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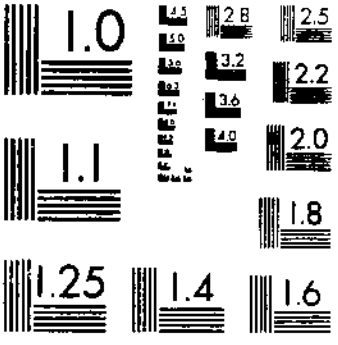

MICROCOPY RESOLUTION TEST CHART
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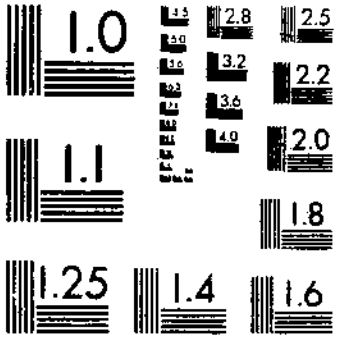

MICROCOPY RESOLUTION TEST CHART
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ECOLOGICAL AND PHYSIOLOGICAL FACTORS INFLUENCING CHEMICAL CONTROL of *HALOGETON GLOMERATUS*

By
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CONTENTS

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ECOLOGICAL AND PHYSIOLOGICAL FACTORS INFLUENCING CHEMICAL CONTROL OF *HALOGETON GLOMERATUS* [1]

By EUGENE H. CRONIN, *plant physiologist, Crops Research Division, Agricultural Research Service*

INTRODUCTION

Halogeton glomeratus (M. Bieb.) C. A. Mey., a poisonous annual weed, was introduced into the cold desert region of North America sometime before 1934. The first collection was made near Wells, Nev., in 1935 (*48*) [2] and the plant has become established on more than 10 million acres of western rangeland.

The plant is poisonous to livestock. The toxic substances in halogeton are sodium and potassium oxalates that account for 17 to 30 percent of its dry weight, depending on the season (*6, 12, 25, 45*). Since the plant is a pioneer invader on disturbed sites in the salt-desert shrub vegetation of the intermountain area, it is an important poisonous weed with which the livestock industry must contend on winter ranges. Fortunately, the palatability of halogeton is extremely low, and it is seldom eaten by livestock except where it is one of the few sources of forage. However, Cook and Stoddart (*7*) found that even when halogeton was a major part of the vegetation, sheep did not consume large amounts unless the seed remained on the plant.

The prolific seed production of the halogeton plant was recognized as early as 1943 by Holmgren (*19*) and later by Bohmont (*4*); Tisdale and Zappettini (*43*); Cook and Stoddard (*7*); Palmer and coworkers (*28*); Rauchfuss, Bohmont, and Beetle (*30*); and many others. The production of two types of seeds, discussed by Zappettini (*48*), Tisdale and Zappettini (*43*), Holl (*18*), and Williams (*45*), is undoubtedly important in the spread and persistence of the plant throughout the cold desert region of North America.

Cook and Stoddart (*7*) found that halogeton seeds retain a high degree of viability even after passing through the digestive tracts of sheep and rabbits, and that these animals are capable of scattering large quantities of seeds over great distances by consuming them and passing them with fecal material. Holmgren (*19*) reports, "As a

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[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, and the Utah Agricultural Experiment Station, Logan, Utah.

Portions of this manuscript were submitted as a dissertation in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Utah State University.

[2] Italic numbers in parentheses refer to Literature Cited, p. 63.

roadside weed, the plant has been rapidly spread by highway equipment, especially road graders, which pick up myriads of seed in heavily infested areas and then plant them along the way." Tisdale and Zappettini (*43*) presented data indicating that movement of the seed by the wind was limited to about 450 feet, and existing vegetation further limited its movement.

I have observed that "whirlwinds or dust-devils," common to the infested areas, can transport small sections of dry-stem material containing large amounts of seed for distances far in excess of 450 feet. In one instance, the distance was probably more than 2 miles. The size of the halogeton infestation and the importance of the area to the livestock industry as winter rangeland make it imperative that methods of controlling halogeton be developed.

Three methods of controlling halogeton exist: (1) Revegetation of infested areas, (2) biological control by grazing management or by introduction of insects or other parasitic organisms, and (3) chemical control. A combination of these methods will probably prove to be the final solution to the problem.

Reseeding some infested areas with crested wheatgrass is feasible and desirable. But Stoddart and coworkers (*42*) and Rauchfuss, Bohmont, and Beetle (*30*) recognized that reseeding the areas where most of the halogeton infestation occurs has not been successful because of low rainfall and the high salt content of the soil (*15, 34*). On sites that are adjacent to areas infested with halogeton, that have at least 12 inches of rainfall, and that support an *Artemisia tridentata* (sagebrush) community, reseeding with crested wheatgrass, when successful, appears to be of value in relieving grazing pressure on infested sites. Revegetation of infested areas with native perennial shrubs would seem even more desirable than with grass species, but little information is available on propagating these shrubs.

Improved grazing management has effectively reduced livestock losses to halogeton. Perennial shrubs and grasses can recover and replace annual weeds in the depleted desert range under proper grazing levels (*20*). Recovery of depleted desert ranges is slow, and the rate of recovery depends on the level of depletion, climatic conditions, and management. Halogeton infestation varies from sites where it is present as a few scattered plants to sites where it is dense and constitutes more than 90 percent of the vegetation. Livestock losses are most likely to occur on sites having dense stands. These sites are usually avoided by herders. Thus, these areas do not contribute to livestock production and serve as a source of seed for other sites where vegetation is removed, reduced in vigor, or otherwise altered. Recent investigations (*13*) have shown that halogeton can alter the physical and chemical properties of the soil. The changes in the soil properties affect germination of seeds (*24, 41*), favoring germination of the less desirable plants, especially the seeds of halogeton.

Chemicals are valuable tools in revegetation since they selectively reduce competition for water by halogeton and other weedy species. This is especially important in revegetation or as an aid to recovery of existing vegetation in areas where water limits growth and survival

of plants. Chemical control of halogeton requires an understanding of the plant's life cycle, its response to various environmental stimuli, its anatomy, and its response to herbicides.

THE LIFE CYCLE OF *HALOGETON GLOMERATUS*

Halogeton is an annual plant that disperses its achenes in the fall. In Utah, germination begins any time after the middle of December when favorable microenvironmental conditions exist. Seedlings (figs. 1 and 2) become established in three general types of habitats: (1) Under mats of the bracts from the black achenes that collect in areas protected from the wind, (2) in cracks between the polygon-shaped soil plates, and (3) on the soil surface. Conditions favorable to germination usually occur first under mats of the bracts, then in cracks, and finally on the soil surface. Seedlings may become established any time during the growing season, provided they receive adequate moisture.

Seedlings established between January and the first of May do not develop beyond the seedling stage until after May 1. During the first

FIGURE 1.—The epigeal cotyledons of halogeton seedlings and head of a straight pin. The lanate hairs in the axil of the first true leaves can also be found in the axil of most vegetative leaves by careful examination.

FIGURE. 2.—The leaves of these plants are starting to enlarge. The photograph was taken during the first week in May 1956.

2 weeks in May, the true leaves enlarge to form a rosette of leaves (fig. 3). Sometime during the last part of May, the lateral branches begin to elongate and form a small decumbent cruciform plant (fig. 4). Through this stage of development, the plant, with its decumbent growth habit, responds to the microenvironment near the soil surface rather than to the macroenvironment. Plants develop more rapidly on warmer sites exposed to the sun and protected from the wind than on shaded sites exposed to the wind.

During late May or early June halogeton makes rapid growth. The lateral branches become negatively geotropic and assume an erect growth habit; the central or fifth branch elongates. Toward the end of June growth slows. The available soil moisture determines both the rate and the amount of growth. Competition with other plants for soil moisture reduces rate and amount of growth.

FIGURE 3.—A rosette of halogeton leaves. The translucent bands, where the "water storage tissue" underlays the epidermis, can be seen on the ventral side of most leaves on this plant.

In late June the plant enters reproductive development, and vegetative growth nearly stops. When bracteoles can be observed with the naked eye, the plant is in the reproductive phase. Within a week a number of physiological changes also occur. These didymous, leaf-like structures are found in the axils of the vegetative leaves but lack the terminal spine. In cross section the bracteoles are reniform to cordate.

The halogeton inflorescence is a glomerate cymose panicle in which the main axis continues to elongate. The terminal glomerules are less developed than those on older portions of the plant.

Causes of flower initiation are not known, but preliminary work by Jansen [3] indicates a complex of photoperiod and night temperatures.

The achenes begin to mature in late August, and the bracts of the black fruit emerge from between the bracteoles about September 10 and nearly cover the plant (fig. 5). By the first half of October, the plant has largely dried. A majority of the black achenes have fallen from the plant by late October or early November. The brown achenes are more persistent and may remain on the plant as late as January or February.

[3] JANSEN, L. L., PERSONAL COMMUNICATION.

FIGURE 4.—Elongation of the lateral branches has started, and the acropetal development of the plant can be observed at this stage. The first branch formed is in the lower left of the photograph, and the second branch is on the opposite side of the plant. Branches 3 and 4 are at right angles to the first two branches and in the upper left and lower right, respectively. Branch 5 is a continuation of the main axis and appears as a whorl of leaves in the center of the plant.

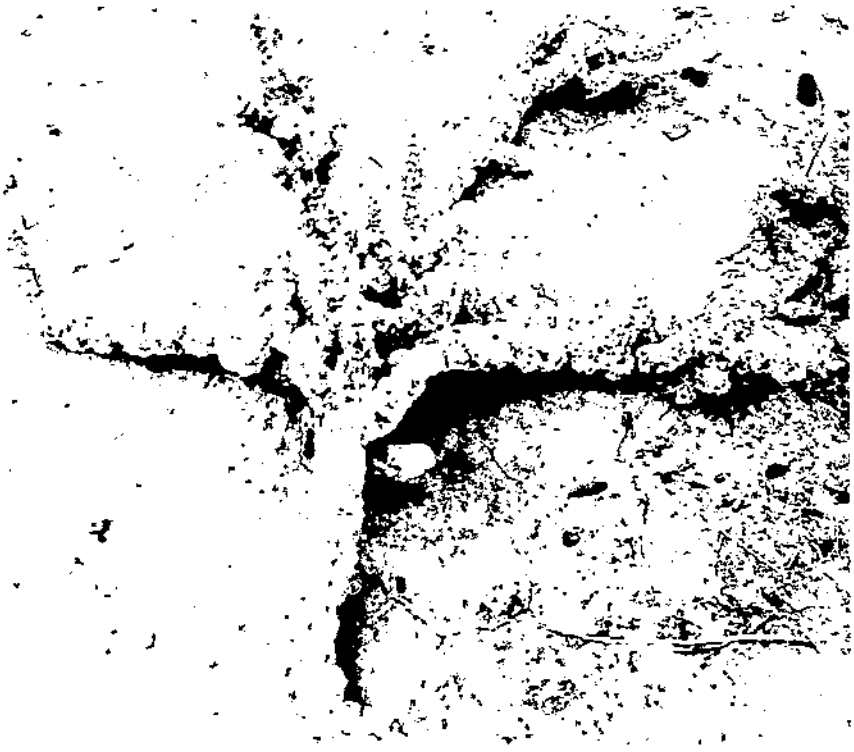

FIGURE 5.—The bracts of the black fruit nearly cover the plant in fall. The decumbent habit of the lateral branches is not typical of the species.

THE HALOGETON FRUITS

The fruits of halogeton are achenes but are popularly classed as seeds. Hereafter they will be referred to as seeds. The two types of halogeton seeds (fig. 6) can be separated according to morphological and physiological characters, as can their embryos (*19, 26, 43, 45, 48*). The seeds are attached to five member bracts, which develop from the calyx. The bracts of the black seeds are scarious (fig. 7) and become detached from the seed before or shortly after falling from the plant. The bracts of the brown seed are coriaceous, tenaciously attached to the seed, and only slightly longer than the seed (fig. 8).

The plant produces brown seeds first. They are the only ones formed until the middle of August (fig. 9), and only black seeds are formed thereafter. Seeds possessing characteristics of both types are to be expected, but the percentage of these intermediates is low.

Black seeds germinate rapidly (*5, 21, 22*). They do not persist for more than one season under field conditions (*18, 43*). The embryos escape from the seeds in as little as 15 minutes after receiving moisture. Black seeds have a short "after ripening" period. From 15 to 20 percent of seeds collected in late October germinate, but 95 to 99 percent of those collected in late November or early December germinate.

The black seeds are named for the color of the coat; the brown seeds for the color of the adhering bracts. Little is known concerning germination of the brown seeds. It is known that they respond to cold treatment, since their embryos are more vigorous after excision than are those of seeds stored at room temperature and then excised (fig. 10). In one instance, plants collected in the field during a December snowstorm were stored in a cold (35° F.), moist environment for 3

FIGURE 6.—The black seeds (upper row) are on a metric scale, and the brown seeds with pentamerous bracts (bottom row) are adjacent to the scale.

FIGURE 7.—Bracts of the black seeds. The seed is attached to the bracts in the lower left of the photograph. (Courtesy of A. H. Holmgren, Curator of the Intermountain Herbarium.)

months. At the end of this period they were covered with molds, dominated by species of *Penicillium*, *Aspergillus*, and *Rhizopus*. When the brown seeds from these plants were exposed to conditions favorable for germination, 98 percent of the embryos escaped from the seeds, uncoiled, and began to elongate. If stored at room temperature, embryos must be excised before they will germinate.

Two 10-year trends of the germination of buried seeds of halogeton are now in progress in four Western States and should result in data needed on the role of the brown seed in the perpetuation of the halogeton plant (7, 8). Germination of brown seeds buried in the soil for at least 1 year varies from 1 to 50 percent (*18*).

Seeds are the principal diet of the western harvester ant, *Pogonomyrmex occidentalis* (Cresson) (*11*), one of the most numerous insects in areas infested by halogeton. I have obtained from 5 to 20 percent germination of brown seeds recovered from the hills of this insect.

FIGURE 8. A portion of a glomerule with the papery bracts of the black seed projecting between the bracteoles, while the brown seed (in bracket) and its bracts is but one-third the length of the bracteoles. The coriaceous bracts adhere tightly to the seed.

The ants remove the bracts from the brown seeds, and this may contribute to increased germination.

L. L. Jansen [4] collected a large number of plants from Utah, Idaho, Nevada, Colorado, Wyoming, and Montana in the fall of 1954. He laboriously counted the black and brown seeds on some of the stems of each collection. He found an average of 27 brown seeds and 47 black seeds on each 1-inch section of the stem. This ratio varied with the location of the collection. In the southern areas the plants usually produced a higher proportion of brown seeds, whereas in the northern areas they produced a higher proportion of black seeds. Jansen also found a large plant that produced more than 40 meters of dried stems. This plant could have produced 42,500 brown fruits and 74,000 black fruits, or a total of 116,500 fruits.

[4] JANSEN, L. L., PERSONAL COMMUNICATION.

FIGURE 9.—Halogeton plant that became established between August 12 and 16, 1954. It was in full bract by the first week in October, when the photograph was made. Only black seeds were produced.

Osmotic pressure of the medium and its relation to seed germination

Halogeton's salt tolerance can be observed on the shores of the Great Salt Lake, where it grows at the base of piles of crude salt (fig. 11). Williams (*46*) found that halogeton made its best growth when supplied with sodium chloride. Much interest exists in the problem of establishing various plants, especially species of grasses, in halogeton-infested areas. Eckert and Kinsinger (*13*) found that the leachate from halogeton deposited the chemical constituents, principally sodium, in the soil.

The objective of the study reported here was to compare the tolerances of germinating seeds of halogeton and some of the associated plants to increasing concentrations of osmotic pressure of the media.

To separate osmotic pressure effects from ion toxicity, the vapor equilibrium techniques of Arcichoviskij and Arcichovaskaja (*1*), as modified and described by Slatyer (*40*), were used. A solution of known osmotic concentration was added to petri dishes until it formed a thin layer over the bottom of the dish. A ring with a plastic screen top was placed in the dish, and the seeds being tested were placed on top of the screen. This arrangement prevented direct contact between the seeds and the solution. The dish was then covered and placed

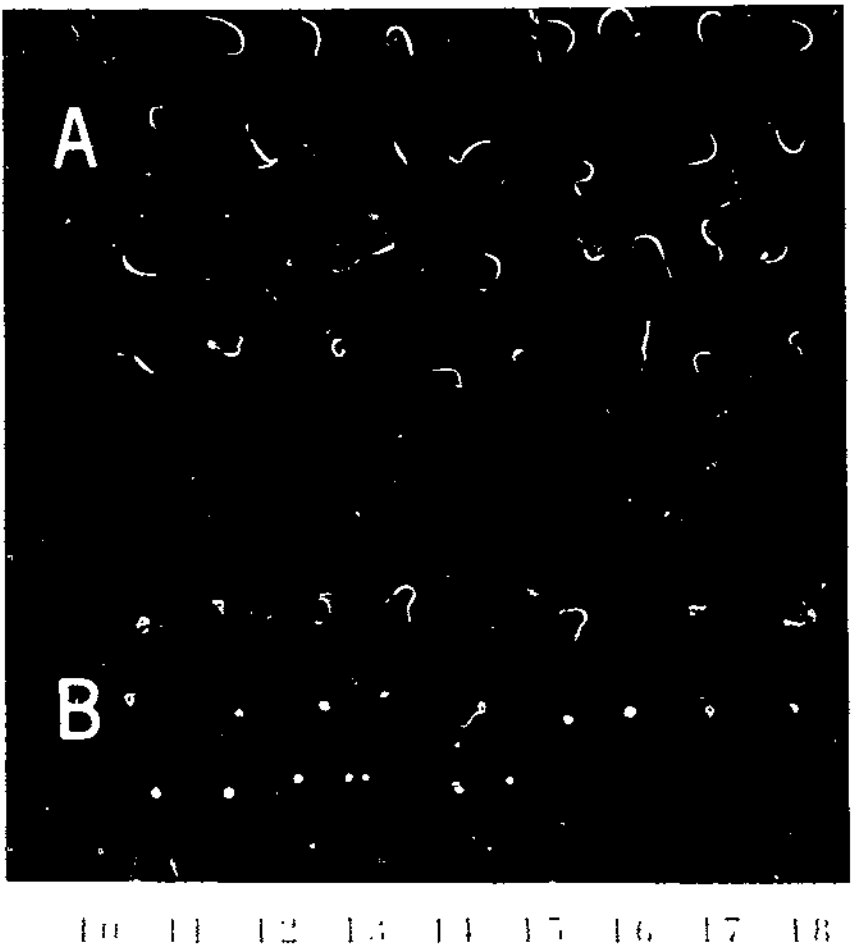

FIGURE 10.—A, Response of embryos of brown seeds of halogeton to cold treatment (stored 1 month at 4° C.); *B*, response of embryos stored at room temperatures. Embryos of both groups of seed were excised and placed on moist filter paper for 4 days. (Millimeter scale is shown at bottom.)

in a well-agitated water bath maintained at 24°±0.01° C. Maintenance within such narrow limits prevented condensation of the vapor in the petri dishes. The seeds remained in this environment for 6 days. A few samples were discarded when rough handling caused moisture to adhere to the screen or to the top of the petri dishes.

Solutions of known osmotic concentrations were prepared with sodium chloride. Concentrations of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 15 atmospheres were used in this study. Ten seeds of a species were placed in each petri dish. The seeds used were Fairway crested wheatgrass (*Agropyron cristatum*) and peppergrass (*Lepidium perfoliatum*) collected in September 1956, and halogeton (seeds) collected in November and December 1956.

FIGURE 11.—Group of halogeton plants growing at the base of a pile of crude salt on the north shore of the Great Salt Lake. The surface inch of soil collected from among these plants contained 4 percent of soluble salt.

After 6 days in the constant temperature water bath, the petri dishes were opened and the seeds divided into the following groups: (1) Seeds dry in appearance, (2) seeds moist and swollen, (3) germination initiated but not complete, (4) germination complete and seedlings normal, and (5) germination complete and seedlings abnormal. The criteria used to separate normal and abnormal seedlings were those outlined by Justice and Reece (*22*). Germination of halogeton and peppergrass was not considered complete until the embryo had escaped from the seedcoat, uncoiled, and started to elongate. Germination of crested wheatgrass was considered complete when root hairs could be observed on the primary root.

To check the viability of the seed samples, a 15-day test at room temperature with 200 seeds of each species was completed. The seeds were placed on blotters in petri dishes moistened with distilled water containing a fungicide (0.002 percent of methyl mercury dicyanidamide). The seeds were incubated at 25° C. for 15 days, and seedlings were counted and removed at 3-day intervals (table 1). To have prolonged the vapor equilibrium test beyond 15 days would have allowed the fungi to destroy many of the healthy seedlings. Repeated handling to remove moldy seed or seedlings would have increased the number of samples that were discarded because of splashing of the solutions in the dishes. Data from the 15-day tests are included to indicate the potential germination of the seed samples. Six days of

favorable temperatures and moisture for germination, or an even shorter period, is not atypical of the environment encountered on the desert. The data from this experiment are tabulated in table 2.

Germination of halogeton seeds collected in November was obtained at concentrations as high as 9 atmospheres. Some of the seeds had initiated germination at all concentrations tested, and most of them were able to absorb water at all concentrations.

Fairway crested wheatgrass did not germinate in the range of 6 to 10 atmospheres concentration, but germination was initiated as high as 9 atmospheres. The seeds were able to obtain water at concentrations as high as 9 atmospheres.

TABLE 1.—*Germination of seeds on filter paper moistened with distilled water containing 0.002 percent of methyl mercury dicyandiamide and incubated for 15 days*

Species [1]	Seeds failing to germinate	Seedlings produced	
		Normal	Abnormal
	Percent	*Percent*	*Percent*
Crested wheatgrass	13. 0	73. 0	14. 0
Peppergrass	17. 0	81. 0	2. 0
Halogeton:			
Seed collected November 1956	31. 5	62. 0	6. 5
Seed collected December 1956	1. 0	86. 5	12. 5

[1] 200 seeds of each species used in test.

Germination of peppergrass was complete only over distilled water. Germination was initiated at concentrations as high as 7 atmospheres, and the seeds were not able to obtain water above 8 atmospheres during the 6 days of the test.

The data from this study imply that halogeton seeds are capable of germinating quicker and with less soil moisture than either Fairway crested wheatgrass or peppergrass. Apparently halogeton seeds would be able to obtain water for germination from more concentrated soil solutions than would the two other species tested and should be more competitive for subsequent available moisture.

Establishment and survival of seedlings of halogeton under field conditions

Field observations indicated that halogeton seedlings became established early in the spring, but the mortality rate for these early seedlings was high. These periods of seedling establishment and mortality were obviously related to the weather. The seeds of halogeton are produced in such large numbers that the plant is capable of perpetuating itself in spite of periods of high or even total seedling mortality. The brown seeds make it possible for the plant to survive through 1 or more years of total seedling mortality.

Since halogeton is an annual dependent on seeds for its perpetuation, a control program should be designed to eliminate the seed-producing crop. Therefore, an experiment was designed to determine the date or dates when the fruit-producing crop becomes established as seedlings in the field. An "established seedling" is used here to designate a seedling that has its root in the soil and its cotyledons expanded. It seems more accurate to discuss "established seedlings" than seed germination, since germination could occur in the soil and could not be observed or recorded.

Permanent plots ranging in size from 3 square inches to 144 square inches were used in the experiment. Each seedling was marked by colored toothpick, pin, or wire to designate the date it was found. It was planned to visit the plots every second week, but sometimes stormy weather made a visit impossible or a period of warm moist weather made it desirable to reduce the interval between visits to 1 week. The work was done in the spring of 1953 and repeated in the springs of 1955 and 1957. Some data are also available for the springs of 1954 and 1956, but they are not complete.

Tisdale and Zappettini (*43*) and Holl (*18*) have reported data concerned with date of germination of halogeton in the field, but not on the methods used to obtain the data. It is probable they used the loop procedure reported by Sharp (*37*).

Of all seedlings marked, 39 percent survived in 1953, 68 percent in 1955, and 43 percent in 1957 (table 3). Plants established after the first week in June represented 0.4 percent of the fruit-producing plants in 1953 and 1.4 percent in 1955. In 1957, six seedlings in the plots became established during the last week in August. I was not in Utah after the 9th of September, and do not know if these plants produced seeds. Since it is doubtful that they could have completed their life cycle, the data are not included in table 3.

The earliest date on which seedlings were found in the plots each year ranged from February 27 in 1953, to April 15 in 1955. Established seedlings were found in the plots on March 2, 1954, on March 16, 1956, and on March 2, 1957. The date of establishment appears to be a response to warm daytime temperatures and enough moisture. The microenvironment is probably more important than the actual weather conditions. Freezing night temperatures do not appear to affect the seedlings adversely, since night temperatures in March and early April are often in the low 20's on the Fahrenheit scale. Dry weather, especially when accompanied by wind, probably accounts for most of the seedling mortality during the spring months.

A majority of the seed-producing plants of halogeton are established in March and April. The first week in June brings a large measure of stability to the population. This stability is probably due more to intraspecific competition than to climatic factors. The plants already established are apparently able to compete much more successfully for the available moisture. This has been demonstrated on many occasions. Where the older plants were removed by mechanical or chemical means, new plants often became readily established. The density of the new population depends on the moisture available on the particular site. Summer rain showers typically permit new plants to become established in the absence of competing plants.

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TABLE 2.—*Germination of halogeton, Fairway crested wheatgrass, and peppergrass seeds as affected by osmotic pressure of the medium*

Kind of seed, and osmotic pressure of medium (atmospheres)	Seeds dry in appearance	Seeds moist and swollen	Germination initiated but not complete	Germination complete		Total seeds tested
				Seedlings normal	Seedlings abnormal	
Halogeton:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*
Collected November 1956:						
0	0	74	12	10	4	50
6	0	74	18	8	0	50
7	0	66	14	20	0	50
8	0	76	22	0	2	50
9	0	70	22	8	0	50
10	2	66	29	0	2	50
Collected December 1956:						
0	0	1	3	95	1	320
1	0	14	5	79	1	160
2	1	11	10	74	4	160
3	0	21	25	52	2	160
4	1	14	19	66	0	160
5	6	17	21	53	3	150
6	3	16	27	53	1	150
7	3	48	25	24	0	150
8	7	46	27	20	0	100
9	1	71	28	0	0	100
10	12	79	9	0	0	100
15	0	91	9	0	0	100

Fairway crested wheatgrass:						
0	0	60	16	24	0	50
6	14	86	0	0	0	50
7	12	82	6	0	0	50
8	30	62	8	0	0	50
9	46	48	6	0	0	50
10	100	0	0	0	0	50
Peppergrass: [1]						
0	4	4	76	16	0	50
6	22	68	12	0	0	50
7	12	86	2	0	0	50
8	54	46	0	0	0	50
9	100	0	0	0	0	50
10	100	0	0	0	0	50

[1] Complete germination of peppergrass occurred only over distilled water.

TABLE 3.—*Halogeton seedlings established in the field on various dates and percentage of survival during 1953, 1955, and 1957, Snowville, Utah*

Date established	Seedlings	Still present at seed stage		Mature population
1953:[1]	*Number*	*Number*	*Percent*	*Percent*
Feb. 27	70	6	9	0.9
Mar. 9	7	0	0	0
Mar. 20	458	190	42	28.0
Apr. 1	173	60	35	8.9
Apr. 16	361	60	17	8.9
Apr. 22	280	207	74	30.6
Apr. 27	71	38	54	5.6
May 6	29	13	45	1.9
May 14	133	77	58	11.4
June 4	141	23	16	3.4
Summer (June 15 to fall)	([2])	3	----------	.4
Total	1,723	677	39	100
1955:[3]				
Apr. 15	531	395	74	38.2
May 7	941	612	65	59.1
May 21	66	13	20	1.3
Summer (July 1 to fall)	([2])	15	----------	1.4
Total	1,538	1,035	67	100
1957:[3]				
Mar. 2	758	330	44	41.7
Mar. 9	501	239	48	30.2
Mar. 16	219	75	34	9.5
Mar. 30	168	55	33	6.9
Apr. 6	94	35	37	4.4
Apr. 13	12	5	42	.6
Apr. 20	10	5	50	.6
Apr. 27	23	9	39	1.2
May 4	3	0	0	0
May 11	18	12	67	1.5
May 25	22	18	82	2.3
June 1	3	1	33	.1
June 8	9	8	89	1.0
Summer (June 15 to fall)	([2])	0	0	0
Total	1,840	792	43	100.0

[1] All seedlings were counted and marked in plots 12 inches square.
[2] Unknown.
[3] All seedlings were counted and marked in circular plots of 3 square inches.

SOME ADAPTATIONS OF HALOGETON TO THE SALT-DESERT SHRUB ENVIRONMENT

Transpiration

Recent studies have indicated that the stomates may be important pathways for penetration by herbicides (*10*). The succulent growth and the accumulation of oxalic acid made it difficult to ignore the possibility that halogeton might exhibit "Crassulacean metabolism." Plants exhibiting this type of metabolism would normally close their stomates during the day. An experiment designed to measure diurnal transpiration was selected because it would determine whether the stomates were open at night or during the day and would indicate the amount of water lost by halogeton each day. The design of the experiment also made it possible to measure the amount of moisture left in the soil after halogeton had died. Field observations had shown that each succeeding generation of halogeton on a site tended to make less growth, which indicated that less moisture was available to the plant each ensuing year.

The study utilized halogeton transplanted from the field into quart plastic pots containing a soil mixture of 4 parts desert soil, 1 part sand, and 1 part black mountain soil. The plants were allowed 3 weeks to establish a new root system and make other adjustments to the new environment. Plants were selected from this group on the basis of uniformity of size, cruciform growth habit, green color of the stems and leaves, and large turgid succulent leaves.

The soil was saturated with water, and the plants were placed in the dark for 24 hours to allow the excess water to drain from the pots. Polyethylene freezer bags were pulled over the pots and tied around the plants immediately below the crown of the plant. Each pot was weighed after the bagging operation, and the individual pots were weighed twice daily at 7 a.m. and 7 p.m. for 6 weeks, or until the plants were dead.

The plants were divided into five groups of six plants, and the groups were placed in various environments. The objective was to measure the variation in transpiration rather than the effects of the environmental factors on transpiration rate. The environments were as follows:

Groups 1 and 2. Plants were placed in the greenhouse where little or no detectable air movement existed. They were exposed to 8 to 9 hours of direct sunlight each day. The air temperatures ranged from a minimum of 58° F. at night to 120° or higher during the day.

Groups 3 and 4. Plants were placed near the outlet of the greenhouse air-conditioning fan where they were subjected to continuous air movement and high humidity. They received about 5 hours of sunlight during the day, and the air temperatures ranged from 50° to 85° F. during the 24-hour period.

Group 5. Plants were placed in a growth room where they received 2,000 foot-candles of light for 16 hours each day and remained in total darkness for 8 hours. The air temperatures were 86° F. during the period of light and 70° during the dark period.

The moisture content of the saturated soils was determined prior to the experiment. Ten pots of soil containing dead halogeton stems were bagged, as were the living potted plants. The percentage of soil mois-

ture in these pots was measured at the end of the experiment to determine whether moisture was lost from the soil because of factors other than transpiration.

The moisture content of the soil from all pots was determined at the end of the experiment. Data on soil moisture are expressed as the percentage of the ovendry weight.

The maximum rate of transpiration for an individual plant during a 12-hour period was 6.577 grams when exposed to 9 hours of direct sunlight and 1.624 grams during a dark period. Most of the moisture loss by the plants occurred during the daylight period, which indicates that the majority of the stomates opened during the daylight and not at night.

The average number of grams of moisture lost by individual plants each day is shown in table 4. Analysis of variance of these data reveals that there are no significant differences between replications, but differences between treatments (groups) are highly significant.

TABLE 4.—*Average amount of water lost daily by individual halogeton plants and average percentage of moisture in the soil after the plants had died* [1]

Treatment (group) [2]	Water lost by individual plants [3]	Moisture in soil after plants had died [4]
	Grams	*Percent*
1	3.5	5.9
2	4.9	3.6
3	4.4	2.0
4	4.9	5.9
5	4.0	5.6
Standard error	2.2	2.9

[1] The plants are presumed to have died from a lack of available moisture.
[2] See text for description of environment for each treatment (group).
[3] The differences between treatments (groups) is significant at the 1-percent level, but there are no significant differences between replications.
[4] There are no statistically significant differences between replications or treatments (groups).

The control pots containing the dead halogeton branches lost 0.72 percent of the moisture from the soil during the experiment. The soil had an average moisture content of 23.96 percent at the beginning of the experiment and 23.24 percent at the end. The average soil moisture for the pots containing living plants was 4.28 percent at the termination of the study.

The percentage of moisture of soil samples from pots containing living plants used for transpiration studies is also listed in table 4. Analysis of variance of these data indicates that differences between treatments (groups) and replications are not statistically significant.

Diurnal activity of the stomates

Studies concerned with transpiration indicated that 75 percent of the moisture lost each day occurred during the daylight hours. Skoss (*39*) and Dybing and Currier (*11*) have demonstrated that the sto-

mates are an important pathway of sprayed substances into the plant. Exact information on diurnal activity of the stomates is pertinent to the study of the response of a plant to any herbicide.

One of the simpler techniques for determining the relative openness of the stomates is using liquids of various surface tensions. In this study, benzene was the only liquid used. Although a number of chemicals (i.e., benzene, gasoline, kerosene, and mineral oil) were used, only benzene resulted in the typical water-soaked appearance of the leaves when the stomates were open. The appearance of the leaves was much the same as when the leaves are lightly pressed between the fingers.

Tests were made on June 22, July 6, July 20, August 3, August 17, and August 31. On the evening before testing, 48 plants were watered and moved to the headhouse. At 7:30 a.m. on the day of the trials, the plants were divided into four groups. One group was placed under 400 foot-candles of light provided by fluorescent light tubes. The second group was placed outside in the shade of the headhouse. Groups 3 and 4 were placed in an area that received full sunlight during the day. Group 4 remained outside with group 3 until the stomates were known to be open, then was moved into the shade with group 2.

Testing was started at 8:30 a.m. and was repeated at intervals of 30 minutes unless there were indications that movement of the stomates was occurring. When movement occurred, the interval was shortened to 15 minutes; however, in many plants the movement of the guard cells had been completed before any changes were detected.

The test used to determine whether the stomates were open or closed consisted of dipping the terminal inch of a branch into a vial of benzene for 5 seconds. One branch from four individual plants in each group was used at each time interval of these trials. These treated tips were observed until the water-soaked appearance could be seen, or for a maximum period of 5 minutes. The treated branch was then removed from the plant.

The stomates at the apex of the leaves opened first and closed last. The stomates of the central and basal portions of the leaves opened more slowly and closed sooner than those on the terminal end of the leaves (table 5). This information was used to determine when the stomates were in the process of opening or closing.

Light intensity appeared to strongly affect stomatal activity, as evidenced by the earlier opening of stomates of plants in direct sunlight. The stomates of plants that were moved from direct sunlight when the stomates had opened tended to close sooner than those of plants that remained in direct sunlight. The stomates of plants that remained in direct sunlight tended to remain open longer than those left in the shade or those that were moved to the shade from full sunlight.

The period of time that all of the stomates remained open varied during the season. It should be emphasized that the plants were watered the evening before these tests were made and should not have been under any internal water stress.

TABLE 5.—*Time of stomatal opening on halogeton leaves exposed to various light and environmental conditions*

Date of trials and treatments	First stomates open	All stomates open	First stomates closed	All stomates closed	Time all stomates remained open
June 22:					*Hours*
Fluorescent light	9:15 a.m.	10:00 a.m	12:15 p.m.	12:30 p.m.	2¼
Shade, outdoors	9:30 a.m.	11:15 a.m.	3:00 p.m.	3:30 p.m.	3¾
Full sunlight, outdoors	9:15 a.m.	11:00 a.m.	3:00 p.m.	3:15 p.m.	4
Sunlight, then shade outdoors	9:15 a.m.	11:00 a.m.	12:30 p.m.	1:15 p.m.	1½
July 6:					
Fluorescent light	9:45 a.m.	11:00 a.m.	----------	[1]11:30 a.m.	[2] ½
Shade, outdoors	9:30 a.m.	11:00 a.m.	12:30 p.m.	3:00 p.m.	1½
Full sunlight, outdoors	9:00 a.m.	10:30 a.m.	12:00 noon	1:00 p.m.	1½
Sunlight, then shade outdoors	9:00 a.m.	10:30 a.m.	11:30 a.m.	12:15 p.m.	1
July 20:					
Fluorescent light	10:30 a.m.	11:45 a.m.	1:30 p.m.	1:45 p.m.	1¾
Shade, outdoors	8:30 a.m.	8:45 a.m.	2:00 p.m.	3:30 p.m.	5¼
Full sunlight, outdoors	----------	8:30 a.m.	3:00 p.m.	3:30 p.m.	6½
Sunlight, then shade outdoors	----------	8:30 a.m.	12:30 p.m.	1:45 p.m.	4
Aug. 3:					
Fluorescent light	9:30 a.m.	11:30 a.m.	12:30 p.m.	1:00 p.m.	1
Shade, outdoors	10:00 a.m.	10:30 a.m.	12:00 noon	12:30 p.m.	1½
Full sunlight, outdoors	9:30 a.m.	11:00 a.m.	1:00 p.m.	1:45 p.m.	2
Sunlight, then shade outdoors	9:30 a.m.	11:00 a.m.	11:30 a.m.	12:45 p.m.	½
Aug. 17:					
Fluorescent light	11:00 a.m.	11:30 a.m.	12:00 noon	1:15 p.m.	½
Shade, outdoors	9:30 a.m.	11:00 a.m.	2:30 p.m.	2:45 p.m.	3½
Full sunlight, outdoors	9:30 a.m.	10:45 a.m.	3:00 p.m.	3:30 p.m.	4½
Sunlight, then shade outdoors	9:30 a.m.	10:45 a.m.	12:30 p.m.	3:00 p.m.	1¾
Aug. 31					
Fluorescent light	10:30 a.m.	11:30 a.m.	12:00 noon	1:00 p.m.	½
Shade, outdoors	1:30 a.m.	11:15 a.m.	2:00 p.m.	2:30 p.m.	2¾
Full sunlight, outdoors	9:00 a.m.	11:00 a.m.	3:00 p.m.	3:15 p.m.	4
Sunlight, then shade outdoors	9:00 a.m.	11:00 a.m.	12:30 p.m.	3:00 p.m.	1½

[1] Stomates had all closed during the normal half-hour interval between inspections.
[2] Stomates open less than ½ hour.

The stomates of halogeton are relatively small and apparently do not open wide since benzene was the only liquid tested with low enough surface tension (about 28.88 dynes per cm.^{2}) to move through the stomates. This agrees generally with data obtained in the anatomical studies of the size of the pore of the stomates.

Changes in the water content of the terminal inch of halogeton stems

The water balance of plants could influence the absorption and translocation of herbicides by its effects on photosynthesis, metabolism, and the activity of the stomates. Redistribution of water is known in some plants when internal moisture stresses occur (*23, 47*). Bartholomew (*2*) found that lemon fruits lost moisture to other parts when the plants were subjected to moisture stress. The older leaves of plants suffering from an inadequate supply of moisture die first, which indicates that the younger leaves are able to obtain moisture at the expense of the older leaves. Most of the younger leaves and bracteoles (a leaflike structure) occur on the terminal inch of halogeton stems. These terminal tips would receive the best coverage by herbicidal solutions applied as a spray.

A change in the percentage of moisture in the terminal inch of stems of halogeton should be indicative of the water balance within the plant. Related changes in the water balance within the plant and its response to 2,4-D sprays should indicate that the water balance of the plant influences its response to applications of the herbicide.

Four sampling sites were chosen that supported healthy, vigorous stands of halogeton and probably formerly supported pure stands of *Kochia americana* (Green Molly). The terminal 1 inch of the primary stem of each halogeton plant was removed with the aid of a pair of scissors and placed in airtight cans whose weight had been measured previously. The volume of each can was 21.2 cubic inches. The stem tips were carefully packed into the cans so as not to crush them, yet to have the largest possible number in each can. Three of these composite samples were collected at each site on each of the five sampling dates. The closed cans of stem tips were transported to the laboratory and weighed; then the open cans were placed in an oven for 48 hours at 105° F., and weighed again. The moisture content of the samples of stem tips was calculated on the basis of their ovendry weight.

The percentage of moisture of the stem tips continued to decrease during the experiment (table 6). The percentage of moisture dropped substantially on each of the four sites sampled. On the first two sites, the drop occurred between June 29 and July 9. On the last two sites similar losses occurred between July 9 and July 23. Bracteoles were observed on all the plants on all four sites when the samples were collected on July 23.

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TABLE 6.—*Change in percentage of moisture of the stem tips of halogeton plants during the growing season of 1957, expressed as the average percentage of the ovendry weight*

Date of sampling	Sampling site				Average
	1	2	3	4	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
June 29	610	494	511	510	531
July 9	395	324	462	438	405
July 23	309	306	303	251	292
Aug. 5	275	252	270	235	258
Aug. 20	248	260	277	228	253

Standard error=15.16.

Anatomy of halogeton

I have studied the anatomical structure of the root, stem, and leaves of halogeton to determine whether the visual aspects of any structure of the plant would suggest the existence of a barrier to penetrating substances or an obstruction to translocation of materials.

The structure of the root (fig. 12) is similar to the root of the common beet, *Beta vulgaris* (*17*). The similarity is most obvious in the

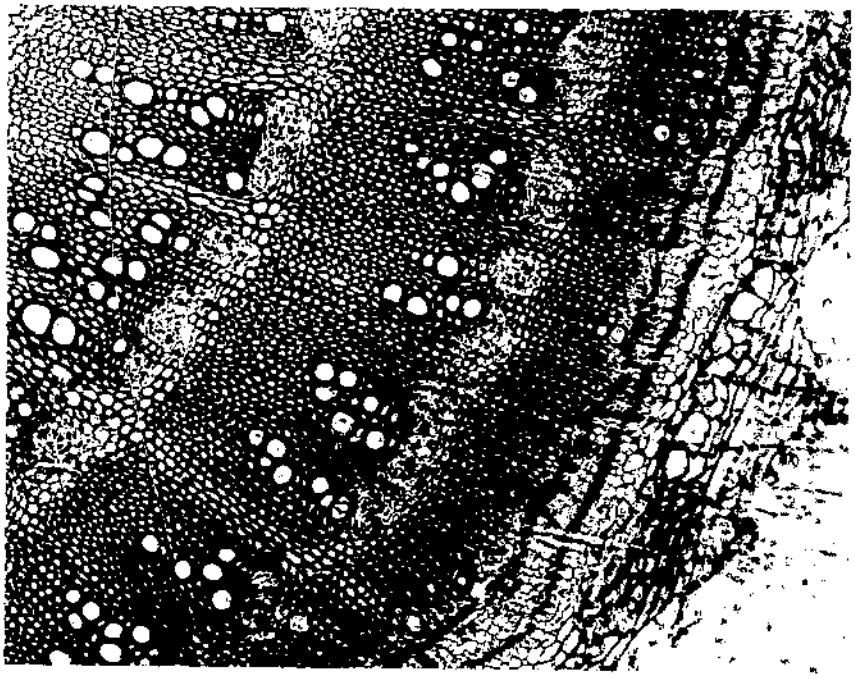

FIGURE 12.—Photomicrograph of cross section of a small root of halogeton showing the concentric rings of tertiary vascular tissue and the disintegrating periderm.

development of the supernumerary rings of vascular tissue and in the periderm region of the more mature roots where the cells are constantly sluffed off as the root grows in diameter.

The stem is an ectophloic polystele (fig. 13). The fascicular cambium and interfascicular cambium form an almost continuous layer in the stem. The pith consists of large parenchyma cells and appears to be the succulent tissue of the stem. Many cells in the pith contain druses. Druses are common in many tissues of the plant and are probably crystals of calcium oxalate.

The papillate epidermis and the cortex of the stem are probably of most interest in relation to absorption of herbicides (fig. 14). The thickened outer walls and anticlinal walls are common in many halophytes. Repp (*31*) indicated that this tissue acts as a barrier to water loss and to the penetration of salt into the tissues of halophytes subjected to salt spray or inundation by sea water. If the cells of the epidermis prevent or inhibit water loss, it is likely that they prevent or inhibit water movement in the opposite direction.

The outer cortex could possibly be classed as a hypodermis, but it is questionable if it should be so termed since it is not definitive. There is a gradual transition from the parenchyma cells of the inner cortex to the thick-walled collenchyma of the outer cortex. While the outer three to five layers undoubtedly act as a supporting layer and may possibly contribute to protecting the plant from water loss and from penetration of extraneous materials, there is no evidence that it contributes to mechanical protection of the plant. The walls of these collenchyma cells contain primary pit fields.

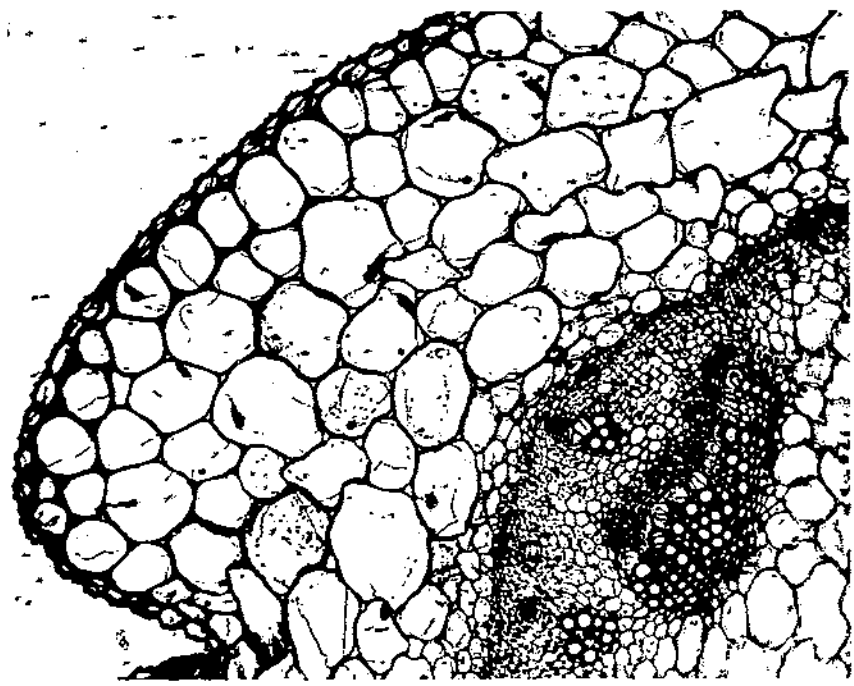

FIGURE 13.—Photomicrograph of a transection of a young stem of halogeton.

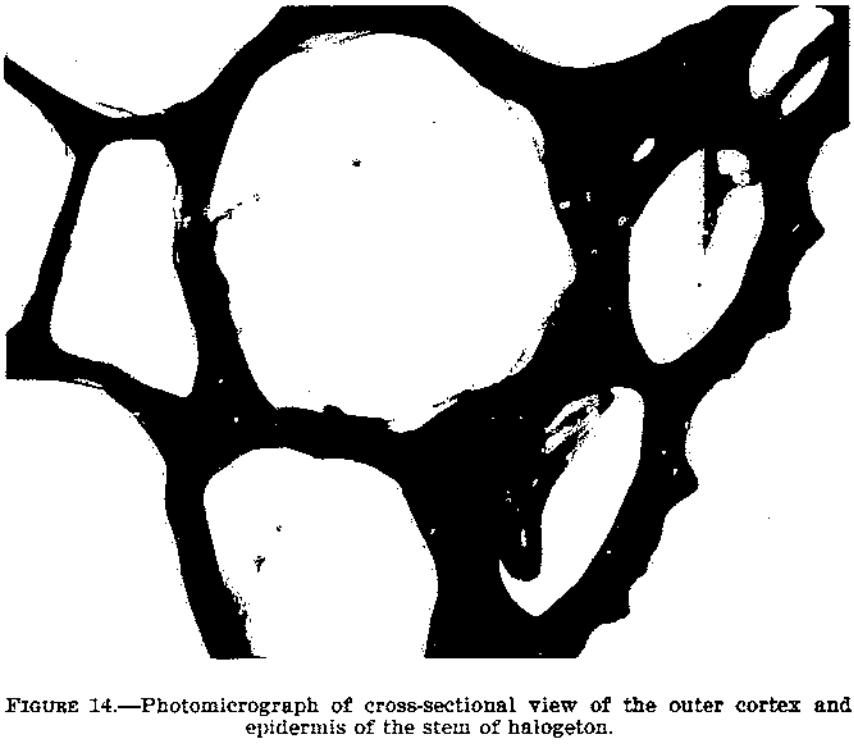

FIGURE 14.—Photomicrograph of cross-sectional view of the outer cortex and epidermis of the stem of halogeton.

The most distinctive feature of the halogeton plant is the leaf, and a transverse view of it is given in figure 15. The venation of the leaf is camptodromous. The core of the leaf consists of a main vascular bundle with the smaller pinnately arranged secondary veins curving outward and upward toward the apex of the leaf. The secondary branches terminate after being adjacent to the spongy mesophyll for a short distance. Tertiary branches and reticulation of the secondary branches have not been observed.

The main vascular bundle is surrounded by leaf cortex, which is responsible for the succulence of the leaf. Repp (*31*) used the term "Wassergewebe" (water tissue) to discuss similar tissue in other halophytes. I prefer the term "water tissue" to "cortex," since the former term not only indicates part of its function but also describes the appearance of the tissue in the halogeton leaf. The cells of this tissue are large, thin-walled parenchyma.

In her summary Repp (*31*) makes the following comments concerning succulents:

> In the habitat under natural conditions the salt enrichment of the plant occurs slowly but unavoidably with age. If the limit is reached, which is determined through the specific plasmic salt hardiness, then the affected plant part dies. Many plant species—and indeed typical halophytes as well as glycophytes also, which are successful on saline soils—have the capacity, by increasing the succulence, to extend the limits of this injurious internal threshold concentration. This increasing succulence depends simply on an enlargement and increased water content of already existing cells, particularly the parenchyma. The capacity for this is developed by plants in very different proportions; it is conveniently produced by already existing succulence (water tissue).

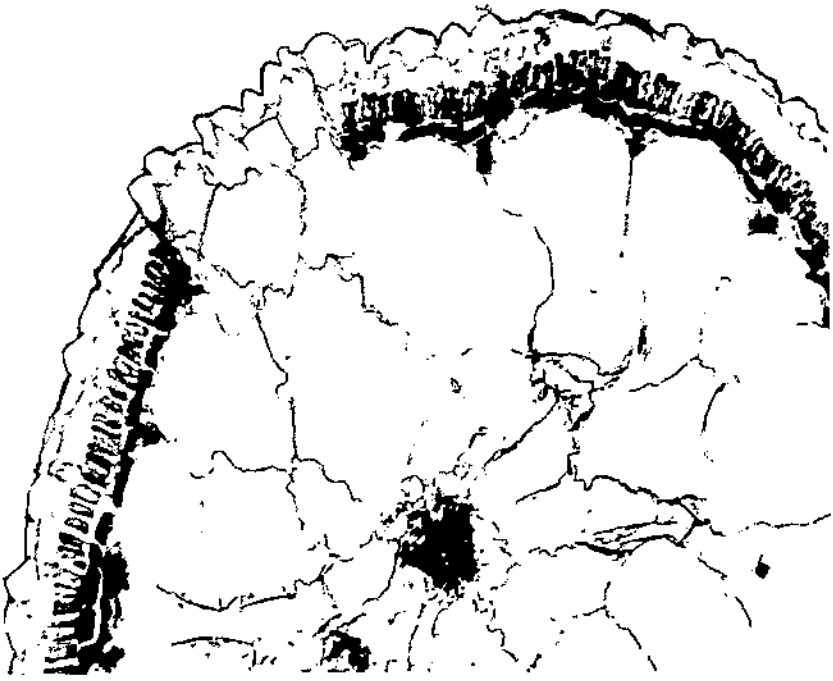

FIGURE 15.—Photomicrograph of a cross section of a leaf on the abaxial side (of a leaf). The main vascular bundle can be seen in the lower center of the figure, and a few of the secondary bundles can be seen under the spongy mesophyll. The spongy mesophyll is the dark layer of cells outside of the water tissue. Note that the water tissue extends to the epidermis on the extreme abaxial side of the terete leaf.

On both the ventral and dorsal sides of the leaf, the water tissue extends from the main vascular bundle to the epidermis. The photosynthetic tissues are on the lateral sides of the leaf except that it becomes connate at the terminal end of the leaf on the abaxial side. It is sometimes united at the apex on the adaxial side, but the distance of connateness would be measured in microns rather than millimeters. These bands of water tissue under the epidermis can be seen on the adaxial sides of the leaves in figures 2, 3, and 4. At the base of the sessile, slightly clavate leaf the bands are broad, but the width is reduced by half within approximately 2 millimeters of the base. Figure 15 illustrates the distribution of the water tissue in the leaf.

The spongy mesophyll is external to the water tissue and is composed of small, densely packed chlorenchyma cells (fig. 16). The tissue is usually one layer, but it is sometimes two cells deep, and the variation has been noted in a majority of the leaf sections examined. Yet it does not appear that there is any specific region in the leaf where the spongy mesophyll is either uniserate or biserate. The concentration of chloroplasts is high in the spongy mesophyll cells and much higher than in the palisade cells.

The palisade tissue is external to the spongy mesophyll and is always uniserate. The tissue is composed of densely packed, elongated chlorenchyma. Between the palisade tissue and the epidermis is a

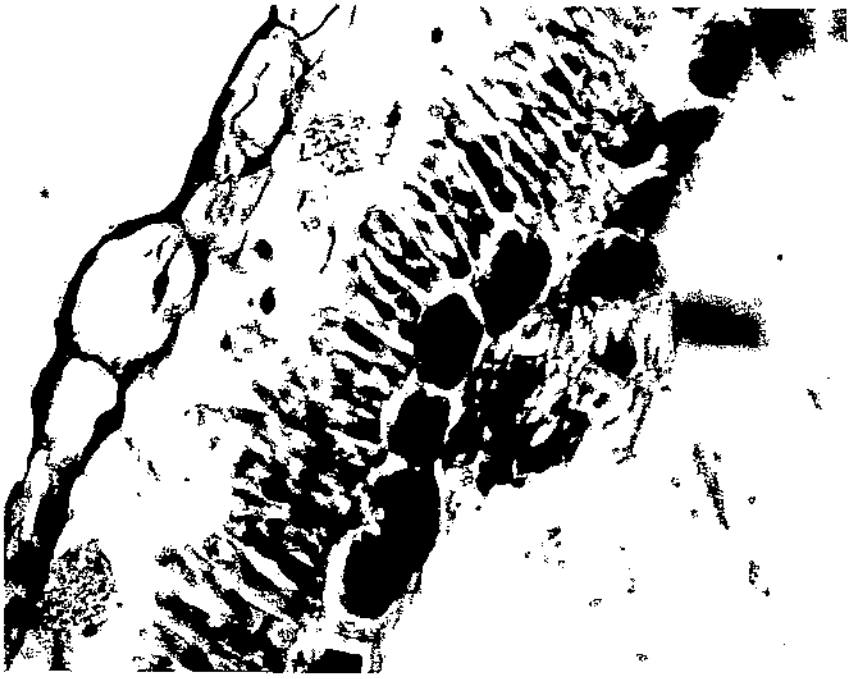

FIGURE 16.—Photomicrograph of photosynthetic tissues, subepidermal tissue, and epidermis of a halogeton leaf. Two large druses can be seen on the upper edge and the lower side of the figure in the cells of the subepidermal tissue.

layer of cells, one to two cells thick, that do not contain chloroplasts. They are thin-walled parenchyma cells, and many of them contain large salt crystals or druses. The number of cells containing druses increases with the age of the leaf. In late August and early September a majority of these cells contain druses. Two druses are shown in figure 16.

The epidermis is composed of cells of many sizes. The outer walls are thicker than the remaining portions of the walls. Stomates are confined to the area of the epidermis over the photosynthetic tissues. The long axis of the pore of the stomates is at right angles to the main axis of the leaf. The enlargement of the subsidiary cells causes the guard cells to appear to rest well below the surface of the leaf (fig. 17).

The stomates were measured by dipping the leaf in a colloidin solution. The colloidin film and the epidermis were then stripped from the leaf and examined under a microscope. The stomates of halogeton, including the guard cells, averaged 35.03×25.59 microns on a large, mature leaf. The pores averaged 11.34×1.14 microns during June, but in early August they did not open as wide; and the average size, when open, was 11.52×0.93 microns. In the epidermis over the photosynthetic tissue, the stomates occurred at the rate of 5,600 per square centimeter. The area of a large vegetative leaf was approximately 0.5 square centimeter.

FIGURE 17.—Transverse view of a stomate from a longitudinal section of a halogeton leaf. The stomates are the sunken type because the subsidiary cells are larger than the surrounding epidermal cells.

The cuticle was difficult to stain. It was successfully stained twice in numerous attempts over a period of 8 years. Attempts to isolate the cuticle have failed, but apparently the cuticle was present and was important in inhibiting movement of water and solutes.

Bracteoles are modified leaves and their anatomical structure is similar to that of the leaf (fig. 18). The photosynthetic tissues occupy a smaller percentage of the area of the bracteoles than they do on the leaves. The water tissue extends to the epidermis on the adaxial side of the bracteole, with the exception of a small area on the terminal end. On the abaxial side the band of this tissue is wider and extends farther up than it does on the leaf (fig. 8).

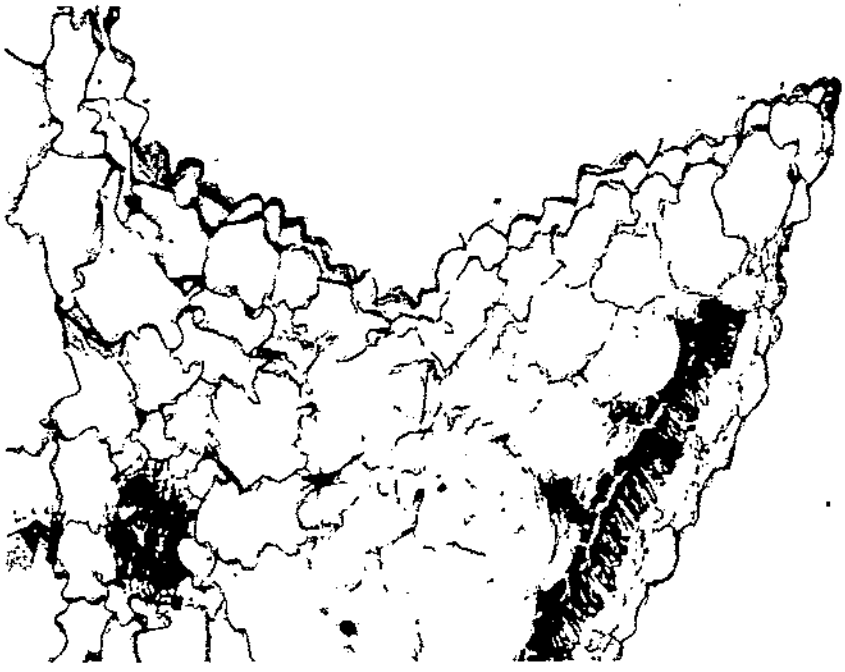

FIGURE 18.—Cross-sectional view of the adaxial side of a bracteole.

RESPONSE OF HALOGETON TO VARIOUS RATES OF 2,4-D ON DIFFERENT DATES

Before 1953, research workers in Idaho had evaluated the effectiveness of applications of various herbicides to halogeton (*26*). They found that the propylene glycol butyl ether ester of 2,4-dichlorophenoxyacetic acid (2,4-D) was one of the most effective herbicides. These research workers were interested in obtaining data on the effectiveness of the chemical at various stages of growth and at divergent geographical locations. I cooperated with them and adapted my experimental design to coincide with theirs, so that a regional comparison could be made. The project was carried out during 1953 and 1954.

A split plot with the whole plot arranged in a 4×4 Latin square was used in 1953. The main plots were 16½×33 feet and were divided into four subplots with dimensions of 16½×8 feet. Two permanent line transects were established on each subplot. The plants in ten 2-inch-square quadrates were counted at regular intervals along each of these transects. The vegetation was sampled before treatment and after the plants had matured in the fall.

Treatments consisted of applications of 1, 2, 4, and 8 pounds of 2,4-D per acre in 15 gallons of spray. Applications were made on June 10, July 10, and August 20. Plots in another group were not treated and were used as controls.

The experimental design in 1954 was changed to a randomized block with four replications of each treatment and eight untreated control

plots. The propylene glycol butyl ether ester of 2,4-D was applied at 1, 1½, and 2 pounds per acre on May 11, June 11, June 24, July 9, July 28; on one set of plots, treatments were applied on June 11 and repeated on August 12.

The treated plots were 12 feet square, but only the center 64-square-foot area was sampled. The vegetation was sampled in the same manner as it was in 1953, except the quadrates were increased from 20 to 36 per plot.

In 1953, the highest percentage of kill (97.2) was achieved with 4 pounds per acre on June 10 (table 7). On the other two dates of application, 8 pounds per acre failed to reduce the population similarly. On the untreated control plots, 11 percent of the plants died between the first part of June and the last part of September.

TABLE 7.—*Halogeton killed with aqueous sprays of the propylene glycol butyl ether ester of 2,4-dichlorophenoxyacetic acid applied at 4 rates on 3 stages of growth in 1953* [1]

Date treated	Stage of growth treated	Kill when 2,4-D was applied at—			
		1 pound per acre	2 pounds per acre	4 pounds per acre	8 pounds per acre
		Percent	*Percent*	*Percent*	*Percent*
June 10	Elongation	70	70	97	97
July 10	Early flowering	11	33	36	55
Aug. 20	Late flowering	9	31	13	57

[1] On the control plots, 11 percent of the halogeton plants died during the experiment.

The sampling method used in 1954 indicated that single treatments on June 11 and on June 24, and double treatments on June 11 and August 12, resulted in complete kill of halogeton. But healthy plants were growing on these plots and simply did not occur within the 2-inch-square samples along the transects on the plots. When the sampling techniques used indicated a 100-percent kill, an actual count of the plants within the 64-square-foot sampling area was recorded. The averages of these data for the four replications are contained in the last three columns of table 8.

Treatments applied June 24 resulted in a high percentage of halogeton kill, while treatments applied 15 days later on July 9 resulted in a relatively small percentage of kill. One important result of the 1954 experiment is that the double treatments (herbicides applied on June 11 and again on August 12) did not result in greater kills than the single treatments applied on June 11 or June 24. During the summer of 1954, almost daily observations of the plots were made. These observations indicated that the vast majority of the plants found in the fall on the plots treated on May 11, June 11, and June 24 were plants that became established after the plots had been treated.

If the plants on the plots receiving a second treatment on August 12 were plants that had become established after the first treatments on June 11, they were less than 2 months old. Certainly the plants

TABLE 8.—*Halogeton killed with aqueous sprays of propylene glycol butyl ether ester of 2,4-dichlorophenoxyacetic acid at 3 rates of application on various stages of growth in 1954* [1]

Date treated	Stage of growth treated	Kill calculated from sampling data when 2,4-D was applied at—			Plants counted on the plots with estimated kills of 100 percent when 2,4-D was applied at—		
		1 pound per acre	1½ pounds per acre	2 pounds per acre	1 pound per acre	1½ pounds per acre	2 pounds per acre
		Percent	*Percent*	*Percent*	*Number*	*Number*	*Number*
May 11	Seedling	53	61	63			
June 11	Cruciform	100	100	100	153	140	12
June 24	Elongation	100	100	100	81	7	21
July 9	Early flowering	35	27	54			
July 28	Flowering	40	42	26			
June 11 and Aug. 12	Cruciform and late flowering.	95	100	100		31	22

[1] Average of 4 replications. A 49-percent increase in the number of plants on the untreated control plots occurred during the period of May through August.

treated on June 24 were 2 months old, and perhaps many of them were more than 3 months old. If chronological age was a factor in the resistance of halogeton to 2,4-D, then these plants on plots treated the second time should have been killed, and the number of plants on these plots should have been substantially less than the number found on plots receiving a single treatment on June 11 or June 24. The lack of real differences between the number of plants on these plots implies that it is not chronological age but physiological age that determines the resistance of halogeton to 2,4-D. Morton, Haas, and Erickson (*26*) concluded that "the tolerance in halogeton to 2,4-D is influenced more directly by physiological development or condition than by chronological age."

FIELD STUDIES OF ABSORPTION-TRANSLOCATION OF 2,4-D BY HALOGETON

1953 experiment

The toxicity of 2,4-D to halogeton decreases sharply around the first of July, when the plant enters its reproductive phase of growth, and continues to decrease over the remainder of the growing season. In the fall of 1952 I visited a number of areas that had been treated with 2,4-D and observed that glomerules on the underside of the stems of the plants often remained green, whereas glomerules on the dorsal side were necrotic and without seed. Some plants on sprayed areas exhibited little or no injury, whereas others were killed almost without regard to the date of treatment. These observations indicated that either the herbicide was not being translocated within the plant, or it was not being absorbed by the plant. It was not clear whether translocation or absorption phenomena accounted for the variable plant responses to 2,4-D during the reproductive phase of growth.

The first attempts to define the factor or factors involved in the resistance of halogeton to 2,4-D during reproductive growth were undertaken during the growing seasons of 1953 and 1954. It was hoped that resolution of these factors might permit development of techniques and materials which would increase the effectiveness of chemical control.

The cruciform growth habit of halogeton is well suited to translocation and absorption studies. Treatment of a single lateral branch with a solution containing 2,4-D should result in symptoms of 2,4-D injury on other branches of the plant, provided the herbicide is absorbed and translocated; and various materials that would aid translocation or absorption of 2,4-D were selected.

During the growing seasons of 1953 and 1954, laboratory and greenhouse facilities were not available, and the first phase of the work was done in the field. Uniform plants were obtained by thinning field populations to one plant per square foot in late May and early June. When the density of halogeton was reduced to this level, vigorous plants with a cruciform growth habit resulted. Dense stands of halogeton often result in plants having only three stems, with the other two stems represented by a rosette of leaves that can develop into glomerules during the reproductive phase of growth.

The herbicide propylene glycol butyl ether ester of 2,4-D,[5] was applied in the various carriers selected for this study. Herbicide solutions of 200 milliliters each were prepared with each carrier containing 750, 1,500, 3,000, 6,000, or 12,000 parts per million. (These concentrations were such that if applied at rates of 80 gallons per acre, the 2,4-D would have been applied at rates of approximately 0, ½, 1, 2, 4, and 8 pounds per acre.)

The carriers used in 1953 were as follows:

(a) Water.
(b) Water plus 1 percent Triton B–1956.
(c) Water saturated with sucrose.
(d) Kerosene.
(e) Ethanol (70 percent).
(f) Lanolin.
(g) Gasoline.
(h) Gasoline saturated with household paraffin.

Water is often used as the carrier for applying 2,4-D under field conditions, and it was selected as a standard for comparing the effectiveness of the other carriers. Triton B–1956 was added to water to improve the wetting properties of the carrier and to obtain maximum penetration of the herbicide.

There is evidence that 2,4-D moves with the carbohydrate stream; and, by supplying sucrose in the carrier (water saturated with sucrose), a more favorable carbohydrate gradient could be created for the translocation of the herbicide (*36, 44*). Rice and Rohrbaugh (*33*) also found evidence that kerosene caused 2,4-D to be translocated readily in destarched plants.

Ethanol was selected as a carrier on the basis of its property of destroying the selectively permeable attributes of the cell membrane. It is also a poor solvent for waxy substances similar to those found in the cuticle. Gasoline is not only a good solvent for wax, but it is also toxic to the cells of plants (*9*). High light intensity, high temperatures, and low humidity in the area where these studies were carried out created conditions favoring a high rate of evaporation of the carriers, especially the more volatile materials like gasoline. For this reason, gasoline was saturated with household paraffin to decrease its volatility. Lanolin was used to determine if the rate of absorption was the limiting factor in the effectiveness of 2,4-D.

The spray solutions were applied with small atomizers (DeVilbiss No. 152) to a single branch to the point of runoff. A tinfoil shield was attached to the treated branch to prevent spray drift to the untreated portions of the plant and to prevent drops from accidentally running down the stem to the crown of the plant. Of course, the lanolin paste could not be used in this manner, so it was applied to the terminal inch of the branch where a large number of young leaves were located. It was also applied as a band around the stem about 1 inch above the crown of the plant. Since only small quantities of the

[5] Furnished by the Dow Chemical Co. The chemical is marketed under the trade name "Dow 10–10." Mention in this publication of firm names or trade products does not imply recommendation by the U.S. Department of Agriculture over others not mentioned.

lanolin paste could be applied, the concentration of the herbicide was increased fourfold in this carrier.

The cruciform branching habit of halogeton made it possible to measure translocation of 2,4-D into branches originating above as well as below the treated branch. One branch of each plant was treated with the spray solution. An effort was made to vary the treated branch systematically within a replication, so that each treatment was applied to a branch in a different position (representing the four positions of the lateral branches on the plant). The spray solutions were applied July 10, July 29, and August 19. Treatments were replicated on three sites, but one replication was lost because of trampling by livestock.

The individual branches were given a "survival rating" 1 week after treatment. The "survival rating" of a replication is the sum of the "survival ratings" of the four branches on each of six plants in a replication.

Symptoms of 2,4-D injury usually appear on branches originating on the central stem above the treated branches. If injury symptoms did not appear on all untreated branches, the branch or branches originating on the central stem below the treated branch usually lacked symptoms of injury. Regardless of the ontogenical position of the treated branch, the branch most likely to exhibit symptoms of injury was branch 5 (a continuation of the central stem above the crown).

The survival rating for the untreated branches is a measure of the herbicide absorbed by the treated branch and translocated into the untreated portions of the plant. But some of the carriers were phytotoxic, and injury caused by these carriers unfortified with herbicide was found on untreated portions of the plant. Some carriers caused injury only to the treated branch.

Analyses of variance of the data for the untreated branches indicate that the differences between survival ratings for carriers, rates, and dates, and interactions of carriers with rates and of carriers with dates all exceed the 1-percent level of significance. The interactions of rates × dates and carriers × rates × dates exceed the 5-percent level of significance.

A summary of the data collected during this experiment is contained in table 9.

Lanolin paste as a carrier did not prove to be satisfactory in this experiment. Little injury was observed on the branches treated at lower concentration on July 29 and August 19.

Water was used as a basis for comparing the effectiveness of the other materials as carriers for 2,4-D. The survival ratings for plants treated with 2,4-D in water indicate that approximately the same conditions existed as on large plots sprayed at various dates with various concentrations of 2,4-D. Increased concentrations of the herbicide did not always result in corresponding increased injury to halogeton, especially on the later dates of application. The toxicity of any given concentration of the herbicide decreases during the season. At the lower concentration of 2,4-D on August 19, the treated branches were not badly injured.

When 1 percent of Triton B–1956 was added to the water, the results were about the same as those with water alone for the untreated

TABLE 9.—*Average total survival rating for 2 replications of 6 field-grown halageton plants, each treated on one branch with various concentrations of 2,4-D in eight carriers, 1953*

Carrier	Date treated	Survival rating when concentration of 2,4-D was [1]—					
		0 p.p.m.	750 p.p.m.	1,500 p.p.m.	3,000 p.p.m.	6,000 p.p.m.	12,000 p.p.m.
Water	July 10	72	72	46	49	20	29
	July 29	72	45	44	43	52	31
	Aug. 19	72	72	72	72	48	72
Water+1 percent of Triton B-1956	July 10	68	44	32	30	19	8
	July 29	70	46	47	49	47	41
	Aug. 19	72	72	72	72	72	64
Water saturated with sucrose	July 10	72	32	38	29	8	30
	July 29	55	51	35	52	38	36
	Aug. 19	72	72	72	72	72	72
Kerosene	July 10	62	11	12	8	8	6
	July 29	64	40	28	20	34	28
	Aug. 19	72	64	68	58	52	51
Ethanol (70 percent)	July 10	56	10	11	6	15	8
	July 29	60	30	28	24	27	28
	Aug. 19	72	72	72	66	67	52
Lanolin	July 10	70	62	58	32	36	30
	July 29	72	70	68	72	66	50
	Aug. 19	72	72	72	72	63	68
Gasoline	July 10	18	10	6	0	0	7
	July 29	60	36	22	32	18	20
	Aug. 19	72	43	70	52	40	50
Gasoline saturated with paraffin	July 10	22	2	0	0	0	0
	July 29	28	19	6	0	0	0
	Aug. 19	38	42	40	28	31	34

[1] Survival ratings are the average of totals in each replication made 1 week after treatment and are derived as follows: 6 plants × 4 untreated branches × the rating value for each branch (0–3 scale).

branches, except on the first date of treatment. The spray solutions were more toxic to the treated branches.

A carrier of water saturated with sucrose did not achieve results that differed substantially from those with water alone as a carrier. Either sucrose did not penetrate into the plant or it did not effect translocation of 2,4-D.

Kerosene was an effective carrier at the earliest date of treatment but failed to maintain its effectiveness at the last date. Whether kerosene functioned to increase the amount of 2,4-D absorbed by the halogeton plant or to increase the translocation of the herbicide is not clear. If the carbohydrate stream does not translocate 2,4-D, kerosene should; and no change in the extent of injury should occur to untreated plant parts. If kerosene transports the 2,4-D across an absorption barrier, a change in the composition of the barrier could be responsible for the change noted with the carrier. Apparently kerosene acts primarily to increase the amount of 2,4-D that penetrates into halogeton during the early part of the reproductive phase of growth. Later the nature of the absorption barrier changes, and kerosene is not effective as a carrier.

Ethanol also was an effective carrier at the first two dates of treatment but proved to be as ineffective as water on August 19.

Gasoline was an effective carrier in plants treated July 10, but results from July 29 and August 19 were erratic and are difficult to interpret. The erratic behavior of gasoline might possibly be due to its rapid rate of evaporation, since gasoline saturated with paraffin did not give erratic results. The latter was more effective than any other carrier, as measured by the results achieved on all three dates of treatment.

If the basis of the resistance of halogeton to spray solutions of 2,4-D is an absorption barrier, as it is possible to infer from this experiment, then some indication of the nature of the barrier can also be deduced from the solvent properties of kerosene, ethanol, and gasoline. Since gasoline is the best solvent for waxy substances, the cuticle would be the obvious source of substances acting as a barrier to absorption.

The toxicity of some of the carriers tended to obscure the effects of the herbicide and caused some difficulty in interpreting the results. This was particularly true of gasoline saturated with paraffin. Since this carrier appeared to be the most effective one used, I decided to repeat the experiment in 1954 using carriers with similar properties.

1954 experiment

Kerosene increased absorption-translocation of 2,4-D in the bean plant but was not particularly effective in increasing 2,4-D injury to halogeton plants when used as a carrier for the herbicide. Kerosene was saturated with sucrose in hopes it would clarify the role of kerosene in the herbicide spray solution. Kerosene appeared to be more effective than water in the 1953 experiment, but it was impossible to determine whether it was acting to increase absorption or translocation.

Water was used as a basis of comparison and the other carriers were selected because they were lipoids and solvents for waxy materials like

gasoline. The carriers used in the 1954 field studies of absorption-translocation were—

(a) Water.
(b) Kerosene saturated with sucrose.
(c) A 1:9 emulsion of gasoline saturated with household paraffin in water.
(d) A 1:1 emulsion of gasoline saturated with household paraffin in water.
(e) Gasoline saturated with household paraffin.
(f) Gasoline saturated with "Sure-seal" crude oil.[6]
(g) Carbon tetrachloride saturated with "Sure-seal" crude oil.
(h) Stoddard solvent saturated with "Sure-seal" crude oil.

Water was used as the standard of comparison of the effectiveness of the various carriers to increase the absorption-translocation of 2,4-D. Treatments were applied on July 12, July 30, and August 20. With the exceptions mentioned, the experiment carried out in 1954 was the same as the one in 1953.

The results of the 1953 and the 1954 experiments cannot be compared directly, since none of the carriers was as effective the second year. Nor do the results from water and from gasoline saturated with paraffin have the same relations, even though they are the only carriers that were used both years.

Statistically, the same factors that were significant in 1953 were significant in 1954. A summary of the data for the three dates of treatment is contained in table 10.

Water as a carrier resulted in less 2,4-D damage to the plants than did similar treatments in 1953, but the relation between concentration of the herbicide and date of treatment is comparable.

Kerosene saturated with sucrose was superior to water as a carrier but was not as effective as other lipoid solvents. The important factor would appear to be that this combination of materials lost its effectiveness as a carrier as the plants aged.

The efficiency of gasoline saturated with household paraffin in 1953 led to the use of this mixture emulsified in water as a carrier in 1954. When 10-percent gasoline saturated with paraffin was emulsified in water, its effectivness was approximately the same as water alone. Water and gasoline saturated with paraffin emulsified together in equal parts was effective on the earliest date of treatment but was no more effective than water on the last two dates of treatment. The results with gasoline saturated with paraffin were only slightly better than those with water on the last two dates of treatment.

Carriers such as gasoline saturated with paraffin, and gasoline, carbon tetrachloride, or Stoddard solvent saturated with "Sure-seal" crude oil are definitely more effective than water as a carrier, especially on the earliest date of treatment. All these carriers failed to maintain a wide margin of effectiveness on the last two dates of treatment, but they did retain some superiority. These carriers are known to move into the plant from the treated branch to the untreated branches and may carry the herbicide with them, but it is probably their penetration of the plant that accounts for their superiority over the other carriers.

[6] A natural crude oil with a composition of 30- to 40-percent paraffin hydrocarbons furnished by the Sure-seal Corporation of Salt Lake City, Utah.

TABLE 10.—*Average total survival rating for 3 replications of 6 field-grown halogeton plants, each treated on one branch with various concentrations of 2,4-D in 8 carriers, 1954*

Date treated and carrier	Survival rating when concentration of 2,4-D was [1]—					
	0 p.p.m.	750 p.p.m.	1,500 p.p.m.	3,000 p.p.m.	6,000 p.p.m.	12,000 p.p.m.
July 12, 1954:						
Water	72	64	58	59	47	46
Kerosene saturated with sucrose	72	62	50	40	43	32
1:9 emulsion of gasoline saturated with paraffin in water	70	72	53	41	46	41
1:1 emulsion of gasoline saturated with paraffin in water	4	9	5	0	3	16
Gasoline saturated with paraffin	11	10	8	5	9	8
Gasoline saturated with "Sure-seal" crude oil	15	16	5	12	9	12
Carbon tetrachloride saturated with "Sure-seal" crude oil	26	21	18	15	8	12
Stoddard solvent saturated with "Sure-seal" crude oil	54	38	13	11	10	3
July 30, 1954:						
Water	72	68	65	68	65	54
Kerosene saturated with sucrose	72	70	68	54	54	51
1:9 emulsion of gasoline saturated with paraffin in water	72	72	66	62	57	53
1:1 emulsion of gasoline saturated with paraffin in water	47	58	57	46	49	42
Gasoline saturated with paraffin	63	50	48	48	50	42
Gasoline saturated with "Sure-seal" crude oil	49	41	36	41	42	37
Carbon tetrachloride saturated with "Sure-seal" crude oil	66	54	57	48	45	37
Stoddard solvent saturated with "Sure-seal" crude oil	62	61	62	46	50	50
Aug. 20, 1954:						
Water	72	72	72	71	65	62
Kerosene saturated with sucrose	72	71	72	71	67	68
1:9 emulsion of gasoline saturated with paraffin in water	72	72	71	67	62	59
1:1 emulsion of gasoline saturated with paraffin in water	54	56	57	57	52	58
Gasoline saturated with paraffin	57	53	54	54	56	49
Gasoline saturated with "Sure-seal" crude oil	65	67	54	52	52	50
Carbon tetrachloride saturated with "Sure-seal" crude oil	61	58	54	53	54	52
Stoddard solvent saturated with "Sure-seal" crude oil	72	65	66	59	54	54

[1] Survival ratings are the average of totals in each replication made 1 week after treatment and are derived as follows: 6 plants × 4 untreated branches × the rating value for each branch (0–3 scale).

ABSORPTION-TRANSLOCATION OF 2,4-D-2-C^{14} IN HALOGETON

Extractions of 2,4-D-2-C^{14} with ethanol from treated plants

Results of preliminary field investigations of the factors involved in the resistance of halogeton to 2,4-D sprays after the plant had entered the reproductive phase of growth suggest that a reduction in the amount of 2,4-D absorbed may reduce the effectiveness of the herbicide. But the use of lipoid solvents as the carrier for the herbicide to overcome the absorption barrier tended also to obscure the symptoms of 2,4-D injury with symptoms of injury by the carrier. It seemed desirable to obtain a more refined measurement of the 2,4-D concentration in the untreated portions of the plant than could be obtained with injury symptoms. The use of C^{14}-labeled 2,4-D appeared to be a tool that would yield such measurements. For these experiments, a small amount of 2,4-D-2-C^{14} was obtained. It had an activity of 0.13 millicurie per gram.

Three carriers were used. Gasoline saturated with household paraffin was selected on the basis of results obtained in the field study. Preliminary trials with water indicated that because of its surface tension it would not adhere to the plant, and it was necessary to add a wetting agent to insure that a known quantity of the herbicide solution remained on the plant. Addition of 1 percent Dynawet [7] to the water carrier resulted in a solution that would adhere to the plant and form a film over the plant surface.

Ennis and Boyd (*14*) and Rice (*32*) have presented data that indicate that the addition of Carbowax to aqueous spray solutions increases absorption of 2,4-D by reducing the rate of evaporation of the spray solution. Apparently, paraffin also does this when it is added to gasoline. Therefore, the third carrier used was water with 2-percent Carbowax 1500.[8]

Single branches of individual plants were treated. Herbicide solutions were mixed so that each branch received 25 micrograms of the labeled herbicide when 0.3 milliliter of the solution was applied to the branch. The solutions were applied with insulin hypodermic syringes and 22-gage 2-inch needles to the glomerules on the upper half of the treated branch. They were applied in 3 aliquots of 0.1 milliliter each at 30-minute intervals to prevent runoff of the solution.

The plants were obtained from the field 26 to 30 days before treatment. They were carefully lifted from the ground in their accompanying soil, which was removed from the roots by a soft spray of water. The plants were then wrapped in wet newspaper and transported to the greenhouse in Logan, Utah, where they were transplanted in quart plastic containers in soil containing 4 parts desert soil, 2 parts sand, and 1 part black loam. After the plants had been transplanted, they were kept in a cold room (35° F.) for 5 days to reduce water losses while new roots formed.

Following the cold treatment, the plants were placed in a portable greenhouse where the air was replaced three times per minute. This

[7] Supplied by the Dow Chemical Co.
[8] Supplied by the Carbide & Carbon Chemical Co.

rapid displacement of air maintained the inside temperature without a substantial reduction in light intensity. The air inlets and outlets were at the same level as the aerial portions of the halogeton plants. This resulted in a strong air movement across the plants, which is not unlike the environment in the field. The appearance of these plants was as similar to plants of field populations as the eye can measure.

The transplanted halogeton plants were watered three times each week. On July 6, July 18, August 6, and August 15, a group of 48 plants were treated. The plants were selected for treatment on the basis of cruciform growth habit, uniform size, a bluish-green color, and full turgid leaves. The plants that were not used were discarded.

The plants selected for treatment were moved to the laboratory, where they were randomly chosen for the various treatments. The branch to be treated was also selected at random and labeled unless it was touching another branch. If so, another branch was chosen and labeled.

Four plants from each treatment were harvested on each of 4 days—1, 2, 4, and 6 days—after treatment by carefully removing the plants from the soil so as to retain a maximum amount of the root system. The soil was washed from the root system with running tapwater, and three replications were dried in a 70° F. oven for 24 hours. One plant (previously selected at random) was placed in a plant press and oven-dried at 70° for 48 hours and used to make radioautographs. The other three plants were dried and then divided into seven samples in the following manner: (1) The treated branch, (2) the crown, (3) the root system, and (4, 5, 6, and 7) the four untreated branches. The branches were cut from the plant about 1 centimeter above the crown. "Crown" is used here to designate the portion of the plant where the four lateral branches originate on the central stem.

The individual samples were broken into small units in a mortar, covered with a minimum amount of ignited sand, and ground into small particles (approximately 20 to 40 mesh) with a pestle. The sand and ground plant mixture was poured into a 30-milliliter vial. The mortar and pestle were rinsed with 5-milliliter aliquots of 80-percent ethanol until no visual traces of the plant material remained. The rinses were added to the proper vial and ethanol was added to fill the vial.

The vials were allowed to stand for 24 hours with intermittent shaking to insure thorough mixing of the solution and the plant material. About 2 milliliters of the resultant steep was decanted into a bottle cap (these caps lacked the cork, glue, and paint found in a typical soft drink bottle cap) and the liquid was allowed to evaporate. The solution in the caps was replenished periodically until the solution had been decanted from the vials. The vials were then refilled with 80-percent ethanol and the evaporation process was repeated. The period required to evaporate the 60 milliliters of ethanol ranged from 7 to 10 days. The caps were stored in trays in a dustproof box until counts of the radioactive materials could be made. A 4-minute count was made for each cap by means of a counting tube with an end window of mica 1.4 milligrams per square centimeter. A 4-hour background count was made each day experimental samples were counted.

The plants used for radioautographs were mounted on 20-weight bristolboard and exposed to "no-screen" X-ray film for 6 months.

The total counts per minute above background for the three plants of each treatment are presented in table 11. Recovery in this experiment ranged from 25 percent to more than 98 percent of the total activity applied.

Absorption and translocation resulted with all three carriers. Statistical analysis of the data revealed that gasoline saturated with paraffin was significantly better as a carrier than either water plus 1 percent of Dynawet or water plus 1 percent of Dynawet plus 2 percent of Carbowax 1500. No statistical significance was found among the other data examined.

The largest amounts of absorbed and translocated radioactive materials were generally found in the crown of the plant and in the roots. In a few plants, activity was higher in the untreated branches than in the crown and roots. Regardless of the carrier, the majority of the radioactivity was always in the treated branch. (These data are not shown because of their large volume.)

The results obtained from the radioautographs (not shown for this experiment) of plants treated with 2,4-D-2-C^{14} agreed with results obtained by making counts of the radioactivity in the plants. The radioautographs indicate that most of the activity not associated with the treated branch was in the crown and root system of the plants. There is little indication that the radioactive material tended to accumulate in the stem tips or in the glomerules on the stem. In nearly every instance there appears to be a gradient from high concentration in the crown of the plant to lesser concentrations in the untreated stems. The lowest concentrations of radioactivity appeared to be in the stem tips, and often there was no indication of radioactive materials in them.

Radioautographs of dissected plants

Crafts (*8*) discussed artifacts created by various methods used in studying movement of (C^{14}-labeled 2,4-D and methods of killing and drying plants. When he compared plants killed and dried by methods similar to those described in the preceding section of this bulletin with plants dried while frozen, he found considerably more transport in the first group. The slower transport in the freeze-dried group was more prominent in plants receiving a short period of treatment (2 hours or less). It appeared that this antifact might possibly be used as a tool to differentiate between absorption and translocation. I repeated the work reported in the preceding section with 2,4-D-2C^{14} and used radioautographs to measure the results. This was done to determine whether the results reported were due to artificial translocation during the drying process.

The data reported in the preceding experiment would indicate that, if absorption of 2,4-D was occurring but the herbicide was not being translocated from the treated branch, it was being moved into the crown and root of the plant by apoplasmic hydrostatic phenomena created during killing and drying of the plant. If the treated branch was cut off before the plant was removed from the soil, there should be no artificial movement of radioactive materials from the treated branch even when the xylem of the plant is under subatmospheric pressure.

TABLE 11.—*Effects of various carriers on absorption-translocation of 2,4-D-2-C^{14} in halogeton at various stages of growth during the first 6 days after application of the herbicide solution to a single branch of the plant* [1]

Composition of carrier	Days between treatment and harvest	Counts per minute of residue from alcohol extracts on [1]—							
		July 6		July 18		Aug. 1		Aug. 15	
		From treated branches	From all untreated portions	From treated branches	From all untreated portions	From treated branches	From all untreated portions	From treated branches	From all untreated portions
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Water plus 1 percent of Dynawet	1	8,940	122	11,405	708	15,283	77	5,750	115
	2	6,903	83	11,422	99	10,464	102	5,668	103
	4	6,119	222	14,227	138	14,447	148	9,098	137
	6	7,694	230	8,490	287	10,836	1,117	4,993	176
Water plus 1 percent of Dynawet plus 2 percent of Carbowax 1500.	1	9,612	150	9,954	180	11,186	95	6,827	106
	2	10,279	99	10,351	51	8,925	149	5,921	112
	4	7,756	93	10,595	251	10,020	116	6,447	85
	6	7,257	714	10,455	298	8,907	209	7,998	75
Gasoline saturated with household paraffin.	1	6,506	1,295	7,142	491	11,592	138	9,578	289
	2	5,592	465	4,794	345	8,989	169	6,219	810
	4	6,218	2,673	5,840	2,670	9,979	205	5,507	269
	6	4,183	2,912	6,378	343	5,529	509	6,240	138

[1] Each figure represents the sum of the counts per minute above background for 3 plants.

Every effort was made to prevent the plants from developing water stress that might increase the amount of movement by hydrostatic phenomena. For this experiment, treated plants were watered the evening before treatment and every other day thereafter until they were harvested. To prevent further development of subatmospheric pressures in the xylem, the plants were placed in a dark, moist chamber the evening before they were harvested.

The treated branches were cut from all the plants as the first step of harvest. The soil was washed from the roots and the other four branches were cut from the plants. The next step was to separate the crown and the roots. Then all branches except the treated branch were sectioned into 2- to 3-inch units and mounted in their relative position on bristolboard and dried between blotters in a 70° F. oven for 24 hours.

Since there was no statistical difference between water plus Dynawet and water plus Dynawet plus Carbowax 1500 in the previous experiment, only water with 1 percent of Dynawet and gasoline saturated with household paraffin were used as carriers in this experiment. The same stages of growth were treated as in the previous experiment, but the plants matured faster during the growing season of 1957, so the dates of treatment are slightly earlier. Plants were treated on July 4, 16, 29, and on August 12. Except as noted, the conditions under which this experiment was conducted were the same as those outlined in the previous experiment.

The results of this experiment are shown in figures 19–22. Diagrammatical drawings have been made of the plant showing the origin of the various branches on the central axis of the plant. The degree of crosshatching indicates the intensity of the reaction between the radioactivity in the plant parts and the X-ray film. The number at the top of each branch corresponds to its order of acropetal development on the central axis. The treated branches of the individual plants can be noted by the fact that they are not sectioned and have been crosshatched in four directions. The concentration of radioactivity in the other branches is indicated by crosshatching in one, two, or three directions. Plant parts that are not crosshatched did not appear to have any radioactive materials.

The results of this experiment indicate that gasoline saturated with paraffin causes not only more radioactive materials to be absorbed into and translocated within the halogeton plant during its resistant phase

of growth (reproductive growth) but also a wider distribution within the plant. Increasing resistance to absorption-translocation of 2,4-D is indicated by the diminishing amounts of radioactivity in untreated portions of the plants with increasing age. The radioautographs indicate that the individual plants vary in their resistance to absorption-translocation of 2,4-D. This is more obvious where water was used as a carrier. This variation in resistance of the individual plants in a population has increased the difficulties of interpreting the results of all experiments concerned with halogeton and 2,4-D.

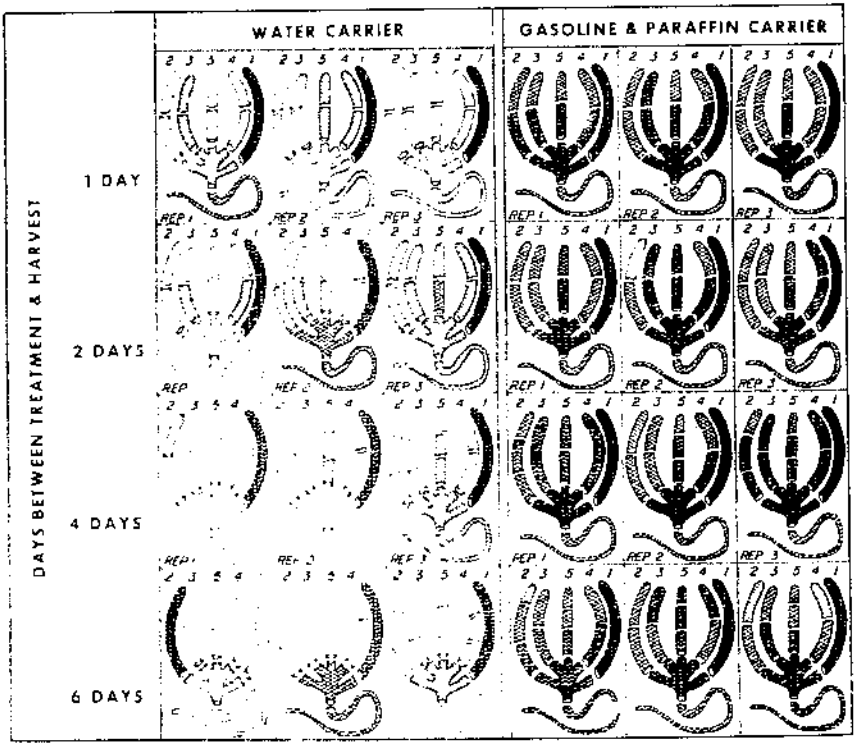

FIGURE 19.—Concentration of radioactivity in plants treated with 2,4-D-2-C^{14} in a water carrier and in gasoline saturated with household paraffin carrier on July 4, 1957.

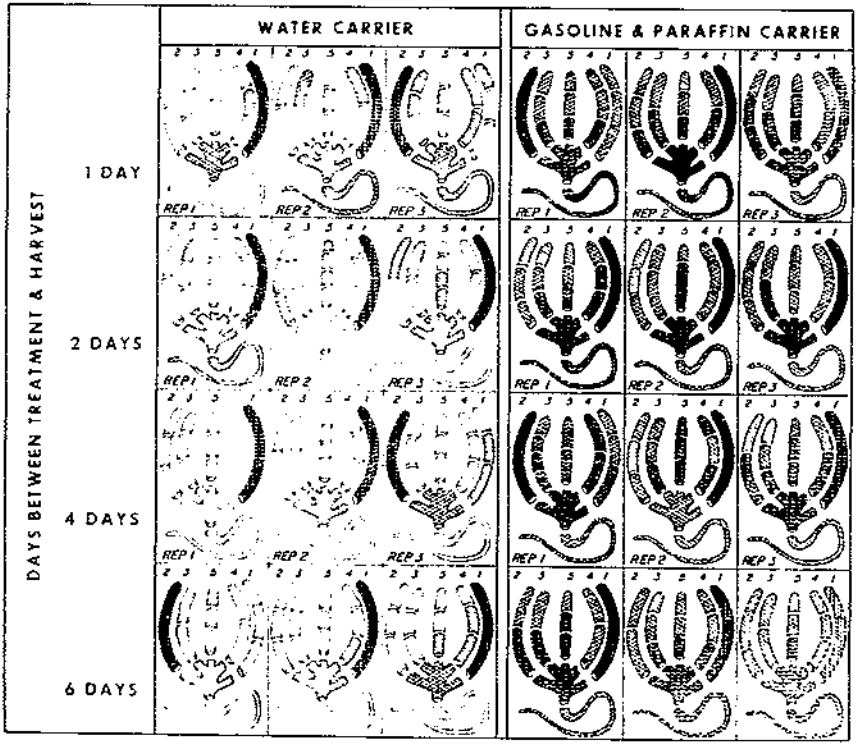

FIGURE 20.—Concentration of radioactivity in plants treated with 2,4-D-2-C^{14} in a water carrier and in gasoline saturated with household paraffin carrier on July 16, 1957.

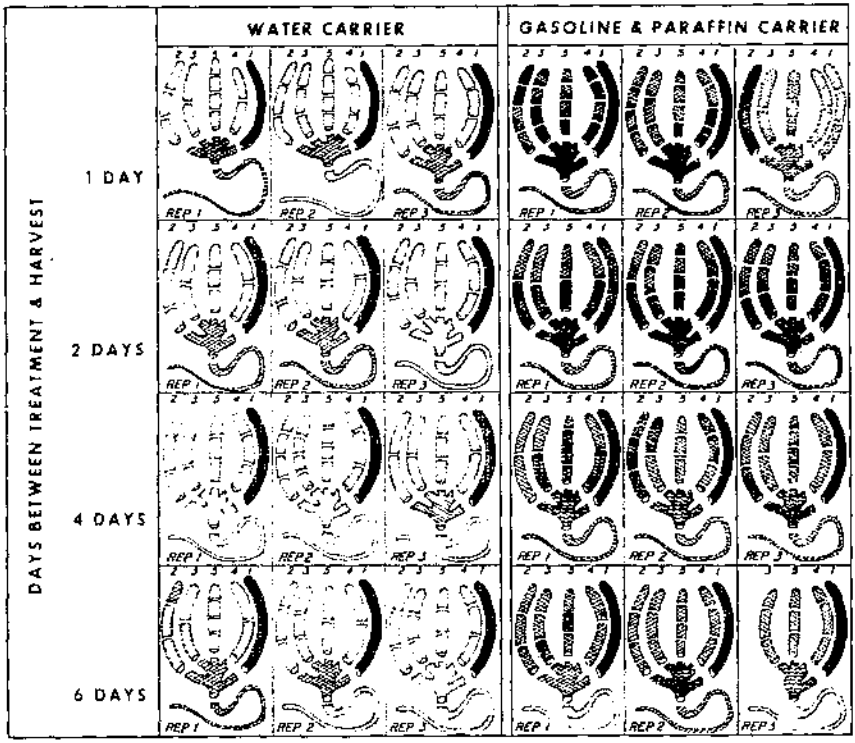

FIGURE 21.—Concentration of radioactivity in plants treated with 2,4-D-2-C^{14} in a water carrier and in gasoline saturated with household paraffin carrier on July 29, 1957.

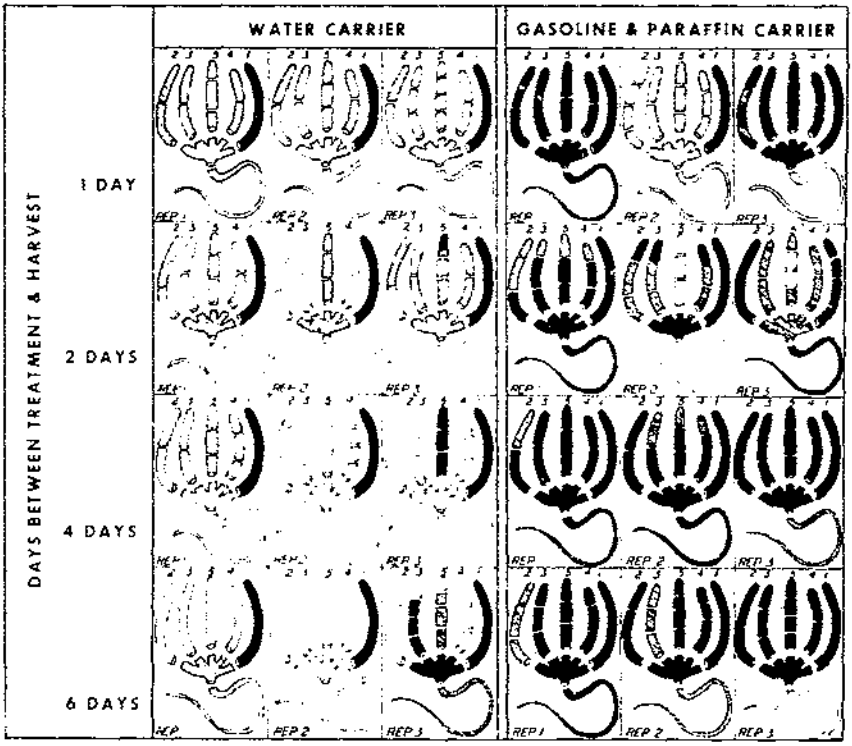

FIGURE 22.—Concentration of radioactivity in plants treated with 2,4-D-2-C" in a water carrier and in gasoline saturated with household paraffin carrier on Aug. 12, 1957.

ABSORPTION AND TRANSLOCATION OF FLUORESCENT DYES

Dybing and Currier (*11*) reported techniques and results involving the use of a water-soluble fluorescent dye to study foliar penetration in plants. These researchers used sodium 3-hydroxy-5,8,10-pyrenetrisulfonate to detect possible pathways of foliar penetration and the effects of some surfactants on the rate of penetration. Dr. Currier furnished a small quantity of the chemical for use in this study.

Aqueous solutions containing 0.1 percent and 0.5 percent of the fluorescent dye and 0.01 percent of Dynawet were used in these tests, which repeated the work reported by Dybing and Currier, who used *Zebrina pendula* (wandering Jew) as the test plants. Under the conditions of this experiment, a longer period of treatment and washing to remove the dye was required to obtain results similar to those reported by Dybing and Currier.

A second dye, bis (*p*-dimethylaminophenyl)-methylenimine (an oil-soluble dye available from biological supply houses under the trade name of "Auramine 0"), was also used to determine the role of the

gasoline saturated with paraffin carrier in absorption and translocation of herbicides. A solution of gasoline saturated with household paraffin and 0.01 percent of Auramine O was used. The dye was not readily soluble in the carrier, and all the dye added to the solution may not have been dissolved.

These tests were conducted during July, August, and October in 1959 with plants held in the reproductive stage of growth by lights supplementing the normal photoperiod. Work with the oil-soluble dye was repeated in August 1960.

Potted plants transplanted from the field during early June were used in the work with halogeton. The terminal inch of a branch was submerged in the test solutions for intervals ranging from 15 seconds to 5 hours. Figure 23, a photograph taken with ultraviolet light, indicates the method of treatment. During the treatment, the plant was placed on its side so the tip of the treated branch was lower than the rest of the branch. This position prevented the dye solution from moving on to the untreated portions of the branch. The plants remained in this position for 1 hour (with the exceptions noted in the results) to insure drying of the dye solution before they were returned to an upright position.

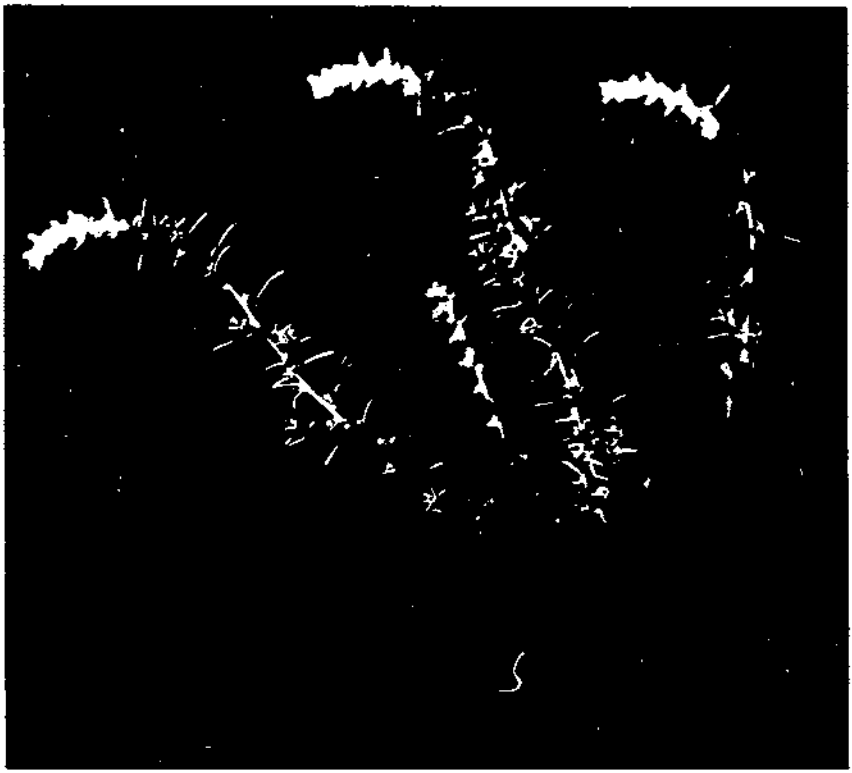

FIGURE 23.—Halogeton plant with three branches treated with fluorescent dye solution. The photograph was taken under ultraviolet light.

The branches treated with the water-soluble dye were washed under running tapwater for periods ranging from 3 minutes to 4 hours. The treated portions were examined under both a dissecting scope and a light microscope with light on the upper surface provided by two adjustable microscope illuminators equipped with H100 SP4 spot-type mercury arc bulbs (GE).

Following periods of absorption ranging from 1 to 48 hours (timed from the moment the branch tip was first submerged in the test solution), the branch was cut off at the base and the tip was removed 2 millimeters below the base. Sections were made of the stem at measured distances from the point where the treated tip was removed. These were examined under a light microscope with the ultraviolet light source. Hand sections of the treated tip were also examined.

Regardless of the time stem tips were immersed in solutions of the water-soluble dye and the time allowed for the material to penetrate the surface of the plant, the dye was not observed to penetrate the surface. The dye could not be washed as easily from the surface of the halogeton plant as from the surface of Zebrina. About 4 hours was required to remove the dye from halogeton by running tapwater over the surface of the treated area. The dye tended to adhere to the terminal area of the vegetative leaves and in the axils of the leaves and bracteoles. After the dye was removed from the surface of the leaves, sections made of the treated leaves and stem showed no evidence of fluorescence: nor was fluorescence ever observed in sections of the untreated portions of treated stems.

Because of the difficulty of removing the dye from the surface of halogeton, it was an arduous task to determine whether the dye was penetrating the stomates. Attempts were made to detect penetration through the stomates by treating plants when the stomates were open and removing the dye after the stomates had closed. Treated plants were placed in the darkroom immediately; this was done to induce the stomates to close quickly, so that the dye could be washed from the surface of the plants before any dye in the stomatal cavities had time to diffuse into the surrounding tissues and become too diluted to be observed. Attempts were also made to strip off the epidermis of the leaf after short periods of 5 to 25 minutes of washing, but the dye moved quickly from the intact epidermis over the area where the epidermis had been removed. As a result, the water-soluble dye did not appear to penetrate the cuticle or the stomatal openings.

Data from the tests with the oil-soluble dye are recorded in table 12, and they probably indicate the penetration and movement of the carrier in the plant. Whether other lipoidal solvents move along these same pathways is not known, but it is assumed that they would follow similar pathways since results were similar when they were used as carriers of 2,4-D.

The dye appeared to enter through the stomates of the leaf and through the cuticle and epidermis of both leaves and stems. Leaves submerged for 2 minutes and longer appeared to have open stomates at the end of the treatment, while periods of 1 minute and less did not appear to cause the stomates to react. The small size of the stomates made it difficult to observe any but the most gross movement of the guard cells. It was much easier to determine the location of the stomates and the position of the guard cells when the stomatal chamber contained fluorescent dye than when the dye was confined to the sur-

TABLE 12.—*Absorption-translocation of Auramine O by halogeton plants treated from Oct. 14 to 28, 1959*

Stems examined (number)	Time stem tip in dye solution	Time for absorption to occur after treatment	Plant structure examined	Distance from treated portion in which dye was observed	Tissue containing fluorescent dye
	Seconds	*Hours*		*Centimeters*	
4	15	1	Leaf	1	Central vascular bundle.
6	15	1	Stem	10	Phloem.
4	15	2	Leaf	1	Central vascular bundle.
4	15	2	Stem	8	Phloem.
4	15	4	Leaf	4	Central vascular bundle.
8	15	4	do	11	Phloem; a few xylem cells.
4	15	8	do	1	Central vascular bundle.
4	15	8	Stem	7	Phloem.
8	15	24	Leaf	0	
8	15	24	Stem	0	
10	30	1	Leaf	1	Central vascular bundle.
4	30	1	Stem	10	Phloem.
8	30	1	do	20	Phloem (small arcs).
4	30	2	Leaf	4	Modified spongy mesophyll.
4	30	2	Stem	6	Phloem.
4	30	4	do	10	Cambium; phloem.
6	30	4	Leaf	2	Central vascular bundle.
4	30	5	do	4	Central vascular bundle.
8	30	5	Stem	5	A few xylem cells.
6	30	5	do	6	Phloem (faint).
4	30	6	Leaf	3	Central vascular bundle.
12	30	6	Stem	10	Phloem (small arcs).
4	30	6	do	10	Phloem (distinct).
8	30	9	Leaf	0	
4	30	9	Stem	10	Secondary xylem.
4	30	9	do	15	A few xylem cells.
8	30	24	Leaf	0	
8	30	24	Stem	0	
8	30	48	Leaf	0	
8	30	48	Stem	0	
	Hours				
4	1	1	Leaf	5	Central vascular bundle.
4	1	1	Stem	8	Phloem.
4	1	4	Leaf	1	Central vascular bundle.
4	1	4	Stem	7	Phloem; a few xylem cells.
4	1	10	Leaf	1	Central vascular bundle.

TABLE 12.—*Absorption-translocation of Auramine O by halogeton plants treated from Oct. 14 to 28, 1959*—Continued

Stems examined (number)	Time stem tip in dye solution	Time for absorption to occur after treatment	Plant structure examined	Distance from treated portion in which dye was observed	Tissue containing fluorescent dye
	Hours	*Hours*		*Centimeters*	
8	1	10	Stem	10	Phloem; areas in xylem.
8	1	24	Leaf	0	
8	1	24	Stem	0	
8	1	48	Leaf	0	
8	1	48	Stem	0	
8	2	1	Leaf	0	Central vascular bundle.
4	2	1	Stem	4	Phloem; cambium.
4	2	4	Leaf	1	Central vascular bundle.
4	2	4	Stem	9	Phloem.
4	2	8	Leaf	5	Central vascular bundle.
6	2	8	Stem	12	Phloem; cambium; a few xylem cells.
8	2	24	Leaf	0	
8	2	24	Stem	0	
8	2	48	Leaf	0	
8	2	48	Stem	0	
8	5	1	Leaf	0	
4	5	1	Stem	5	Phloem.
4	5	2	Leaf	5	Central vascular bundle; one lateral branch of vascular bundle.
5	5	2	Stem	8	Phloem; small area of secondary xylem.
4	5	4	Leaf	1	Central vascular bundle.
4	5	4	Stem	10	Phloem.
4	5	8	Leaf	1	Central vascular bundle.
4	5	8	Stem	12	Phloem.
4	5	10	Leaf	2	Central vascular bundle.
6	5	10	Stem	8	Phloem; a few xylem cells.
4	5	12	Leaf	1	Central vascular bundle.
4	5	12	Stem	5	Phloem; camium.
6	5	12	Leaf	3	Central vascular bundle.
8	5	14	Stem	0	
10	5	18	Leaf	0	
10	5	18	Stem	0	
8	5	24	Leaf	0	
8	5	24	Stem	0	
8	5	48	Leaf	0	
8	5	48	Stem	0	

face or when the plant was treated with the carrier alone. Some of the oil-soluble dye was flushed from a small area of the leaf surface with 95-percent ethanol. Such treatment was helpful in determining whether the dye was in the substomatal chambers and the anticlinal walls of the epidermis cells.

When it could not be determined that the stomates were open and the dye had moved into the substomatal chambers, sections of treated leaves were made. These sections generally indicated that movement through the epidermal tissue was largely confined to the anticlinal walls of the epidermis, but the dye was also observed in the lumen of the cells. Penetration through the epidermis cells and through the pores of the stomates normally occurred in the same plants. Movement through the tissue of the leaf other than the epidermis did not appear to be confined to the cell walls; rather, the intensity of the fluorescence was commonly greater in the protoplasm.

The time required for the dye to penetrate into the treated portions of the plant and move into the untreated portions of the stem appeared to be about 1 hour. There was some variation among individual plants. The 1-hour period appeared to be required for the materials to penetrate into the conductive tissue of the plant. Once translocation was initiated, it was apparently rapid. The dye and the carrier appeared to be confined to the phloem for a short distance below the treated section of the stem. The dye was observed in the xylem 5 centimeters below the treated portion of the stem but not above this point.

Concentration of the dye was reduced beyond visual recognition with both distance from the treated stem tip and time. The fact that the dye was never observed in the stem or leaves of the untreated portions of the stem more than 12 hours after treatment is thought to indicate that penetration occurs over a relatively short period—much shorter than 12 hours. Penetration time was probably related to the volatile nature of the carrier. The fact that the dye was not observed beyond 14 to 20 centimeters below the treated tip is thought to be due to a simple dilution factor.

While the dye was present in all tissues of the treated portions of the stem, it was confined to the phloem, xylem, and cambium of the untreated portions. It was not observed in the interfascicular cambium, pith, or cortex—an indication that intratissue movement was extremely slow or nonexistent in these tissues.

In treated leaves, the dye was observed in all tissues except the water tissue (cortex). This was interpreted to indicate that the dye was stable in the tissues of the halogeton plant even when the tissue containing the dye was subjected to sectioning operation.

ABSORPTION AND TRANSLOCATION OF 2,4-D-2-C^{14} AND WATER-SOLUBLE FLUORESCENT DYE THROUGH THE ROOTS OF HALOGETON

In an effort to gain some indication of the translocatability of 2,4-D and the water-soluble fluorescent dye, both compounds were supplied to the root system of the plant on the assumption that absorption would not be restrictive in the roots. Plants for this study were obtained from field populations and grown in culture solution

FIGURE 24.—Photograph taken under ultraviolet light of a halogeton plant after its roots had been exposed to 0.01 percent solution of a water-soluble fluorescent dye for 24 hours.

until they had developed a new absorbing root system (about 4 weeks).

The labeled 2,4-D was supplied to the root system in the culture solution at concentrations of 250, 500, 1,000, and 4,000 parts per million. The plants were exposed to the solution for 2 to 6 hours, sectioned into short lengths, mounted on blotters, ovendried, and radioautographed. Plants to be supplied with fluorescent dye were removed from the culture solution and placed in a 0.01-percent solution of the water-soluble fluorescent dye. These plants were moved to the dark room and observed at hourly intervals under ultraviolet light.

Radioautographs of the plants exposed to labeled 2,4-D indicated that time of exposure had little effect on the amount of radioactivity or its location in the plant. This is somewhat surprising in relation to the concentration, but the time of exposure varied only 4 hours. Increased concentrations of the herbicide may have adversely affected the permeability of the roots.

The water-soluble dye could be observed within the intact plant 8 hours after it was placed in the dye solution. Figure 24 is a photograph of a plant taken under ultraviolet light 24 hours after being placed in the dye solution. Both the radioactive 2,4-D and the water-soluble fluorescent dye tended to concentrate in the adaxial base of the leaves and bracteoles.

FIELD PLOT TRIALS WITH GASOLINE SATURATED WITH PARAFFIN AS A CARRIER FOR 2,4-D

Gasoline saturated with household paraffin was used as a carrier for 2,4-D on large field plots since it had appeared to be effective as a carrier in experiments concerned with absorption and translocation. Because it is flammable, the mixture was emulsified in water at the following concentrations: 0, 5, 10, 20, and 50 percent by volume. The mixture plus the 2,4-D was applied to plots at the rate of 15 gallons per acre. The propylene glycol butyl ether ester of 2,4-D was applied at rates of 0, ½, 1, and 2 pounds per acre.

The study was conducted on a random block design with plots 12 feet square with four replications for each treatment. The treatments were applied on July 10, July 31, and August 21, 1954. These treatments were compared to an application of 2 pounds per acre in a carrier of water and to 16 plots receiving no treatment. Results were evaluated by visual estimates.

Halogeton was in the reproductive phase of growth when treatments were applied and was resistant to 2,4-D, as indicated by 52-percent kill obtained with the herbicide in a water carrier treated July 10 (table 13). The percentage of kill increased when gasoline saturated with household paraffin was incorporated into the spray mixture. Either 20 or 50 percent of gasoline saturated with paraffin and 1 or 2 pounds per acre of 2,4-D were the best spray mixtures used in this experiment. The effectiveness of the carriers and 2,4-D still depended on the stage of growth of halogeton.

TABLE 13.—*Average kill of the original population of halogeton plants on plots treated with various rates of 2,4-D in various concentrations of gasoline saturated with paraffin emulsified in water* [1]

Date treated and amount of 2,4-D applied per acre (pounds) [2]	Kill obtained when concentration of gasoline saturated with paraffin emulsified in water was—				
	None	5 percent	10 percent	20 percent	50 percent
July 10:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0		2	0	2	2
½		20		28	44
1		59	54	86	75
2	52	69	80	50	74
July 31:					
0		0	0	2	0
½		5	2	5	10
1		2	18	6	7
2	18	20	20	38	30
Aug. 21:					
0		0	0	5	0
½		5	0	2	1
1		0	0	12	12
2	0	12	2	0	8

[1] An average of 4 percent of the halogeton plants died on the 16 plots receiving no treatment.

[2] Applied in a spray mixture at the rate of 15 gallons per acre.

DISCUSSION

It is fortuitous when one finds an undesirable plant susceptible to control measures at a stage of development that is critical in the completion of its life cycle. Controlling halogeton is more complicated. It is susceptible to applications of 2,4-D during its vegetative stage of growth, but to disrupt the life cycle of halogeton it would be necessary to prevent all seed production on a control area. Halogeton plants may become established after summer showers (*18*, *28*, *42*, *43*). Morton, Haas, and Erickson (*26*) and I have noted plants that became established after the middle of August and produced seeds by the end of the growing season.

The prolific seed production of halogeton is important in considering control of the plant. It is possible to calculate the theoretical potential production of the seed on an inch of halogeton stem. As Jansen's [9] data indicate, each 1-inch portion of halogeton stem produces an average of 27 brown seeds and 47 black seeds. Justice and Reece (*22*) found that 67 percent of the black seeds produced normal seedlings when the pH of the medium was 8.0. (Gates, Stoddart, and Cook (*15*) found that the pH of the surface soils (0 to 6 inches) investigated in the salt-desert shrub area varied from 8.1 to 8.4.) My data, from three seasons, indicate that the average survival of established seedlings is 53 percent. If the "normal" seedlings of Justice and Reece would be "established" seedlings under field conditions, the

[9] JANSEN, L. L. PERSONAL COMMUNICATION.

implications are that the black seeds from 1 inch of halogeton stem would be potentially capable of producing 17 mature plants during the following growing season. The progeny from a single inch of stem could produce sufficient plants to infest many acres within 2 to 3 years.

Tisdale and Zappettini (*43*) have stated that a large plant may produce as many as 25,000 seeds, whereas a plant germinating in August produced 270 seeds. They have also compiled data that indicate that a vigorous stand of halogeton can produce 200 to 400 pounds of seed per acre with approximately 571,760 black seeds and 167,850 brown seeds per pound of seed produced.

There are insufficient data on the germination of the brown fruit to calculate their potential, but they should not be dismissed as unimportant. Holl (*18*), Robocker and Kerr (*35*), and Sharp (*38*) have data indicating that the brown fruit are viable and will germinate. Williams (*45*) has grown plants to maturity from the embryos excised from the brown seeds and found them indistinguishable from plants originating from black seeds. If all seed production was prevented during any single growing season on any specific site, a source of new plants from the brown seeds from previous crops would remain in the soil. It is also possible for seeds to be transported into an area by wind (*18*), by animals (*7*), and by man and his machinery (*19*).

The leachate from halogeton apparently changes soil properties (*13*) and creates an environment more favorable for the germination of seeds of halogeton and other undesirable weeds (*24*). Halogeton seeds appear to be able to germinate in media of higher osmotic pressure than the seeds of some of the associated plants used in this study. The implications are that once halogeton infests a site, it changes the environment to make it more suitable for itself. The change may be as simple as increasing the osmotic pressure of the soil solution.

Morton, Haas, and Erickson (*26*) and I found that halogeton becomes resistant to aqueous sprays of 2,4-D around the first of July. The onset of resistance to the herbicide occurs about the time the plant enters its reproductive phase of growth. The percentage of moisture in the terminal inch of the stems drops sharply at about the same time.

Early observations indicated that the basis of resistance could be due to a lack of translocation or to poor penetration of the herbicide. Little or no evidence is contained in the data compiled here to indicate that the herbicide is not translocated readily in the plant. The roots of halogeton are apparently permeable to the solutes in the soil moisture (*26*, *46*), and the water-soluble fluorescent dye and C^{14}-labeled 2,4-D supplied to the root system were translocated rapidly into the other parts of the plant.

Evidence is substantial that 2,4-D does not penetrate easily into the plant during reproductive growth. Although the anatomical studies were limited in scope, they do permit speculation on some aspects of absorption of 2,4-D in halogeton. There appear to be no anatomical structures in the root to prevent absorption.

The thick-walled cells of the epidermis of both stem and leaves and those of the outer cortex of the stem suggest a relatively high degree of impermeability to aqueous solutions (*31*). The cortex of the leaf proved extremely difficult to dehydrate and infiltrate in the paraffin method; it similarly may retard entrance of the herbicide. The biser-

nate layer of cells of the leaf between the epidermis and chlorenchyma contains cells similar in appearance to those in the leaf cortex, implying that the bisernate layer might inhibit penetration of herbicides.

Repp (*31*), who worked with the halophytes of the coastal area of northern Denmark, has suggested that the halophytes of the salt marshes almost always possess on their aerial parts an arrangement of hairs, cuticle, and thickened outer walls of the epidermis that protect against wetting and penetration from salt water. The halogeton plant possesses all these structures; the epidermis has already been discussed.

Lanate hairs are found in the axils of the leaves and appear to inhibit wetting of the lower portions of the leaves. When a surfactant is added to an aqueous spray, a disproportionate amount of the water collects in the axils of the leaves.

The cuticle also resists wetting. It is thought to be the most important individual structure of the halogeton plant that limits penetration by externally applied liquids. Attempts to isolate the cuticle for study have been unsuccessful. The cuticle responded with no consistency to lipoid stains. In 8 years the cuticle was observed on only two plants as a result of treatment with Sudan IV. The cuticle deposited on the surfaces of the halogeton plant probably varies in amount [10] and composition (*27*) with the date and site of collection and among individual plants.

Separation of the phenomena of penetration and translocation is not an easy task with any plant, and with the methods used to study these factors in relation to halogeton it has been extremely complex. Because of the small leaves of halogeton, it has been necessary for the materials to be translocated before absorption could be detected. No individual experiment concerned with absorption and translocation can be used to determine which factor is responsible for the resistance of halogeton to 2,4-D during its reproductive phase of growth. When data from all the experiments are considered together, much evidence exists that lack of absorption is the primary reason for the exiguous response to 2,4-D.

Field trials with the various carriers for 2,4-D, conducted in 1953 and 1954, indicate the relative importance of absorption and translocation of the herbicide during resistant stages of growth of halogeton. When 2,4-D was applied in aqueous carriers, there was little damage to the untreated portions of the plant except at high concentrations of the herbicide. As resistance of the plant to 2,4-D increased, even the treated branch tended to show fewer symptoms of injury, although at the higher concentrations it was killed.

Lipoid solvents as carriers for the herbicide resulted in the death of the treated branch. With some of the more phytotoxic substances, there was severe injury to the untreated branches of the plant. This is interpreted to indicate that materials such as kerosene, ethanol, gasoline, carbon tetrachloride, and Stoddard solvent are translocated within the plant. Increased injury to untreated portions of the plants was noted when 2,4-D was added to these carriers. The increased injury must be due to the 2,4-D. Whether the herbicide is carried to the untreated portions of the plant by these carriers or is trans-

[10] ORGELL, W. H. THE ISOLATION AND PERMEABILITY OF PLANT CUTICLE. Doctorate thesis, Univ. Calif., Davis, Calif. 1954.

located independently of these carriers is not known, but 2,4-D probably moves with lipoid carriers.

Carriers of water and of gasoline saturated with paraffin performed at the same levels relative to each other in experiments in which C^{14}-labeled 2,4-D was used in the same way as in the field studies. With the labeled herbicide it was possible to obtain data on the concentration of the radioactive materials (assumed to be 2,4-D) in the untreated parts of the plant. The radioactivity found in the untreated parts of the plant was significantly greater with the carrier of gasoline saturated with paraffin than with the water carrier. This study also indicated that adding Carbowax 1500 to water (*14*) did not increase the amount of 2,4-D that penetrated the treated branch and was translocated within the plant. Apparently, evaporation of aqueous carriers, which is presumably reduced by Carbowax 1500, is not important so far as absorption-translocation of 2,4-D in halogeton is concerned. This is not true with many of the lipoid solvents, since the addition of paraffin to gasoline increased the effectiveness of this substance as a carrier.

Skoss (*39*) collected data that indicated that the stomates are important pathways of penetration for herbicides in some plants. I did not observe any of the water-soluble fluorescent dye in the stomatal chamber even when the stomates were known to be open at the time of treatment, but I did observe the oil-soluble fluorescent dye in the stomatal chambers. This was true with plants known to have open stomates at the time of treatment and with plants known to have closed stomates. The stomates are probably not an avenue for entry of 2,4-D with aqueous carriers but are utilized with lipoid solvents. The lipoid solvent appears to destroy the control of the guard cells over the pore opening.

Experiments to study absorption-translocation by means of fluorescent dyes cannot be assumed to be directly related to the absorption and translocation of 2,4-D. The dyes indicated the movement of the carrier. The water-soluble fluorescent dye was not observed to penetrate the surface of any halogeton plant during the experiment, but it was tenaciously adsorbed on the surface of the plant. In both field and laboratory studies of absorption-translocation of 2,4-D, there was often evidence that the herbicide applied in aqueous carriers was absorbed and translocated; the amount appeared to vary with the individual plant. Evidence from these three types of experiments indicates that water may not move into the aerial portions of the plant while 2,4-D is absorbed and translocated independently, at least by a portion of the population. The solubility properties of 2,4-D are probably the basis of differential absorption of the herbicide and the carrier. If this assumption is correct, it follows that translocation of 2,4-D in halogeton is not a problem.

Gasoline saturated with paraffin was used as a carrier for an oil-soluble fluorescent dye. Evidence from this experiment was that the carrier is able to penetrate the cuticle and tissues of both the leaves and stems and to gain entry into the plant via the stomates. It is likely that other lipoid carriers, especially those that are solvents for waxy materials, would behave in a similar manner.

Movement from the surface into the vascular system appears to require about 1 hour. Once the material is in the phloem, it is ap-

parently translocated rapidly. Nor does the evidence indicate that it depends on the phloem as a pathway of distribution in the plant. It diffuses from phloem to xylem after moving a short distance (5 cm.) and after having been in the phloem for only a short time. This agrees with data reported by Radwan, Stocking, and Currier (*29*) and also agrees with evidence obtained in both field and laboratory studies where symptoms of injury by the carrier were observed on untreated branches of the plant.

The lipoid solvents and 2,4-D are undoubtedly closely associated in the processes of penetration and translocation. Penetration of 2,4-D into a population of halogeton apparently is enhanced with, if not dependent on, a lipoid carrier; but it is doubtful that translocation of these carriers and 2,4-D is a dependent reaction. Hay (*16*) and Rohrbaugh and Rice (*36*) have used both kerosene and sucrose to facilitate the transport of herbicides from destarched bean leaves. In halogeton, the primary function of kerosene appears to be to lessen the impediments to the penetration of 2,4-D. An aqueous solution of sucrose as a carrier did not appear to be as effective as water. I assume that sucrose did not enter the plant and did not affect the carbohydrate gradient. When sucrose was added to kerosene, the results were not sufficiently different from the expected results with kerosene alone to indicate that sucrose affected the translocation of the herbicide.

Regardless of the carrier and the date of application, 2,4-D applied to the aerial portions of the plants appears to be absorbed and translocated by, at least, a fraction of the population. The proportion of the population that will absorb and translocate the herbicide can be substantially increased with the use of lipoid carriers that act as solvents of waxy materials. These phytotoxic carriers can penetrate through the stomates and can apparently destroy the control of the stomatal opening through their toxic action on the guard cells.

When halogeton first enters its reproductive phase of growth, its response to 2,4-D is suddenly and dramatically reduced. The toxic action of 2,4-D is continually but gradually reduced as the season continues. Even the wax solvents were not as effective as carriers on the last date of treatment as they were during earlier stages of reproductive development.

Apparently the resistance of the plant to 2,4-D is the result of an absorption barrier: and the cuticle, judged by the results of the experiment with the water-soluble fluorescent dye, would appear to be the absorption barrier to aqueous solutions. Aqueous herbicide solutions start to lose their effectiveness in late June and for all practical purposes are completely ineffective by late July. The percentage of moisture in the stem tips drops sharply during this time, which may be indicative of the hydration of the cuticle. Early in the reproductive phase of growth, all of the lipoid solvents were more effective than water as a carrier of 2,4-D; but later only solvents of waxy substances were effective. Even the wax solvents lost much of their effectiveness as carriers on the last date of application. Changes in composition of the cuticle probably occur throughout the reproductive phase of growth.

The rate of growth by halogeton changes over the season, especially during the later stages of reproductive development. The low rate of

growth may be a factor in the loss of effectiveness by the wax solvents on the last date of treatment.

Detailed information is needed on the composition of the cuticle and on the changes that occur during the growing season. Some of the changes in composition are suggested by the data presented here. Preliminary studies concerned with evaluating chemicals for their toxicity to halogeton have shown that some chemicals are able to penetrate into the plant when applied as aqueous sprays during reproductive growth. Halogeton killed by these chemicals ranges from 60 to 90 percent. These chemicals have side chains of alkylamino radicals and include 2,4-D, triazines, polychlorobenzoic acids, and trichlorobenzoic acids. Not all chemicals with these side chains are effective, but all effective chemicals contain an alkylamino side chain.

SUMMARY

Halogeton glomeratus (M. Bieb.) C. A. Mey. was introduced into the cold desert region of North America sometime before 1934. This poisonous annual weed now infests more than 10 million acres of western rangeland. The toxic substances in halogeton are sodium and potassium oxalates. These compounds account for 17 to 30 percent of its dry weight. It not only is lethal to livestock but also removes heavily infested sites from production because livestockmen are afraid to allow their animals to graze the sites.

Seedlings of halogeton may become established anytime from February through August and complete their life cycle during the growing season. Seedlings begin rapid vegetative growth in May. Near the first of July the plants cease vegetative growth and reproductive growth begins. The seeds begin to mature in late August and early September. In October the seeds are mature, but the best germination is obtained from seeds collected in late November and December.

Halogeton produces two types of seeds that can be separated on morphological and physiological characters. The plant produces brown seeds first. They are the only ones found on the plant until the middle of August. Plants that become established after the middle of August will produce only black seeds.

The black seeds germinate readily and do not persist in the soil for more than one season. The brown seeds have not been germinated in the laboratory, but mature plants have been grown from the excised embryos. It is known that they germinate under field conditions and that they persist in the soil for many years.

Field studies on the date seed-producing plants became established revealed that most of them became established in April. They became established as seedlings as early as February 28 and as late as the middle of August.

Black seeds of halogeton were germinated at much higher osmotic concentrations than were seeds of peppergrass (a native annual) or crested wheatgrass (an introduced perennial grass used to revegetate arid rangeland).

Halogeton opens its stomates for a short time in the forenoon. An individual plant, with 5 stems about 6 inches long, lost about 4 grams of water each day.

The water content of the stem tips drops at the same time the plant enters reproductive growth and becomes resistant to 2,4-D.

Microscopic examination of the anatomical structure of halogeton suggests the cuticle, epidermis, and water tissue as possible sites of an absorption barrier. When open, the pores of the stomates averaged 11.3×1.1 microns in June and 11.5×0.9 microns in August. The stomates were found over photosynthetic tissue. Their density was 5,600 per square centimeter on large, mature leaves that averaged 0.5 cm^2 in surface area.

Rates of 1, 1½, and 4 pounds per acre of 2,4-D applied on June 24 resulted in nearly complete kill of halogeton. Fifteen days later, on July 9, even the highest rate killed less than 50 percent of the plants. Repeated treatments on the same plots on two dates (June 11 and August 12) gave about the same percentage kill of halogeton as the single treatment on June 11.

Various carriers were used to study absorption and translocation of 2,4-D in the field. The effectiveness of the carriers was measured by the extent of 2,4-D injury on the untreated branches of the plant. Lipoid solvents, especially wax solvents, resulted in the greatest injury to the untreated branches. Sucrose and kerosene, which have been found to increase translocation in the bean plant, were only a little more effective than water. Injury was noted first and was the most severe on untreated branches originating higher on the central stem than the treated branch.

Radioactive-labeled 2,4-D was used to treat halogeton. The highest concentrations of radioactivity were found in the untreated portions of the plant when gasoline saturated with household paraffin was used as the carrier. Household paraffin was added to gasoline, a good wax solvent, to reduce its rapid rate of evaporation. The highest concentrations of radioactivity were found in the root and in the crown of the treated plants.

A water-soluble fluorescent dye was not able to penetrate into the plant, but an oil-soluble dye readily moved into the plant. It was possible to follow the movement of the oil-soluble dye within the treated branch of the plant.

Both radioactive-labeled 2,4-D and the water-soluble fluorescent dye were quickly absorbed by the root and transported to other parts of the plant.

CONCLUSIONS

The qualities that adapt halogeton to the harsh, uncertain environment of the salt-desert shrub range are also the qualities that make control of the weed difficult and exacting. The prolific seed production, the persistence of the brown seeds in the soil, and the numerous ways that the seeds are transported are factors that insure its survival. Control of halogeton requires its eradication unless other plants can occupy the space left when halogeton plants are removed by chemicals or other methods.

Halogeton is susceptible to applications of 2,4-D in the vegetative phase of growth; but when reproductive growth begins, resistance to 2,4-D occurs. Seedlings that become established after the first part of July quickly enter into reproductive growth and attain the same resistance exhibited by the chronologically older plants.

The resistance to 2,4-D displayed by halogeton is primarily due to the lack of absorption. Carriers such as gasoline saturated with paraffin can increase the effectiveness of the herbicide. The wax solvent properties of these carriers are believed to increase their effectiveness.

The cuticle is probably the barrier to penetration by aqueous solutions, but anatomical studies suggest that the epidermis of both the stem and the leaf, the outer cortex of the stem, and the subepidermal tissue of the leaf may act to inhibit penetration.

Absorption and translocation of C^{14}-labeled 2,4-D and water-soluble dye were rapid when these substances were supplied to the root system of halogeton plants.

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