounds in certain Canada thistle selections. Only those physiological responses obtained

without the herbicide factor are noted here. Additional coverage is given under the next heading, "herbicide studies."

The foliar growth of the six Canada thistle ecotypes responded differently to red, green, blue, and warm white light. Stem elongation occurred in all plants grown under red light. However, the leaf and stem diameters of plants grown under this light were less than those of plants grown under other lights. The total foliar growth was promoted more by green, blue, and white light than by red light.

Under the red light, four of the Canada thistle ecotypes yielded significantly smaller amounts of alcohol insoluble solids (AIS) than under green, blue, or warm white light. More AIS were produced under warm white light in all thistle ecotypes except one. These light responses and the morphological differences existing between individual ecotypes indicated that physiological variation can be expected as a corollary of phenotypic variation.

The roots gave a different response than the foliage in the amount of AIS produced. Some individual ecotype differences also were noted.

Light quality had an effect on the metabolism of pectic substances and caused a highly significant difference in the foliage pectic content in all thistle ecotypes except two. In all selections, blue and warm white light resulted in the least production of pectic substances in the foliage.

In direct opposition, blue and warm white lights gave the greatest pectic substance production in the roots of Canada thistle.

The pectic substances were calculated from three arbitrary classes, according to solubility in three different substances. A number of differences which were noted between selections were attributed to genetic variability.

Herbicide studies. The Wyoming 1962 regional research progress report (W-63) contains the following statement: "Selections of a species may vary in their physiological bases. Therefore, a particular selection (ecotype) may be more susceptible to 2,4-D than another. This may explain the variation in the susceptibility of the selections (ecotypes)." The physiological responses noted above and the interactions between light and herbicides and other data on herbicidal results reported in the following paragraphs are verification of this premise.

Hodgson reported (1963) a range of survival one year after treatment among the 10 ecotypes of thistle ranging from 5 to over 100% for 4 pounds per acre of amitrole. When 1.5 pounds of 2,4-D were applied at either the bud or bloom stage of growth, survival ranged from about 40 to 100%.

In the Wyoming studies, the phytotoxicity of 2,4-D at the 2- and 4-pound per acre active-ingredient applications was greater under red

100

light than under green, blue, or warm white light. Differences were noted between all ecotypes in the amount of fresh foliage and root weight produced under different chemical-light treatments. Thus, the foliar growth of all ecotypes was significantly reduced when plants grown under red light were sprayed with 2 pounds per acre (a.i.) of 2,4-D. In one ecotype more foliage weight was produced under blue light when treated with 2,4-D than under red, green, or warm white light. In all other ecotypes, blue light was second only to red light in reducing foliage production. As noted earlier, red light was more effective than other types of light in stimulating stem elongation. The lowered resistance of plants to the combination of 2,4-D and red light may arise from this effect.

Treatment with 2,4-D did not cause a difference in the amount of pectic substances produced in the foliage or roots of any of the ecotypes. Light quality was the only factor causing significant differences in pectic substances between ecotypes noted earlier. However, these differences are indicators of the existence of physiological variations between the ecotypes which may be related to the variation observed in 2,4-D response.

Work was done in Utah with two strains of Canada thistle. One strain of plants was taken from an area that had been treated with 32 pounds of amitrole per acre (a.i.), eliminating all but a few plants. The second strain consisted of the plants surviving on a plot which had been treated with a low volatile ester of 2,4-D applied at 4 pounds per acre (a.i.). The plants remaining alive were increased asexually and used for further testing. The results indicated that this strain was quite tolerant to amitrole.

Environmental studies. The Wyoming studies were conducted under a controlled environment and concentrated on some basic physiological phenomena related to light. They showed that light quality can change the tolerance limits of some ecotypes to 2,4-D and that some ecotypes were more influenced than others.

The Utah studies showed that the factor of soil moisture could be critical in its effect on plant tolerance to herbicides. One field study indicated that amitrole gave better control on plants growing in soils having a high moisture content, as indicated by number of irrigations.

Genetic significance. These studies are good evidence that genetic resistance to chemical treatment exists within species as well as between species. In addition, morphological and physiological differences between ecotypes have been identified that might eventually become a basis for identifying specific resistance factors within the species.

The Utah study also gives some indication that the asexually propagated progeny of plants surviving chemical treatment are more resistant to the chemical than the majority of the plants in the species population originally treated with the same chemical.

Field bindweed

Regional studies on the variation in field bindweed (*Convolvulus arvensia* L.) were made in New Mexico and Washington.

Herbicide studies. Studies made at Pullman, Washington, showed that the roots of field bindweed plants, susceptible and resistant to 2,4-D, reacted differently to foliar applications of the chemical. Using excised stem segments of the two strains in a sterile nutrient medium, it was possible to show a much greater sensitivity to 2,4-D for the stem tissue of the susceptible strain.

In additional root studies, using whole plants grown in glass-faced boxes with bands of 2,4-D-impregnated agar placed at right angles to the rapidly growing roots, it was possible to show the following effects of 2,4-D on roots:

1. The closer 2,4-D was applied to the root tip, the more effect the treatment had on the roots.

2. In no case was any response to 2,4-D noted in the root or foliage above the point of application.

3. When the agar strips containing 2,4-D were applied directly to the growing points of the roots, the susceptible strain showed a greater sensitivity.

4. Warburg respiration studies with finely segmented root tips also indicated a greater sensitivity in the susceptible strain.

The effect of 2,4-D on roots also was measured in a sensitive wheat variety. These studies indicated that 2,4-D in the concentrations used:

1. Increased the volume of the cortical cells in the region of elongation at a certain state of their development. The major swelling was due to this form of cellular enlargement.

2. Brought about numerous divisions in the pericycle, resulting in the formation of abnormal lateral root primordia.

All root tip enlargements, therefore, were caused by the stimulus of the growth regulator (2,4-D) on the actively growing root.

These studies indicated that part of the damage to plants from foliar applications may be due to root effects even though the foliage may appear to be undamaged.

In the New Mexico study, three strains of bindweed were selected for investigation on the basis of their previously determined reaction

to 2,4-dichlorophenoxyacetic acid (2,4-D). These strains were classified as resistant, intermediate, and susceptible.

The first results showed that the translocation of 2,4-D occurred with equal rapidity in both the susceptible and resistant strains of field bindweed. This was demonstrated by the swelling of root tips, cessation of root growth at equivalent periods of time after treatment, and by autoradiographs made of plants treated with radioactive 2,4-D. Other results were as follows:

1. The radioactivity in water extracts of treated bindweed plants was associated with the supernatant fluid containing the microsomes. However, on further centrifugation, the bulk of the activity was found to be in the supernatant (cell sap) fraction.

2. In electrophoresis, the major radioactivity in the microsome fraction was determined to be adjacent to or in association with the proteins and nucleo proteins.

3. No radioactivity was found in association with the amino acids.

4. Addition of iron to nutrient of stem section or to foliage of intact plants did not alter the uptake or response of the plants to 2,4-D.

5. A chromatograph of extracts from treated plants indicated the amount of asparagine from the susceptible plants was nearly double that from the resistant plants, and pipecolic acid was present on the chromatogram from the susceptible plant.

6. Coenzyme A added to nutrient agar did not modify or affect the uptake or response of stem sections of bindweed to 2,4-D.

7. A growth factor analog (benzimidazole), when added separately from 2,4-D, caused an increase in the growth of the axillary buds of stem sections from susceptible bindweed plants.

Genetic significance. The studies made on field bindweed are classic examples of the importance of establishing a physiological-biochemical basis for genetic variation. The completion of these studies and additional studies on other species may eventually lead to the development of a series of laboratory procedures whereby the chemical resistance of certain species of plants can be predicted in advance and chemical control measures prescribed exactly in relation to the undesirable species and its associated desirable or crop species.

These studies clearly indicate that genetic variation can be defined in terms of biochemical-cellular difference.

Discussion

From a taxonomic viewpoint, these studies on variation should be an invaluable addition to the knowledge of the composition of a species. Further, these studies point up some of the shortcomings of the "old school" taxonomy. Species can no longer be thought of, nor described, in terms of one or a few "typical" specimens filed away in a herbarium. To be usable and truly representative, a herbarium should include representative specimens of all of the morphological (and identified physiological) variants known. True, this sort of herbarium collection might complicate the identification of some species by wiping out the "clear-cut" type of specimen, but it would eliminate a lot of present-day confusion and present species in a truer light than ever before.

Perhaps what is needed is a new type of regional herbarium. These regional herbaria would be repositories of variation. Plants and physiological-biochemical, cytological, morphological, ecological, and genetic information would be collected by taxonomic specialists in each field for each species of the region. This information, consisting of specimens, samples, pictures, and written data, would be continuously collected and screened to extend our knowledge of species and species variation. All information contributing to this knowledge of species would be filed in a way it could be readily used by researchers and others needing the accuracy or true taxonomic representation of the species described.

The ecological-genetic information gathered in connection with this project has added to our understanding of a plant species in relation to its environment. It also has given us a great deal of insight into the role of environment and herbicides (as a part of the environment) in plant selection and evolution.

The information on physiological-genetic variation has added to our knowledge of how basic plant processes, involved in the life cycle of plants, have varied between and within species. This information also has added to our knowledge of how environment can affect the tolerance of a species to a chemical.

Ultimately, from the practical viewpoint of controlling the distribution of undesirable species, a great deal has been added to our physiological-ecological-genetic knowledge of plants; this information may enable us in the future to predict a species response to a particular herbicide.

Also, these studies point up the danger of complacency in herbicidal studies. The herbicide that will give effective control today or this year may not work next year or 5 or 10 years from now. Because

of genetic changes that can take place in plants and because of the selective force exerted by a herbicide as a part of the environment, chemical resistance for a particular herbicide may become a genetic characteristic of the species. This chemical resistance could be expected to develop much faster in a herbaceous species that completes its life cycle on an annual basis than in a woody shrub or tree-like perennial species that takes years to complete a life cycle.

In terms of future emphasis, it is an unfortunate fact that we know almost nothing of the genetic variation that exists in our wild plant populations. However, we need this information to make good land-management decisions in relation to agricultural management, forest and watershed management, range management, and wildlife management. Trial and error, rather than basic knowledge, will continue to guide our decisions until a better understanding is acquired of genetic-physiological-ecological relationships. This project has added to our basic understanding in these areas, but it has opened a tremendous area of ignorance which needs a great deal of concentrated study.

References

Alley, H. P. 1960, 1961, 1962. Annual reports of cooperative regional project W-63. Effect of certain climatic and soil factors on the response of plants to herbicides. Wyoming Agric. Expt. Sta.

Andersen, R. N., and E. A. Helgeson. 1958. Control of wild oats by prevention of normal seed development with sodium 2,2-dichloropropionate. Weeds, 6: 263-270.

Arneklev, D. R. 1963. Variability studies on leafy spurge (*Euphorbia eoula*). M.S. thesis, Montana State College, Bozeman.

Baker, L. O. 1959. Herbicidal control of wild oats as affected by environmental factors and ecotypes. Contributing project to Regional Research Project W-63. Montana Agric. Expt. Sta.

Baker, L. O. 1960, 1961, 1962. Annual reports of cooperative regional project W-63. Effect of certain climatic and soil factors on the response of plants to herbicides. Montana Agric. Expt. Sta.

Cain, S. A. 1944. *Foundation of Plant Geography*. New York: Harper and Bros.

Carder, A. C. 1956. Panel discussion of the control of wild oats. Canada National Weed Committee, West. Sect. Proc., *10*:41.

Clausen, J. 1954. Partial apomixes as an equilibrium system in evolution. Caryologia, Suppl.

Clausen, J., D. D. Keck, and W. M. Hiesey. 1940. Experimental studies on the nature of species. I. The effect of varied environments on western North American plants. Carnegie Inst. Wash. Pub. 520.

Crafts, A. S. 1961. *The Chemistry and Mode of Action of Herbicides*. New York: Interscience Publishers.

Freed, V. H. 1960, 1961, 1962. Annual reports of cooperative regional project W-63. Effect of certain climatic and soil factors on the response of plants to herbicides. Oregon Agric. Expt. Sta.

Haun, C. R. 1956. Dormancy and germination studies of the wild oat (*Avena fatua* L.) M.S. thesis, Montana State College, Bozeman.

Heslop-Harrison, J. 1953. *New Concepts in Flowering-Plant Taxonomy*. London : Heinemann.

Hodgson, J. M. 1963. Annual report to the United States Department of Agriculture.

Hodgson, J. M. 1964. Variations in ecotypes of Canada thistle. Weeds, *12*: 167-171.

Josephs, M. J., D. R. Mussell, J. K. Leasure, and R. L. Warden. 1957. A chemical for the control of wild oats (*Avena fatua*) : 1,2,4,5-tetrachlorobenzene. 14th North Central Weed Control Conf. Proc., *14*:37-38.

Klingman, G. C. 1961. *Weed Control: As A Science*. New York: John Wiley and Sons, Inc.

Leggett, H. W. 1957. Summary of wild oat research. *Research Report, Canada National Weed Committee, West. Sect.* pp. 35-36.

Leighty, D. H. 1958. Dormancy and germination studies of the wild oats (*Avena fatua* L.) M.S. thesis, Montana State College, Bozeman.

Lindsay, D. R. 1956. Taxonomic and genetic studies on wild oats. Weeds, *4*:1-10.

Lute, A. M. 1930. Germination characteristics of wild oats. Assoc. of Seed Anal. No. Am. Proc., *23*:70-73.

Muzik, T. J. 1961. Annual report of cooperative regional project W-63. Effect of certain climatic and soil factors on the response of plants to herbicides. Washington Agric. Expt. Sta.

Schmutz, E. M., and D. W. Whitham. 1962. Shrub control studies in the oak-chaparral of Arizona. Jour. Range Mgmt., *15*:61-67.

Stebbins, G. L. 1950. *Variation and Evolution in Plants*. New York: Columbia University Press.

Thurston, J. M. 1953. The biological approach to the problem of wild oat control. British Weed Control Conf., *1*:240.

Toole, E. H., and F. A. Coffman. 1940. Variations in the dormancy of seeds of the wild oat, *Avena fatua*. Jour. Am. Soc. Agron., *32*:631-638.

Turesson, G. 1922. The genotypical response of the plant species to the habitat. Hereditas, *3*:211-250.

Turesson, G. 1930. The selective effect of climate upon the plant species. Hereditas, *14*:99-152.

Turesson, G. 1936. Rassenokologie und Pflanzengeographie. Bot. Notiser: 420-437.

Wagle, R. F. 1958. Early growth variation in bitterbrush and its relation to environment. Ph.D. dissertation, Univ. of Calif.

Wagle, R. F. 1960, 1961, 1962. Annual reports of cooperative regional project W-63. Effect of certain climatic and soil factors on the response of plants to herbicides. Arizona Agric. Expt. Sta.

Wagle, R. F., and E. M. Schmutz. 1963. The effect of fenuron on four southwestern shrubs. Weeds, *11*:149-157.

Whitham, D. W. 1960. An ecological and economic study of the effect of various herbicidal treatments on grass-shrub relationships in the Arizona chaparral. M.S. thesis, Univ. of Ariz., Tucson.

Whitworth, J. W. 1960, 1961, 1962. Annual reports of cooperative regional project W-63. Effect of certain climatic and soil factors on the response of plants to herbicides. New Mexico Agric. Expt. Sta.

CHAPTER FIVE

The Soil Behavior of Herbicides as Influenced by Their Physical Properties

V. H. Freed, J. B. Vernetti, and M. L. Montgomery

The number of herbicides for application to the soil increases every year. It has been found that a higher degree of selectivity and a longer period of weed control may be achieved with chemicals applied to the soil in contrast to those applied to foliage (Klingman, 1961; Robbins, Crafts, and Raynor, 1952). However, those chemicals applied to the soil are more subject to varied degrees of changes in environment which determine the effectiveness of the treatment than are those chemicals applied to foliage (Freed, 1958; Klingman, 1961). For this reason, it is important to develop a basic understanding of the laws and principles governing the behavior of these chemicals. A thorough understanding of such phenomena would permit development of a program for more rational use of these chemicals and the attainment of a higher degree of effectiveness under more varied conditions.

Factors Influencing the Effectiveness of Chemicals

It has been established that the type of soil to which the chemical is applied, the amount of moisture in the soil at the time of treatment and subsequent to it, and various other factors may determine the ultimate biological effectiveness of the chemical (Freed, 1951; Frissel, 1961; Hill *et al.*, 1955; Ogle and Warren, 1954; Robbins, Crafts, and Raynor, 1952; Shaw *et al.*, 1960; Sheets and Danielson, 1960). The interaction of a chemical with these factors of the environment may be adduced to be due to the physical and chemical properties of the material applied (Freed, 1958). This may be accepted as a general premise from which it is then possible to derive a logical explanation for the observed behavior of the chemical. It is the purpose here to examine some of the observed behavior patterns of the chemicals and from them to adduce chemical and physical explanations for their characteristic behavior.

Experience has shown that there are at least five major means by which the effective concentration may be reduced. They are: adsorption, leaching, volatilization, microbiological breakdown, and chemical

breakdown (Audus, 1951; Blouch and Fults, 1953; Bollen, 1961; Burschel and Freed, 1959; Hill *et al.*, 1955; Ogle and Warren, 1954; Sherburne and Freed, 1954; Upchurch and Pierce, 1957.) For purposes of this discussion, the sixth manner by which an effective concentration of the chemical may be reduced, namely, removal of the chemical by plants, is excluded. With any given chemical, any two or more of these factors may be operative simultaneously.

In the usual situation, the herbicide is applied to the soil and then at the end of a finite time its effectiveness is evaluated. Because there may be a lapse between time of application and when the weeds germinate and the young plant is exposed to the chemical, the rate at which the effective concentration of the chemical is being reduced by various factors is of importance.

An attempt is made here to evaluate certain factors and processes in relation to the activity of soil-applied chemicals. Particular attention is given to chemical factors that may be useful in predicting the behavior of a chemical.

The solubility of a compound in water is of utmost importance in soil behavior, as this property often will determine the rate at which the chemical will leach and is also related to adsorption of the compound by soil constituents. The structure of a compound can give some indication as to the adsorbability of the material (Glasstone, 1946; Leopold *et al.*, 1960). For example, compounds belonging to the open chain series of organic chemicals would not be expected to be adsorbed as strongly as the aromatic compounds which possess strong van der Waal forces. If, on the other hand, the chain compound contains a functional group that may react with soil constituents, the adsorption may be manifold greater. This latter case is particularly evident in the case of the heterocyclic compound 3-amino 1,2,4-triazole, where the amino group is apparently absorbed on the sites at which ammonia and nitrogen become attached. In the breakdown of chemicals, the bond energy involved in the functional group most likely to be attacked is of importance. For example, if the ester or amide derivatives of a compound have equal biological activity, it would be expected that the amide would have a longer residual life in the soil. This arises from the fact that the bond energy of the amide is greater than that of the ester; consequently, hydrolysis of the ester proceeds with much more facility than hydrolysis of an amide. Ionizable functional groups, particularly those capable of salt formation, are also responsible in markedly modifying the behavior of a chemical in the soil.

Adsorption of Herbicides in Soils

Thermodynamics of adsorption

The adsorption of chemicals by soil has long been recognized as a factor in the biological activity of soil-applied pesticides. Adsorption of herbicides, such as arsenic, by soils was described many years ago (Robbins, Crafts, and Raynor, 1952). Sorption of a compound by a solid surface is a complex phenomenon that may consist of nonspecific adsorption which is due merely to the general attraction of the surface and molecules for one another, or it may involve chemisorption in which bonds between the chemical and solid surface are established (Hollingsworth, 1954). In most of the considerations given to the adsorption process here, we are dealing principally with nonspecific adsorption except where noted.

Adsorption is an equilibrium process wherein the system of the chemical either in solution or in vapor state comes to equilibrium with the chemical adsorbed on the surface of the soil constituent.

$$(\text{Chemical})_{\text{equil. conc.}} + \text{Soil} \rightarrow (\text{Chemical})_x \text{ Soil} + \text{Chemical} \tag{1}$$
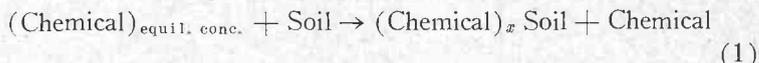
In a specified system at a given temperature and pressure and concentration, the ratio of the amount of chemical adsorbed to that in the equilibrium solution is constant, as shown in equation 2.

$$K = \frac{(\text{Chemical})_{\text{adsorbed}}}{(\text{Chemical})_{\text{equil. conc.}}} \tag{2}$$

This constant ratio is not a meaningful constant because the activities of the chemical, the chemical soil complex, and the soil have not yet been specified. If these activities (A) are specified as indicated in equation 3, a thermodynamic equilibrium constant is obtained.

$$K = \frac{A_{(\text{Chemical})x \text{ Soil}}}{A_{\text{Chem. in sol.}} \, A_{\text{soil}}} \tag{3}$$

Using the relationship developed in equation 3, it is possible to write an equation for the free energy of the adsorption reaction, which is the driving force of the reaction, as shown in equation 4.

$$\triangle G^0 = RT \ln K \tag{4}$$

The superscript of the free energy $(\triangle G^0)$ in equation 4 indicates that $\triangle G$ is obtained under standard conditions. It is the reference point used to determine the free energy at any other temperature or under any other condition. The standard conditions are usually at 25°C, one atmosphere of pressure, and when the reactants are at unit activity.

In order to determine the free energy under other conditions, it is necessary to make use of the relationship given in equation 5.

$$\triangle G = \triangle G^0 + RT \, ln \, \frac{A_{(\text{Chemical})x \, \text{Soil}}}{A_{\text{Chem. in sol.}} \, A_{\text{soil}}} \tag{5}$$

Turning now to the matter of activities, convention specifies that under standard conditions of 25°C and one atmosphere of pressure, solid substances have unit activity. Thus, in a heterogeneous system of soil and chemical either in solution or vapor phase, the soil surface is considered to have an activity of unity. Similarly, the complex (Chemical)$_x$ Soil formed as indicated in equation 1 is an insoluble solid and its activity is taken as unity. On this basis, we then write equation 6 in the form given below.

$$\triangle G^0 = RT \, ln \frac{1}{A_{\text{Chem. in sol.}}} \tag{6}$$

This may be written then as shown in equation 7a.

$$\triangle G^0 = RT \, ln \, A_{\text{Chem.}} \tag{7a}$$

Since in most instances very dilute solutions are being dealt with, it may be assumed that the activity is approached by the molarity of the solution and that the molarity is a close approximation to the molality. In which case we then obtain equation 7b.

$$\triangle G^0 = RT \, ln \, M_{chem.} \tag{7b}$$

It has been shown that the adsorption reaction is one of extent, i.e., fractional area covered (Jurinak and Bauer, 1956). It therefore becomes necessary to evaluate $\triangle G^0$ at a specified extent of area covered. The symbol θ is used to indicate the fractional area covered by the chemical. Since the amount adsorbed (in say, $\mu g/g$ soil) is directly proportional to θ, the actual numerical value of θ need not be known to evaluate the free energy change. It is possible then to select a point at which a given number of micrograms (or micromoles) of chemical are adsorbed per gram of soil and evaluate the free energy change at this fractional coverage at different temperatures.

The free energy change in this adsorption reaction indicates the extent to which the reaction goes. However, it does not follow that because an appreciable amount of chemical is adsorbed that it is also tightly bound to the surface. It is quite possible to have a substantial quantity of the chemical adsorbed without its being bound very tightly.

In order to determine the effective binding of the chemical by the surface, it is necessary to look for other parameters that offer a more reliable indication of binding. Examination of the problem reveals

that there are two factors involved. One is the extent of binding, the second is the degree of order that is attained during the binding process. If a high degree of order is achieved during the binding process, then the binding will, of necessity, be strong. This consideration immediately leads to the examination of the change of entropy, which is a measure of the degree of order imposed during the reaction. Entropy is determined from the rate of change of the free energy with a change in temperature, as shown in equation 8.

$$\frac{\partial G^0{}_\theta}{\partial T} = \triangle S_\theta \tag{8}$$

With this information, it is possible to derive a new relationship which is more indicative of the energy with which a compound is adsorbed to a surface. This relationship is shown in equation 9.

$$\triangle H^0{}_\theta = \triangle G^0{}_\theta + T \triangle S^0{}_\theta \tag{9}$$

From the data calculated with the foregoing equation, it is possible to tell whether the chemical is tightly bound by the surface of soil constituents. There should be a large change in the amount of chemical adsorbed at different temperatures if the binding is strong. That this is the case is illustrated in Table 1.

Table 1. VARIATION OF ADSORPTION WITH TEMPERATURE

Chemical	Temperature	Percent adsorbed
	°C	%
CIPC	5	56.6
	30.5	51.5
Amitrole	20	50.3
	28	47.8

At first glance, the differences in Table 1 may seem small, but it must be remembered that these changes are evaluated in terms of absolute temperature $(273 + °C)$. In this light, the magnitude of change becomes appreciable for amitrole, indicating considerable strength of binding to the soil.

Effect of soil type

Using the variation of change in adsorption with temperature, the "entropy" factor $(\triangle S)$ may be calculated. This has been done for monuron on two different soil types (Table 2).

Table 2. THE ENERGY OF ADSORPTION BY DIFFERENT SOIL TYPES (MONURON)

Soil	Energy (K cal)	ΔS (entropy units)
Sandy	1.25	0.5
Muck	5.60	15.5

Clearly, the muck soil with its higher affinity for monuron adsorbs the monuron in a manner that results in a higher degree of order of arrangement of the adsorbed monuron.

Adsorption of chemical by soil may be a very important factor in the biological effectiveness of that chemical. For example, it is a matter of common knowledge that a chemical which is strongly bound by soil colloids is less effective at a given rate of application as one goes from a light sandy soil to increasingly heavier soils and finally to muck. This point has been demonstrated repeatedly with the urea herbicides. While the adsorption process is an equilibrium process, if the chemical is strongly bound by a colloid, the effective concentration in the soil solution is materially reduced at any given instance. The stronger the binding, the less the amount of chemical in the soil solution available for action against the plant. The strength of this binding varies among the constituents of the soil—sand, silt, clay, and organic matter. As a general rule, clay and organic matter are the portions of the soil that adsorb most strongly.

Another instance where adsorption by the soil is a factor is in the leaching process. In leaching, as water enters the soil and moves down through its horizon, the chemical is dissolved and carried along with the water stream but is always in contact with soil surfaces. The chemical dissolved in this percolation water equilibrates between the colloid surfaces and the water. If the chemical is tightly bound by the soil colloids, the amount of chemical leached to a given depth in the soil and the rate at which it is leached is markedly reduced. It has been found that the heat of binding may be useful to describe the leaching process. Loss by evaporation or steam distillation is another process in which adsorption plays an important role. As the strength of binding increases, less and less of the chemical is volatilized from the soil surface.

Relation of adsorption to latent heat of solution

Adsorption, as pointed out earlier, is a complex phenomenon involving everything from simple nonspecific adsorption to chemisorption such as is found in ion exchange. Certain correlations have been found between chemical structure and adsorbability as well as between water solubility and adsorption (Freed, 1958; Frissel, 1961; Glas-

stone, 1946). In general, it might be stated that compounds of an aromatic nature or containing groups capable of polarization will tend to be more readily adsorbed. Similarly, compounds having high intramolecular attraction are readily adsorbed. It has been found, in the case of gases for example, that the most readily liquefiable gases are adsorbed to a greater extent (Glasstone, 1946). This comes about from the high intra- and intermolecular attraction in these materials. Table 3 shows how water solubility gives a rough degree of correlation with the extent of binding of compounds. It should be noted, however, that extent of binding and strength of binding are not necessarily correlated.

Table 3. Amount of Chemical Adsorbed as a Function of Water Solubility

Chemical	Water solubility (ppm, 25°C)	Fraction of available chemical adsorbed
Amitrole	28.0	0.10
2,3,6-TBA	7,700	0.20
2,4-D	605	0.55
Fenac	203	0.60
CIPC	103	0.60
Monuron	230	0.65
Atrazine	70.0	0.70
Simazine	5.60	0.85
Casoron	45.0	0.48
Diuron	42.0	0.80
Dacthal	0.56	0.90

It was noted that the more readily liquefiable gases are adsorbed to a greater extent. In studying this phenomenon, the concept of integral heat of adsorption was introduced. This value is the heat of adsorption found as the amount of chemical adsorbed increases. The integral heat of adsorption was found to approach but not necessarily equal the latent heat of evaporation as multimolecular layers built up on the adsorbing surface. Examination of the situation in which multimolecular layers are built up reveals that not only is there attraction between the surface and the adsorbed species but also an attraction between the adsorbed molecules. It is reasonable to assume, therefore, that the integral heat of adsorption should approach the latent heat of evaporation in this case. In light of this information, it would be expected that there should be a parallel between the behavior of gases and the behavior of solutes in a water solution. Examination of several cases

revealed that there is a reasonably good correlation between the strength of binding and the latent heat of solubility of many of the herbicides (Table 4).

Table 4. Adsorption as a Function of the Latent Heat of Solution

Chemical	Heat of solution (K cal)	Leaching	Adsorption, indicated by soil type response
2,3,6-TBA	1.6	Readily	Small
Fenac	6.7	Resistant	Large
Amiben	2.8	Readily	Intermediate
2,4-D	6.1	Intermediate	Large
2,4,5-T	8.9	Resistant	Large
Simazine	9.0	Resistant	Large
Fenuron	3.9	Intermediate	Intermediate
Monuron	>6.0	Resistant	Large
CIPC	<4.9	Moderate	Intermediate
Casoron	2.8	Moderate	Small
Dacthal	12.4	Resistant	Large

The seeming correlation between latent heat of solution and adsorption, while good, is not sufficient proof that this is a valid relationship. If an organic compound could be found whose latent heat of solution were negative, i.e., one less soluble at high temperature than low and whose adsorption behavior was in accord, the relationship could be established. Such a compound was found in EPTC. The solubility of this compound decreases as temperature increases (375 ppm at 25°C and 636 ppm at 3°C), giving a negative heat of solution. Similarly, as shown in Table 5, the amount of chemical adsorbed decreases as the temperature increases. This data, therefore, confirms the correlation between the latent heat of solution and adsorption.

In considering the adsorption of herbicides by soils, it is necessary to think in terms of the variation in extent and binding of the

Table 5. Relation of Heat of Solution to Adsorption and Leaching in Soils

Compound	Heat of solution	Percent adsorption		Percent leaching	
		25° C	3° C	25° C	3° C
		%	%	%	%
CIPC	+4.9	60	71	0.2	0.0
EPTC	−3.9	61	52	0.6	5.2

chemical with varying soil types. Examination of the amount of chemical adsorbed by different soil types has shown that there is a regular progression in the extent of adsorption as well as binding, starting with the light sandy soils and progressing on through the muck or high organic soils (Ashton and Sheets, 1959; Blouch and Fults, 1953; Coggins and Crafts, 1959; Gantz and Slife, 1960; Jurinak, 1957; Lichtenstein, 1959; Smith and Ennis, 1953; Warren, 1956). This is shown by the data given in Table 6.

The differences in amount of chemical adsorbed by soils and clays at different pH's have been reported by Frissel (1961). There can be no doubt that the various clays found in different soils will vary markedly in their ability to adsorb chemicals (Coggins and Crafts, 1959). This is shown in Table 7.

Table 6.   ADSORPTION AS A FUNCTION OF SOIL TYPE

Chemical	Fraction of available chemical adsorbed		
	Sandy	Loam	Peat
2,3,6-TBA	.14	.45	.40
Monuron	----	.47	.69
Amitrole	.05	.20	.90

Table 7.   ADSORPTION OF CIPC BY VARIOUS CLAYS

Adsorbent	Temperature	Adsorbtion
	$°C$	$\mu g/g$
Sandy soil	24	10.3
Kaolin	30	30.1
Bentonite	30	77.0
Illite	30	77.5

The foregoing data probably reflect not only the difference in specific adsorptive capacity but differences in surface area as well. It also has been noted that change of ionic environment, e.g. salts and pH, cause a marked variation in adsorption. This may arise from the effect of other ions such as Ca^{++}, H^+, or OH^- on the clay itself or on the organic species being adsorbed.

It is seen then that adsorption phenomena are of utmost importance in the soil behavior of chemicals (Ennis, 1954; Fang, Theisen, and Freed, 1961; Hartley, 1960; Hill et al., 1955; Upchurch and Pierce, 1957). These particular phenomena play a role not only in determining the concentration of chemical in the soil solution but may

also modify leaching (Upchurch and Pierce, 1957), volatilization (Fang, Theisen, and Freed, 1961), and rate of breakdown.

Vapor Phenomena in Soil Behavior of Herbicides

Relation of vapor pressure to herbicide loss

Many of the modern organic herbicides have measurable vapor pressures which play a role in the behavior of the chemical in the soil (Ashton and Sheets, 1959; Massini, 1961). The tendency of the chemical to vaporize may be an important factor in the biological activity, as was thought to be the case with EPTC, or it may be an important source of loss as in the case of IPC. The behavior of herbicides in the gaseous state is a complex one involving an equilibrium between the vapor, the solid surface of the soil, and the solution of the chemical in the soil water (Goring, 1957; Jurinak, 1957).

In the gaseous state, molecules behave as if they have little attraction for each other and are in a perfect solution of the diluting gas. As a consequence, the molecules tend to rapidly occupy all space available to them. Distribution of vapors of a herbicide in soils at a given temperature is dependent on this tendency of a gas molecule to diffuse in all directions but is limited or restricted by the ease of diffusion through a porous solid, the effect of gravitational attraction on the gas molecule, the amount of moisture and the solubility of the gas in that moisture, and the adsorption of the chemical by the soil surface.

The tendency of a chemical to change into the vapor state is indicated by its vapor pressure. Vapor pressure is described conveniently by the following equation.

$$ln\ P = \frac{\triangle H}{P} \cdot \frac{1}{T} + C \qquad (10)$$

It will be noted in this equation that there is a constant ($\triangle H$) of evaporation which is a parameter of the material under consideration. The constant ($\triangle H$) is of importance because it determines the change of vapor pressure with temperature. $\triangle H$ may be defined as the quantity of heat in calories required to convert one mole of liquid at a given temperature to one mole of gas at the same temperature. If $\triangle H$ is small, then the rate of change of vapor pressure with temperature will be small. Conversely, if $\triangle H$ is large, a large change of vapor pressure with temperature will be noted. The rate at which a chemical volatilizes should then be some function of its latent heat of evaporation and the rate at which the heat is supplied to the system from the outside. The relation of this physical constant to vapor loss of herbicides from soil is shown by the data in Table 8.

116

Table 8. Latent Heat and Vapor Loss

Compound	Vapor pressure (mm Hg)	$\triangle H$ (K cal/mol)	Percent loss
Isopropyl ester of 2,4-D....	1.5×10^{-2}	24.0	13.0
EPTC	2.0×10^{-2}	14.5	18.8
PEBC	4.8×10^{-3}	12.0	16.7

Co-distillation

In dealing with herbicides of a volatile nature, the addition of nonvolatile solvents or solid materials will modify the vapor loss (Sheets, 1959). This phenomenon is expressed in Raoult's law, where the vapor pressure (P) of a component (in this case, the solvent) is equal to the product of the vapor pressure of the pure solvent and its mole fraction. Raoult's law generally applies to the solvent in very dilute solutions.

$$P = P_0N \tag{11}$$

The vapor loss of EPTC as influenced by an additive is shown in Table 9.

Table 9. Vapor Loss of EPTC From Soil

	Percent loss in 24 hours	
	Dry soil	Moist soil
	%	%
EPTC	18.3	33.4
EPTC plus oil	5.6	23.4

When dealing with gases that dissolve in a liquid, particularly in water, Henry's law is usually applied (Goring, 1957). In this case the pressure of the solute in the aqueous solution is equal to a constant times the mole fraction of the solute present. This relationship is shown in equation 12.

$$P_{solute} = KN_{solute} \tag{12}$$

When considering the behavior of a volatile herbicide in soil and its relationship to water content, there is another way of expressing Henry's law. This is shown in equation 13.

$$K = \frac{\text{solute as vapor}}{\text{solute in aqueous phase}} \tag{13}$$

The foregoing indicates that with a given amount of herbicide as a vapor in the soil, increasing the amount of moisture in the soil will reduce the amount of chemical in the vapor state. This follows because the numerator of the equation decreases as the concentration of chemical in solution decreases.

Another factor in soil behavior of volatile herbicides is the matter of steam distillation or co-distillation. Organic compounds having a measurable vapor pressure will distill with water. This deduction follows logically from application of Dalton's law of partial pressures and Henry's law, both of which indicate there should be a measurable vapor pressure of a volatile solute above an aqueous phase. Under ideal conditions these volatile materials will co-distill with water in a proportion expressed in equation 14.

$$\frac{W_2}{W_1} = \frac{P_2 M_2}{P_1 M_1} \tag{14}$$

A number of herbicides are known to distill with water; among them are dinitrophenol, EPTC, and the N-phenyl carbamates. The rate of loss as influenced by co-distillation is apparent from the previous two tables but is shown more conclusively in Table 10, where evaporative efficiency was measured. In this instance the loss of chemical as a function of water loss was measured at the same temperature. It is apparent that with rapid water evaporation the system failed to attain equilibrium between the vapors, with a consequent reduction of efficiency.

Table 10.   Co-distillation Efficiency of EPTC, Isothermal

Percent H_2O loss	Percent EPTC loss
%	%
100	8.6
85	14.1
65	25.8

In considering the loss of a herbicide from the soil due to evaporation and distillation phenomena, the rate of removal of the vapor from the few millimeters above the soil surface becomes important. If it were possible, for example, to allow the herbicide to attain its equilibrium vapor pressure immediately above the soil surface and prevent the escape of these vapors, the loss would stop. However, diffusion into the air and removal by air currents and wind proceeds continuously at the soil surface. In this case the loss of chemical is

accelerated by causing the concentration of vapors to approach zero, with a subsequent shift of the equilibrium to more and more material vaporizing.

The movement of herbicides in soils is of importance in their activity. The two major methods of movement are as a solution or as a vapor. The subject of diffusion and leaching of a material in the soil solution will be taken up later; the movement of the material as a vapor will be considered here. The movement of a vapor in a porous solid such as soil is going to be influenced by the porosity or amount of free space in the solid, the size of the pores, the amount of adsorption of the vapors by the solid surface, and the homogeneity of the medium. In a given soil the porosity and pore size will be determined in some measure by the amount of moisture. It has been found, for example, that vapor movement is much reduced in a wet soil. The diffusion or movement of a vapor in soil can thus be described by the following equation, which was derived from Fick's law of diffusion:

$$\frac{N}{At} = CD \frac{dp}{dL} \tag{15}$$

where N is the mass of the diffusing molecules, A is a cross-sectional area, t is time, $\frac{dp}{dL}$ is the rate of change of pressure along some axis, C is a constant for the soil, and D is a diffusion constant.

Examination of this equation shows us immediately that if the medium into which the gas is diffusing is not isotropic the factor $\frac{dp}{dL}$ may approach a constant in one direction but the change in another direction could be essentially zero. Thus, if a vapor-tight barrier was encountered by the diffusing gas, the concentration of vapor would rapidly build up to where the pressure at the barrier was the same as the pressure at the point from which the gas was diffusing. Under this situation, the gas then would tend to diffuse in the line of least resistance and a greater amount of it would diffuse in this direction. In the soil, one may find hard pans and clay barriers below the point of application which have reduced permeability to the gas. In this case, the downward movement of the gas is restricted so that more and more of the gas will tend to diffuse toward the soil surface and escape there through air movement.

It is sometimes assumed that vapors of herbicides, because of their greater molecular weight than air, rapidly settle to lower elevations. This concept is supported by the barometric formula

$$P = P_0 e^{\frac{-Mgx}{RT}} \tag{16}$$

where P is pressure at some point x, P_0 is the vapor pressure, M is the molecular weight, g the acceleration due to gravity, x the distance or length, R the gas constant, and T the temperature in degrees absolute.

This equation indicates that a gas will distribute itself in a uniform gravitational field according to its molecular weight. Thus, the greater concentration of gas of higher molecular weight will be found at lower elevations. However, it is important to note that due to the kinetic motion of the gas molecules, a finite pressure will be found at any height. Hence, while gases do tend to concentrate in a gravitational field, one does not find as distinct layering as is encountered with heavy immiscible liquids when added to water. Thus, the concept that vapors of gases readily sink down through the soil displacing the soil atmosphere is much too naive.

Adsorption as it relates to diffusion of gases is a matter of reducing concentration or of material available for movement. The adsorption phenomenon described in the foregoing section under adsorption applies equally well to vapors as to solutes. Of particular interest in the case of the gases, however, is the fact that many of the aliphatic compounds have but weak adsorption forces to interact with the soil surfaces and are therefore readily displaced by water.

Leaching of Herbicides

The movement of herbicides in soil in association with water is a phenomenon of considerable importance in determining the effectiveness of these materials (Del Pozo, 1959; Gantz and Slife, 1960; Hanks, 1946; Hartley, 1960; Holstrum and Loomis, 1956; Logan, Odell, and Freed, 1953; Massini, 1961; Ogle and Warren, 1954; Thornwaite, Mather, and Nakamura, 1960; Upchurch and Pierce, 1957). For the most part, the downward movement or leaching of a chemical is given the first consideration. However, lateral movement in the soil with water and even upward movement is of great importance. The upward movement, which is a result of mass transfer of water upward under the influence of evaporation from the surface, may concentrate a chemical at the soil surface, thus effectively removing it from the root zone. This concentration at the surface may result in poor weed control or, depending on the chemical, may result in a situation where the phytotoxicity remains too long. Concentration at the surface as a result of evaporation of water is not infrequently experi-

enced in row or furrow irrigation or where subsoil water is abundant. The movement of water downward in soil is thought to be in the form of film and is produced by the combined effects of capillary forces and gravitational forces. Upward movement of the water, as in the case of evaporation from the surface, is the net difference between capillary forces and the gravitational forces. Of particular pertinence to the problem considered here is the rate of permeation and leaching of a given mass of water.

The chemicals of interest in this discussion are applied to the surface of the soil or mixed rather shallowly in the soil. As water arriving at the surface of the soil penetrates, it encounters the sheet of applied chemical, dissolving and carrying the chemical with it as it percolates through the soil. The displacement of the chemical under rapid percolation of water is predominantly with the bulk of the water solution. Counteracting this downward movement is the tendency of isodiametric diffusion of the chemical in solution. Where the water percolation is rapid, the bulk movement of chemical will be in direction of water flow, but as water percolation becomes slower and slower, that is, the mass of water passing through a given point in the soil, diffusion becomes an even greater factor in determining the ultimate distribution of the chemical in the soil profile.

As the chemical is carried through the soil profile by movement of water, it is in localized equilibrium between the dissolved phase and the adsorbed phase. That is to say, some of the chemical molecules in solution are adsorbed on the surface of the soil particles and others are released. This equilibrium is being constantly re-established as the chemical moves down through the soil. However, it should be noted that at no time will all of the chemical be removed from the solution by adsorption on the surface of the soil particles.

The process of leaching a chemical through the soil profile may be considered to be analogous to chromatography. By this analogy, it would be expected that after a given amount of solvent had passed a given point in the soil profile, it would have carried at least a portion of the chemical down through the soil profile. In this instance there should be some point in the soil profile where a maximum concentration of the chemical would be found.

Such has been found to be the case with several chemicals as reported by different authors (Gantz and Slife, 1960; Sherburne, Freed, and Fang, 1956; Smith, Feldman, and Stone, 1957; Upchurch and Pierce, 1957). The "wave" movement of a solute through the soil profile by water, giving rise to bands of high concentration, has been noted with chemicals other than herbicides. The "velocity" of this movement in terms of depth of penetration as a function of

amount of applied water was described for monuron in terms of the following equation (Freed, 1958):

$$y = x \, e^{\frac{-\Delta H}{RT}} \tag{17}$$

where y = depth in inches for maximum concentration, x = inches of water, ΔH = enthalpy of adsorption, T = temperature (degrees absolute), and R = gas constant in calories.

The movement of strontium 90, on the other hand, has been described in terms of the number of cycles of water (Thornwaite, Mather, and Nakamura, 1960).

The most rapid and probably most predominant movement of dissolved solutes in the soil is with the mass water movement. It would seem to follow from this that both the direction and path of the movement of chemicals in the soil will be those followed by the water. Thus, it would be expected that the bulk chemical travel would be in the water-flow lines found in the soil.

The amount of herbicide carried into the soil by water passing through the sheet of chemical applied to the surface will depend upon the solubility of the chemical (Hartley, 1960), the amount of water available to effect solution, and the rapidity with which the water moves through the chemical zone. The solubility of a chemical under conditions of equilibrium with the solid or liquid phase is a function of temperature and latent heat of solubility.

$$Log \, S = \frac{\Delta H}{RT} + C \tag{18}$$

S = solubility at equilibrium, ΔH = latent heat of solubility, T = absolute temperature, and R = molar gas constant in calories.

The rate or speed at which the chemical dissolves under field conditions is a function of not only the foregoing factors but the state of dispersion as well. Thus, with the same amount of chemical, increasing the extent of surface area, as with small particles, will increase the rate of solution and thus enhance the rate of penetration into the soil.

The amount of chemical carried in the soil in any case is going to be proportional to the amount of water available to dissolve the chemical and carry it into the soil. Temperature will be a deciding factor in the solution of material. In an instance such as with EPTC, a lower temperature will mean a greater amount of chemical will be dissolved. The converse is true of most other organic herbicides.

Consideration of the leaching process suggests that since the solution of at least trace amounts of chemicals is very rapid, limited quantities of the chemical should be found near the front of the perco-

lating water. This has been difficult to demonstrate because of the relative insensitivity of many of the methods of detection available for this type of study. The advent of radiochemical assay and extremely sensitive chemical methods has permitted the demonstration of the validity of this assumption. Table 11 clearly supports this point of view in presentation of data in a case of triazines and chlorinated benzoic acid. It may be noted also that the findings of Upchurch and Pierce (1958) in the study of leaching of monuron in soil further support the data presented in Table 11. Thus, it becomes apparent that a small amount of chemical does follow the water front. The point of highest concentration, however, will be a function of both soil type and the strength of the bond between the chemical and the soil.

Table 11. LEACHING OF CHEMICALS IN SOIL

Compound	Soil type	Water added	Depth maximum penetration	Depth maximum concentration
		inches	*inches*	*inches*
Monuron	Loam (15% H_2O)	1	1.75	0.25
	Loam (15% H_2O)	3	5.75	2.0
	Clay (15% H_2O)	1	1.65	0.25
	Clay (15% H_2O)	3	4.50	1.75
2,3,6-TBA	Loam	3	12.0	8.0
	Peat	3	12.0	2.0
Simazine	Loam	12	7.0	1.0
Atrazine	Loam	12	12.0	7.0

However, it should be noted that the zone of higher concentration becomes more diffuse as the amount of leaching increases. Thus, at the surface of the soil prior to leaching, one might find an extremely high concentration in the surface quarter inch. Following leaching by one inch of water, the zone of highest concentration may be at the two-inch depth, where the concentration will be approximately one-half that of the zero-depth concentration. After leaching with four inches of water, one might find a maximum concentration six inches deep in the soil profile but at about one-eighth the original surface concentration. However, throughout the entire soil profile one would find varying amounts of chemical both above and below the zone of maximum concentration.

The depth of maximum concentration of chemical in the soil profile would be predicted to be independent of the surface application on the basis of the theory of leaching. This is true with benzoic acids, as illustrated in Table 12. In this instance identical columns of soil were treated with one, two, and four x rates of 2,3,6-TBA. The columns

were leached under identical conditions with the same amount of water, and the percentage of the amount of chemical in three-inch sections of the column was determined. The percentage distribution was then calculated from the total amount of chemical in the column. It is apparent from Table 12 that the penetration is about equal for all rates of application. Likewise, Phillips (1959), in studying the distribution of benzoic acids under field conditions, found this to be true. The absolute amount of chemical at this zone of maximum concentration is, of course, directly dependent upon the surface application.

Table 12. Depth of Penetration as a Function of Rate of Application of 2,3,6-TBA[1]

Column section	Rate	Percentage of total
		%
1	1 ×	-----
2		20.4
3		20.4
4		59.2
1	2 ×	15.4
2		10.5
3		26.8
4		47.3
1	4 ×	20.1
2		12.3
3		31.5
4		36.1

[1] Del Pozo, 1959.

Under static conditions of soil moisture or where the percolation rate is very slow, diffusion may tend to become a very important factor in determining the distribution of the chemical in the soil profile. Thus diffusion would be expected to cause a redistribution in the soil profile, perhaps flattening the curve of maximum concentration and giving a more uniform distribution throughout the soil profile. Such an interpretation appears to be the case in the work presented by Logan, Odell, and Freed (1953) with IPC. It was demonstrated that the IPC was redistributed upon standing after leaching the columns.

The intensity and frequency of moisture or rainfall would markedly influence the distribution of chemical in the soil profile (Thornwaite, Mather, and Nakamura, 1960). Thus, the amount of chemical and the depth to which the chemical penetrates depend upon the amount of water percolating through the soil profile to the depth of

reference. If the intensity factor is low, the rate of percolation of water will be low also; there will be ample time for redistribution of the concentration of chemical through the diffusion process. The frequency with which water is applied to the soil surface will also be a factor in the sense that as the interval between water applications increases there is more opportunity for diffusion and for redistribution through evaporation of water from the soil surface. The very excellent treatise of Upchurch and Pierce (1958) on leaching of monuron clearly demonstrates this to be the case.

Soil type is an extremely important factor in determining the rate and extent of leaching of a given chemical. Thus, in the equation given by Sherburne, Freed, and Fang (1956) (see also Freed, 1958), the distribution in the soil was determined by the strength of binding through adsorption. Hence, with a given amount of moisture to leach the chemical in different soil types, it is obvious that the greater amount of chemical will leach to a greater depth in sandy soils than in clays or mucks. However, closer examination of this situation suggests the possibility that when starting with air-dry soil, if sufficient moisture is applied to wet the soils to field capacity to an equal depth, then the chemical will penetrate to about an equal depth in all soil types. Del Pozo (1959) confirmed this deduction in a study of the leaching process.

Examination of the foregoing data suggests that by specifying soil type, amount of moisture, rate of percolation, and certain physical properties of the chemical, the leaching of the chemical through the soil profile may be predicted at least in a qualitative way. Thus, the velocity of leaching is determined by the amount of water for leaching and the binding energy of adsorption, which was shown previously to be related in a general way to the latent heat of solubility. The absolute amount of chemical carried into the soil under a given set of conditions will be dependent upon this factor and on the absolute solubility of the parent compound.

Loss of Herbicides Through Decomposition in the Soil

The final consideration in the behavior of herbicides in soil has to do with loss of these materials through decomposition. This decomposition may arise from photodecomposition in surface applications, chemical reaction, or biological attack (Audus, 1951, 1952; Blouch and Fults, 1953; Bollen, 1961; Hill et al., 1955). The susceptibility of a compound to photodecomposition is dependent on the compound's make-up, which determines its ultraviolet-absorbing ability and the effect of this added energy on different bonds in the molecule. Hetero-

cyclic compounds and compounds containing nitrogen appear to be particularly susceptible to this type of attack.

Loss of biological activity of a chemical in the soil through chemical reaction is determined by the relative susceptibility of that chemical to undergo reactions either in solution or in the adsorbed phase. Many chemicals will undergo oxidation or hydrolysis under these conditions. The case for microbiological decomposition is well documented for a number of herbicides. Such compounds as the aryl carbamates, phenoxyacetic acids, phenylureas, and triazines have all been shown to undergo microbiological degradation.

The point to be discussed relative to decomposition of herbicides in soil is that of the physical chemistry of the process. It was reasoned by Burschel and Freed (1959) that this loss should follow a first-order rate law. It may be adduced that since the soil, the moisture, and the microorganisms of the soil are in such abundance, the rate limiting concentration should be that of the herbicide itself. Exploration of this concept for IPC, CIPC, and amitrole demonstrated that it was valid for these cases. It was shown that a parameter, namely the enthalpy of activation, could be derived from the data obtained. This parameter can be used in predicting the extent or length of residual life of a compound in the soil where decomposition was a major factor in loss of activity. This has been subsequently substantiated using other materials (Burnshide, Schmidt, and Behrens, 1961; Burschel, 1961).

References

Ashton, F. M., and T. J. Sheets. 1959. The relationship of soil adsorption of EPTC to oats injury in various soil types. Weeds, 7:88.

Audus, L. J. 1951. The biological detoxication of hormone herbicides in soil. Plant and Soil, 3:170.

Audus, L. J. 1952. The decomposition of 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid in the soil. Jour. Sci. Food and Agric., 3:268.

Blouch, R., and J. Fults. 1953. The influence of soil type on the selective action of chloro-IPC and sodium TCA. Weeds, 2:119.

Bollen, W. B. 1961. Interactions between pesticides and soil microorganisms. Annual Review of Microbiology, 15:69.

Burnshide, O. C., E. L. Schmidt, and R. Behrens. 1961. Dissipation of simazine from the soil. Weeds, 9:477.

Burschel, P., and V. H. Freed. 1959. The decomposition of herbicides in soils. Weeds, 7:157.

Burschel, P. 1961. Experiments on the retention of simazine in the soil. Weed Res., 1:131.

Coggins, C. W., Jr., and A. S. Crafts. 1959. Substituted urea herbicides: their electrophoretic behavior and the influence of clay colloid in nutrient solution on their phytotoxicity. Weeds, 7:349.

Del Pozo, J. 1959. Determination of the adsorption and movement of 2,3,6-trichlorobenzoic acid in soil as influenced by soil type and water percolation. M.S. thesis, Oregon State Univ., Corvallis.

Ennis, W. B. Jr. 1954. Some soil and weather factors influencing usage of pre-emergence herbicides. Proc. Soil and Crop Sci. Soc. Fla., 14:130.

Fang, S. C., P. Theisen, and V. H. Freed. 1961. Effects of water evaporation, temperature and rates of application on the retention of ethyl-N,N-di-n-propylthiocarbamate in various soils. Weeds, 9:569.

Freed, V. H. 1951. Some factors influencing the herbicidal efficacy of isopropyl N-phenylcarbamate. Weeds, 1:48.

Freed, V. H. 1958. The chemistry and reactions of herbicides as related to their use. Proc. Western Weed Control Conf., 16:18.

Frissel, M. J. 1961. The adsorption of some organic compounds, especially herbicides, on clay minerals. Versl. Landbouwk. Onderz., 67:3, 54 pp.

Gantz, R. L., and F. W. Slife. 1960. Persistence and movement of CDAA and CDEC in soil and the tolerance of corn seedlings to these herbicides. Weeds, 8:599.

Glasstone, S. 1946. Textbook of Physical Chemistry, 2d ed. New York: D. Van Nostrand Company.

Goring, C. A. I. 1957. Factors influencing diffusion and nematode control by soil fumigants. Dow Chemical Company ACD Info. Bull. 110.

Hanks, R. W. 1946. Removal of 2,4-dichlorophenoxyacetic acid and its calcium salt from six different soils by leaching. Botan. Gaz., 108:186.

Hartley, G. S. 1960. In Herbicides and the Soil, E. K. Woodford and G. R. Sagar, eds. Oxford: Blackwell Scientific Publications.

Hill, G. D., J. W. McGahen, H. M. Baker, D. W. Finnerty, and C. W. Bingeman. 1955. The fate of substituted urea herbicides in agricultural soils. Agron. Jour., 47:93.

Hollingsworth, E. B. 1954. Effect of soil pH on activity of DNOSBP. Proc. Southern Weed Conf., 7:212.

Holstrun, J. T., Jr., and W. E. Loomis. 1956. Leaching and decomposition of 2,2-dichloropropionic acid in several Iowa soils. Weeds, 4:205.

Jurinak, J. J., and N. Bauer. 1956. Thermodynamics of zinc adsorption on calcite, dolomite and magnesite-type minerals. Soil Sci. Soc. Am., 20:466.

Jurinak, J. J. 1957. Adsorption of 1,2-dibromo-3-chloropropane vapor by soils. Jour. Agric. Food Chem., 5:598.

Klingman, G. C. 1961. Weed Control: As A Science. New York: John Wiley and Sons, Inc.

Leopold, A. C., P. van Schaik, and M. Neal. 1960. Molecular structure and herbicide adsorption. Weeds, 8:48.

Lichtenstein, E. P. 1959. Absorption of some chlorinated hydrocarbon insecticides from soils into various crops. Jour. Agric. Food Chem., 7:430.

127

Logan, A. V., N. R. Odell, and V. H. Freed. 1953. The use of C^{14} in a study of the leaching rate of isopropyl N-phenyl carbamate. Weeds, 2:24.

Massini, P. 1961. Movement of 2,6-dichlorobenzonitrile in soils and in plants in relation to its physical properties. Weed Res., 1:142.

Phillips, W. M. 1959. Residual herbicidal activity of some chloro-substituted benzoic acids in soil. Weeds, 7:284.

Ogle, R. E., and G. F. Warren. 1954. Fate and activity of herbicides in soils. Weeds, 3:257.

Robbins, W. W., A. S. Crafts, and R. N. Raynor. 1952. *Weed Control,* 2nd ed. New York: McGraw-Hill Book Co., Inc.

Shaw, W. C., J. L. Hilton, D. E. Moreland, and L. L. Jansen. 1960. *In* Nature and Fate of Chemicals Applied to Soils, Plants, and Animals, USDA, Agric. Res. Serv., 20-9:119.

Sherburne, H. R., and V. H. Freed. 1954. Adsorption of 3-(p-chlorophenyl)-1, 1-dimethylurea as a function of soil constituents. Jour. Agric. and Food Chem., 2:937.

Sherburne, H. R., V. H. Freed, and S. C. Fang. 1956. The use of C^{14} carbonyl labeled 3-(p-chlorophenyl)-1,1-dimethylurea in a leaching study. Weeds, 4:50.

Sheets, T. J. 1959. Effects of soil type and time on the herbicidal activity of CDAA, CDEC, and EPTC. Weeds, 7:442.

Sheets, T. J., and L. L. Danielson. 1960. Herbicides in soils. *In* Nature and Fate of Chemicals Applied to Soils, Plants, and Animals, USDA, Agric. Res. Serv. 20-9:170.

Smith, A. E., A. W. Feldman, and G. M. Stone. 1957. Mobility of N-1-naphthylphthalamic acid in soils. Jour. Agric. Food Chem., 5:745.

Smith, R. J., Jr., and W. B. Ennis, Jr. 1953. Studies on the downward movement of 2,4-D and 3-chloro-IPC in soils. Southern Weed Control Conf. Proc., 6:63.

Thornwaite, C. W., J. R. Mather, and J. K. Nakamura. 1960. Movement of radiostrontium in soils. Science, 131:1015.

Upchurch, R. P., and W. C. Pierce. 1957. The leaching of monuron from lakeland sand soil. Part I. The effect of amount, intensity, and frequency of simulated rainfall. Weeds, 5:321.

Upchurch, R. P., and W. C. Pierce. 1958. The leaching of monuron from lakeland sand soil. Part II. The effect of soil temperature, organic matter, soil moisture, and amount of herbicide. Weeds, 6:24.

Warren, G. F. 1956. The relative adsorption of several herbicides by widely differing soils (abstract). No. Cent. Weed Control Conf., 13:5.

Glossary

Amiben	3-amino-2,5-dichlorobenzoic acid
Amitrole	3-amino-1,2,4-triazole
Atrazine	2-chloro-6-ethylamino-4-isopropylamino-1,3,5-triazine
Barban	4-chlorobut-2-ynyl N-(3-chlorophenyl) carbamate
Bromoxynil	3,5-dibromo-4-hydroxybenzonitrile
Casoron	2,6-dichlorobenzonitrile
CIPC	Isopropyl N-(3-chlorophenyl) carbamate
Dacthal	Dimethyl tetrachloroterephthalate
Dalapon	2,2-dichloropropionic acid
2,4-D	2,4-dichlorophenoxyacetic acid
DATC	2,3-dichloroallyl di-*iso*propylthiolcarbamate
Diuron	3-(3,4-dichlorophenyl)-1,1-dimethyl urea
DMSO	Dimethyl sulfoxide
DNBP	4,6-dinitro-2-*s*-butylphenol
Endothal	7-oxabicyclo [2,2,1] heptane-2,3-dicarboxylic acid
EPTC	Ethyl N, N-di-*n*-propylthiolcarbamate
Fenac	2,3,6-trichlorophenylacetic acid
Fenuron	NN-dimethyl-N'-phenyl urea
IAA	Indole-3-acetic acid
IPC	Isopropyl N-phenylcarbamate
MH (Maleic hydrazide)	1,2-dihydroxypyridazine-3,6-dione
MCPA	4-chloro-2-methylphenoxyacetic acid
Monuron	3-(4-chlorophenyl)-1,1-dimethyl urea
PBA	Polychlorobenzoic acid, 2,3,6-trichlorobenzoic acid; 2,3,4,5-tetrachlorobenzoic acid and isomers
PEBC	Propyl ethyl-*n*-butylthiolcarbamate
Silvex	2-(2,4,5-trichlorophenoxy) propionic acid
Simazine	2-chloro-4,6-bis(ethyl amino)-1,3,5-triazine
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
2,4,5-T LVE	2,4,5-trichlorophenoxyacetic acid, low volatile ester
2,3,6-TBA	2,3,6-trichlorobenzoic acid

Administrative and Research Personnel

ARIZONA AGRICULTURAL EXPERIMENT STATION
R. K. Frevert, Director, Tucson, Arizona
R. F. Wagle, W-63 Committeeman, Tucson, Arizona

CALIFORNIA AGRICULTURAL EXPERIMENT STATION
C. F. Kelly, Director, Berkeley, California
C. L. Foy, W-63 Committeeman, Davis, California

COLORADO AGRICULTURAL EXPERIMENT STATION
Rue Jensen, Director, Fort Collins, Colorado
M. A. Ross, W-63 Committeeman, Fort Collins, Colorado

IDAHO AGRICULTURAL EXPERIMENT STATION
J. E. Kraus, Director, Moscow, Idaho
L. C. Erickson, W-63 Committeeman, Moscow, Idaho

MONTANA AGRICULTURAL EXPERIMENT STATION
J. A. Asleson, Director, Bozeman, Montana
L. O. Baker, W-63 Committeeman, Bozeman, Montana

NEVADA AGRICULTURAL EXPERIMENT STATION
D. W. Bohmont, Director, Reno, Nevada
and Administrative Adviser of W-63
H. P. Cords, W-63 Committeeman, Reno, Nevada

NEW MEXICO AGRICULTURAL EXPERIMENT STATION
P. J. Leyendecker, Director, University Park, New Mexico
J. W. Whitworth, W-63 Committeeman, University Park, New Mexico

OREGON AGRICULTURAL EXPERIMENT STATION
G. B. Wood, Director, Corvallis, Oregon
V. H. Freed, W-63 Committeeman, Corvallis, Oregon

UTAH AGRICULTURAL EXPERIMENT STATION
K. W. Hill, Director, Logan, Utah
D. C. Tingey, W-63 Committeeman, Logan, Utah

WASHINGTON AGRICULTURAL EXPERIMENT STATION
Mark T. Buchanan, Director, Pullman, Washington
T. J. Muzik, W-63 Committeeman, Pullman, Washington

WYOMING AGRICULTURAL EXPERIMENT STATION
Neal W. Hilston, Director, Laramie, Wyoming
E. Chamberlain, W-63 Committeeman, Laramie, Wyoming

UNITED STATES DEPARTMENT OF AGRICULTURE
A. J. Loustalot, Cooperative State Research Service, Washington, D. C.