AN ABSTRACT OF THE THESIS OF

TOMAS POLLAK for the Master of Science
(Name of student) (Degree)

in Farm Crops presented on August 14, 1970
(Major) (Date)

Title: THE EFFECT OF 2, 4'-DINITRO-4-TRIFLUOROMETHYL-DIPHENYLETHER (C-6989) ON BEANS (*Phaseolus vulgaris* L.) UNDER CERTAIN SOIL AND LIGHT CONDITIONS

Abstract approved: Redacted for Privacy
Dr. William R. Furtick

Oregon is the largest producer of green beans for processing in the United States.

Because the repeated use of a single herbicide may lead to changes in the weed population, build up of residues, and other undesirable factors, a continuous search for new herbicides is taking place.

C-6989[1/] has been registered and used in soybeans; however, the response of green beans to the chemical has not been studied in detail.

In this work the following factors were studied: The tolerance of beans and other plants to C-6989, site of uptake, type and site of

---

1/ Trade name is Preforan. Chemical name is 2, 4'-dinitro-4-trifluoromethyl-diphenylether.

action of the herbicide and the extent to which mechanical conditions of the soil surface is responsible for herbicidal injury to bean seedlings. An evaluation was made of the residual effects of C-6989 in rotation crops.

Results indicated that the herbicide is taken up by roots, shoot or combination of both, depending upon species. Applied at high rates on beans, the effects observed were that of a contact type herbicide. It appeared to block the photosynthetic process by destroying chlorophyll pigments. Under field conditions, a rate of 4 lb/A applied preemergence controlled a broad spectrum of weeds without harmful effects to five varieties of bean. Delays in the application of the herbicide after bean planting, as well as delays in irrigation, tended to cause crop injury. The residual life appears to be short enough under summer conditions to allow the planting of rotation crops without injury.

The Effect of 2, 4'-dinitro-4-trifluoromethyl-diphenylether (C-6989) on Beans (*Phaseolus vulgaris* L.) Under Certain Soil and Light Conditions

by

Tomas Pollak

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1971

APPROVED:

Redacted for Privacy

Professor of Farm Crops
in charge of major

Redacted for Privacy

Head of Department of Farm Crops

Redacted for Privacy

Dean of Graduate School

Date thesis is presented August 14, 1970

Typed by Velda D. Mullins for Tomas Pollak

ACKNOWLEDGMENTS

The author wishes to express his gratitude to the following persons and institutions:

Dr. William Furtick for his guidance and advice throughout the graduate program, as well as his helpful suggestions in the completion of this work.

Dr. Garvin Crabtree for his generous assistance.

Dr. Arnold Appleby for his useful ideas.

Dr. Richard Ilnicki for his valuable advice and help.

CIBA Corporation for financing these studies and to Mr. William Anliker for his continuous interest in the progress of the investigation.

Mr. James Moran for his assistance in the use of the CDC-33000 computer.

The author is deeply grateful to his parents for their financial support throughout his graduate education.

TABLE OF CONTENTS

THE EFFECT OF 2, 4'-DINITRO-4-TRIFLUOROMETHYL-DIPHENYLETHER (C-6989) ON BEANS (*Phaseolus vulgaris* L.) UNDER CERTAIN SOIL AND LIGHT CONDITIONS

INTRODUCTION

Oregon leads the nation in the production of green beans for processing. The yearly harvested area is close to 30, 000 acres which represent a total value of nearly 16 million dollars (35).

In the U.S., the yearly loss in green bean production due to weeds is 8 million dollars (34). The use of herbicides has become widespread, if not indispensable, in the production of vegetable and field crops. Because of the minimization of permissible residues of pesticides, the new mechanization techniques and the repeated use of one herbicide that will lead to important changes in the weed population, a continuous search for new materials and their combinations is taking place.

The introduction by CIBA Company of a new herbicide, 2, 4'-dinitro-4-trifluoromethyl-diphenylether, Preforan T.M.[1/] or formerly C-6989, has appeared promising in controlling weeds

[1/] Trade Mark of CIBA Agrochemical Co., Vero Beach, Florida.

in beans. Up to now C-6989 has been registered and used in soybeans in the U.S. However the response of green beans to the chemical has not been studied in detail.

These studies were conducted to: a) determine the tolerance of green beans and other plants to C-6989; b) site of uptake, type and site of action of the herbicide; c) investigate some of the factors responsible for herbicidal injury to bean seedlings including mechanical conditions of the soil surface, d) time of application in respect to the bean plant emergence, and irrigation management, and e) to evaluate the residual effects in rotation crops.

LITERATURE REVIEW

Beans

Green beans belong to the Leguminoseae family. The plant, as other legumes, fixes atmospheric nitrogen (12). The seeds are an excellent source of food, proteins, vitamins and minerals.

According to the state at which they are harvested, they may be used as "dry", "green-shelled" or "snap" (11).

In Oregon, most of the bean crops are processed (11). Bush beans represent the largest acreage, and the growing of the crop is highly mechanized. Pole bean acreage is a smaller percentage of the total, and the crop requires large amounts of labor (11).

It is generally agreed that the first four weeks of the bean growth are the most critical for weed competition as reflected in crop yields (2). Past methods of weed control have been centered about the cultivator and hoe (2).

An early reference to chemical weed control in beans (12), suggests the use of 4, 6-dinitro-o-sec. -butylphenol (dinoseb or DNBP) for broadleaf weeds, 2-chloroallyl-diethylditiocarbamate (CDEC) for annual grasses, and ethyl N, N-dipropylthiocarbamate (EPTC) for perennial grasses.

In the last 10 years some new herbicides for use in beans have appeared in the market. The most popular ones at the present date are as follows: α, α, α, -trifluoro-2, 6-dinitro-N, N-dipropyl-p-toluidine (trifluoralin), 4-(methylsulfonyl)-2, 6-dinitro-N, N-dipropylaniline (nitralin), and 3-amino-2, 5-dichlorobenzoic acid (amiben), in lima beans only.

Diphenylether herbicides

The diphenylether herbicides have been on the market since 1968 largely developed by the Japanese chemical industry (2).

In Japan and southeast Asia, they are widely used pre- and post-emergence and/or post-transplanting in rice regardless of flooded conditions of the soil (2).

These compounds have been described as very versatile, suitable for use in several crops, in tropical, subtropical, and mediterranean type climates (31, 6).

In the U.S. two diphenylether herbicides are being marketed at the present time. One is 2, 4-dichlorophenyl-4-nitrophenyl ether or nitrofen, the other is C-6989. Both act mostly by contact on plants and are essentially insoluble in water. Both control nearly the same range of plant species (31, 6). Mechanisms of action are still under study.

Following, is a chronological review of this family of herbicides in general and C-6989 in particular.

In 1967, Arai, Chisaka, and Kataoka (3) reported nitrofen very effective while searching for a chemical efficient in breaking dormancy of barnyardgrass seeds (Echinochloa crusgalli L. Beauv.).

Ukei and Shimizu (33) in 1967, reported the ineffectiveness of diphenylether derivatives, kinetins and potassium nitrate in breaking dormancy of seeds of Gallium sp. They attributed this to the histological conformation of the seed coat.

Studies done by Hayasaka (17), also in 1967, suggested for the first time that a diphenylether compound (HE-314), showed a selective herbicidal effect on rice (Oryza sativa) and barnyard-grass. These effects were more marked when applied at the 2 to 3-leaf stage of rice, as well as under flooded conditions. According to Hayasaka, the herbicide acted upon both, plumule and roots of susceptible species. He also speculated that when the herbicide was applied under flooded conditions it was dispersed on water resulting in contact activity. After settling on the soil it showed pre-emergence activity.

Matsunaka and Inada (22) in 1967, provided data about physiological and biochemical behavior of this family of herbicides. They classified diphenylether herbicides in two groups: one without the ortho substituent on one benzene ring active under light or dark conditions. The second group, having ortho substituent(s) requires light for activation.

Furuya and Arai cited by Matsunaka and Inada (22) also showed that the ortho substituted diphenylether herbicides required light for activation. Compounds with meta or other substitutions are active in light or dark. Table 1 shows the classification given by these authors.

Table 1. Common and Chemical Names of Two Types of Diphenylether Herbicides.

Common name	Chemical name
Ortho-substitution	
Nitrofen (NIP or TOK)	2, 4-Dichloro-4'nitro-DPE [a/]
KK-60	4 Chloro-2, 4'-dinitro-DPE
C-6989 (Preforan)	2, 4'-Dinitro-4-trifluoromethyl -DPE
TCPE	2, 4, 4'-Trichloro-DPE
CNP(MO-338)	4'-Nitro-2, 4, 6-trichloro-DPE
MO-263	2, 4-Dichloro-6-methyl-4'-nitro-DPE
MO-500	2, 4-Dichloro-6-fluoro-4'-nitro-DPE
Meta-substitution	
HE-314	3-Methyl-4'-nitro-DPE
--	3-Chloro -4'-nitro-DPE
NW-40187 (HE-306)	3, 5-Dimethyl-4'-nitro-DPE
--	3, 5-Dichloro-4'-nitro-DPE
MO-600	3-Chloro-4-fluoro-4'-nitro-DPE
HE-306	3, 5-dimethylphenyl-4'-nitro-DPE

a/ DPE: Diphenylether.

In 1967, Chandra and Grover (5) were investigating the effect of several herbicides on soil micro-organisms. Nitrophen was included in the experiments. They found no significant differences in micro-organism populations between treated and untreated soils.

In 1968 several diphenylether compounds were evaluated in Oregon (23). Among them, C-6989 was successful in controlling broadleaf weeds, but was reported somewhat weak on the control of grasses at 3 lb/A. When used in green beans, crop injury was reported (9).

Ebner, Green and Pande (15) the same year, working with C-6989, suggested that crop injury may occur at low temperatures. They also presented evidence for a contact type of action (15). They stated: "Initial damage to some crops can be observed, as the rolling shoot in pushing through the treated soil surface it can be scorched by the herbicide giving a broken line of spots across each of the first 3 or 4 leaves. This damage is outgrown and never gives rise to permanent stunting being unnoticeable after 5 or 6 weeks". This strongly suggests that there is no visible evidence of translocation.

Walter, Eastin and Merkle (36) agree with Ebner, Green and Pande (15) in that C-6989 effect is mostly due to contact action. However, when they followed the translocation pattern C-6989 C^{14} within several plant species, i.e., soybean (Glycine max L.), grain sorghum (Sorghum vulgare Pers.), peanut (Arachis hypogaea L.), morning glory (Ipomea hederacea L. Jacq.) they found higher concentrations of C-6989 in lower stems of morning glory and grain sorghum, than in any other species.

Walter and coworkers (36) observed that more herbicide was translocated following root than foliar applications. Since morning glory ana grain sorghum seedlings are more susceptible to C-6989 than soybean and peanut, there is an apparent correlation between the extent of translocation and the susceptibility of the plant species.

Rogers (29) confirmed the findings of Walter, Eastin and Merkle (36) when he observed that in soybean the herbicide tends to be absorbed by roots, and he did not observe any significant translocation towards the shoot of the plant.

Eastin (13) working with peanuts, reported that C-6989 is rapidly absorbed from nutrient solution but only 6.5% of the radio-labled chemical absorbed, is translocated to the shoot after 144 hours.

Concerning the persistence of C-6989 in the soil, Walter, Eastin and Merkle (36) reported that at rates of 3 and 4.5 lb/A in a clay soil, less than 10% of the herbicide was present 5 months after application. In a sandy loam soil, at the same rates 3 months after application less than 50% of the herbicide was present on the soil. Incorporation had little effect on the persistence. Also leaching from the upper soil profile is negligible. These workers also reported that the dissipation of C-6989 at the soil surface was rapid, suggesting that volatility or photodecomposition could be involved.

Reviewing the work on mechanism of action, evidence was found that the basic diphenylether moiety is very stable in biological systems (20). However, some authors reported metabolites and proposed degradation pathways (13, 14, 22).

Rogers (28) working with soybean and cucumber (*Cucumis sativus* L.), reported that a large amount of the radiolabeled herbicide (approximately 50%) persists in the intact herbicide fraction, and this was found in the roots. Further, Rogers (28) suggested that soybeans have a detoxifying mechanism, in which the first step is the cleavage of the ether linkage followed by the formation of several degration intermediates. These include the following: a) 4-amino-2-nitro-4-trifluoromethyl-diphenylether; b) 4-hydroxy-3-aminobenzoic acid; c) 2-hydroxy-5-trifluoromethylaniline; d) 2, 4-diamino-4-trifluoromethyl-diphenylether; and e) an unidentified compound. The same author indicates that cleavage of the ether linkage, occurs after the reduction of the nitro substituent on the trifluoromethyl-containing portion of the molecule.

Reduction of nitro substitutents, has been reported to be involved in the degradation of other herbicides (2).

Results obtained by Rogers (28) are consistent with those obtained by Eastin (13) in 1969, with peanut seedlings and later (14) with cubumber seedlings.

Eastin (13, 14) observed that the major pathway of degradation of C-6989 was: hydrolysis of the ether linkage and reduction of the 4-nitrophenol to form 4-aminophenol. As an alternate pathway, he proposed reduction of one of the nitro groups and hydrolysis of the ether linkage. It was also indicated that major detoxication of C-6989 occurred in the roots of peanut seedlings the metabolites being translocated later to the shoot. Eastin (13) failed to detect the metabolites reported by Rogers (28).

It is concluded from both works, that the rate of degradation of C-6989 in soybean and peanut seedlings is sufficient to serve as a protective mechanism, if the metabolites formed are relatively non-phytotoxic.

Geissbuhler, cited by Eastin (13) noted that in corn (_Zea mays_), C-6989 was rapidly absorbed by roots and then rapidly metabolized, the major intermediates being the same as those isolated by Eastin.

Matsunaka in 1969 (20, 21), proposed another explanation for the mechanism of action of diphenylether herbicides. It was indicated that diphenylether herbicides must pass through a photobiochemical activation after absorption into the plant tissue. This activation would be independent of the Hill reaction, since no interference in the effects of 2-chloro-4, 6-bis (ethylamino)-_s_-

triazine (simazine), 3-p-chlorophenyl-1, 1-dimethyl urea (mornuron), 3, 4-dichloropropionanilide (propanil) (Hill reaction inhibitors), when used together with nitrophen were found.

As previously reviewed, Matsunaka (22) classified diphenylether herbicides in two groups, in accordance to their light necessities for action. Matsunaka (21) seeking an explanation for these findings, worked with normal etiolated (treated with 3-amino-1, 2, 4-triazole, (amitrole) seedlings of rice, and found that the xanthophyll pigments, were specially important in photo-activation of diphenylether herbicides. Two different models were proposed based on xanthophylls as acceptors of light energy in the photoactivation of diphenylether herbicides: a) the light energy absorbed by xanthophylls will be used in the activation of this herbicide, thereby causing phytotoxicity. This was based on the fact, that activation was not a simple conversion to a toxic compound by light; and b) there is consistent evidence that light acts upon the hormonal balance in higher plants (2, 4, 18). It was suggested that plants in which the hormonal level is affected by light energy absorbed through pigments, i.e., xanthophylls, may be very susceptible to the ortho substituted diphenylether herbicides. Therefore, there would be no conversion of the herbicide into toxic compounds.

GENERAL MATERIAL AND METHODS

Experiments in the field and in the greenhouse were conducted in the spring and summer of 1969.

The greenhouse experiments were conducted with facilities of Oregon State University. Preliminary tests were established to determine the rate-response curve of the plants to be used.

The applications of the herbicide in the greenhouse were made with an overhead track-mounted sprayer, using an 8003-E[2/] nozzle and water as a carrier with a volume of 50 gpa. The pressure was approximately 40 psi.

Analyses of the soil used in the greenhouse were performed (Table 2). This soil prior to being used, was autoclaved for 4 hours, to prevent damping-off.

Table 2. Analyses of a Sandy Loam Soil Used in the Greenhouse Studies.

Soil pH	OM [a/] %	CEC [b/] me/100g	Sand %	Silt %	Clay %
6.14	1.42	17.66	69.75	19.52	10.73

a/OM = Organic matter.
b/ CEC = Cation exchange capacity.

2/ Spraying System Co., 3204 Randolph St., Bellwood, Ill. 60104.

Plastic pots, 4 x 4 x 5 inches with perforated bottoms were used. These pots were placed in metallic trays 15 x 10 x 1.5 inches in size.

Subirrigation was provided to keep the soil as near as possible to field capacity.

The greenhouse temperature was 20 ± 7° C.

Fluorescent and incandescent lights were supplied 14 out of every 24 hours. The intensity of light was approximately 300 fc.

Plants were clipped at the soil surface and dried for 48 hours at 80° C. Dry weights were recorded.

Unless otherwise stated, a completely randomized design was used. The data were statistically analyzed and least significant differences determined whenever F was significant. The least significant difference test (LSD) was used, at 1% and 5% probability levels. The coefficient of variation (CV), was calculated for each experiment. The analyses of variance are presented in the Appendix.

The field trials were established to study the herbicide behavior under field conditions.

Some properties of the soil where the field experiments were performed are indicated in Table 3.

Table 3. Analyses of a Chehalis Silty Clay Loam Soil.

Soil pH	OM %	CEC me/100 g	Sand %	Silt %	Clay %
6.0	3.43	30.5	1.49	64.13	34.38

The plot size was 10 x 16 ft with 5 rows. Only the 3 center ones were considered, the outer two being guard rows.

Plots were rated visually for herbicide injury to crop and weeds, using the scale 0 - 100, in which 0 indicates no observable effect, 50 still an acceptable stand and 100 total destruction. This scale was found to agree accurately with the results obtained by taking fresh plant weights. Any deviations from these general materials and methods will be covered under the specific experiments.

EXPERIMENT I. SITE OF UPTAKE OF C-6989 by FOUR PLANT SPECIES

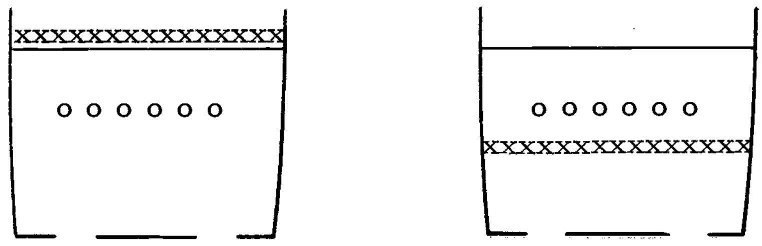
The use of preemergence herbicide applications to soil has increased considerably over the past few years. Consequently, the localization of the herbicide in the soil and the exact site of uptake by the young seedlings are receiving considerable attention. These considerations are particularly important when "depth protection" is used as a means of obtaining herbicidal selectivity in the field. The optimal placement of herbicide with regard to selectivity of the plant will depend on whether its most effective route of entry is through the roots or through the shoot.

The purpose of this experiment was to evaluate the relative importance of root versus shoot uptake, measured by toxicity to plants when compared to the untreated control or check.

Materials and Methods

Bean, cucumber, rice, and barnyardgrass were the plant species used. The seeding rate was respectively 5, 15, 20 and 30 seeds per pot.

The herbicide was placed above and below the seed for each plant species as described in Figure 1. The rates of C-6989 used in this experiment were 0, 4, 8, 12 and 16 lb/A. Each treatment was replicated four times.



xxxxx Herbicide

0 0 0 Seeds

Figure 1. Cross section of pots showing seed position and placement of herbicide.

Plants were harvested at intervals. First beans, then cucumbers, then rice and finally barnyardgrass.

Results were statistically analyzed as a separate experiment for each plant species.

Results

The results of this experiment are given in Tables 4, 5, 6 and 7 for bean, rice, barnyardgrass and cucumber, respectively.

From the analyses of bean results, it was noted that the average dry weight of plants for all rates when the herbicide was placed on the surface, was 1.460 gm. When the herbicide was placed below the surface the mean dry weight was 1.276 gm. The small relative difference of 0.194 gm between placements, was enough to be significant statistically at the 5% level of probability. However, as was visually observed, these differences were too small, and further studies seeking for a particular site of uptake would be desirable. Results are presented in Table 4.

Table 4. The Effect of C-6989 at Four Rates and at Two Soil Placement Levels on Average Dry Weight of Bean Seedlings. a/

Rate	Herbicide placement	
lb/A	Surface	Below seed
0	1.560	1.560
4	1.301	.879
8	1.228	1.257
12	1.519	1.400
16	1.694	1.285

a/ Average dry weight (gm) of plants from 4 replications.

$LSD_{05} = .449$

Rice responded markedly to the soil placement of C-6989. Uptake was primarily through the roots. Differences in dry weight between the two placement levels were statistically significant (1% probability). Placement of C-6989 on the surface of the soil did not produce as much injury to rice seedlings as when it was placed under the roots. The herbicide at 8 and 12 lb/A particularly, caused more injury to the plants when placed below the seed than when placed on the surface of the soil. These results are summarized in Table 5.

Table 5. The Effect of C-6989 at Four Rates and at Two Soil Placement Levels on the Average Dry Weight of Rice Seedlings. a/

Rate	Herbicide Placement	
lb/A	Surface	Below seed
0	.982	.982
4	.888	.401
8	.901	.368
12	.913	.315
16	.850	.370

a/ Average dry weight (gm) of plants from 4 replications.

LSD_{05} = .099 LSD_{01} = .134

C-6989 apparently entered barnyardgrass seedling through coleoptiles. As evident in Table 6, when the herbicide was applied to the surface of the soil, it caused a considerable reduction in dry weight as compared to the placement below the seed. The dry weight of plants in which the herbicide was below the seed was more than twice that when placed on the surface at the same rates. Plants reflected a marked decrease in dry weight when compared to untreated checks. This seems to indicate, that to a minor extent, the herbicide was absorbed through the roots. With the surface placement, at rates of 8 and 12 lb/A plants were very sparse and were never larger than 2 inches. At 16 lb/A the stand was very poor and the soil almost bare. At the lowest rates, plants never succumbed and tended to recover. This did not occur with rates over 8 lb/A.

Table 6. The Effect of C-6989 at Four Rates and at Two Soil Placement Levels on the Average Dry Weight of Barnyardgrass Seedlings. a/

Rate	Herbicide placement	
lb/A	Surface	Below seed
0	1.004	1.004
4	.304	.748
8	.253	.605
12	.279	.518
16	.507	.434

a/ Average dry weight (gm) of plants from 4 replications.

$LSD_{05} = .209$ $LSD_{01} = .281$

In cucumber the same symptoms and intensity of symptoms appeared, regardless of whether C-6989 was placed on the soil surface or below the seed. The plant injury observed was nearly the same for all rates. Although there was no statistically significant evidence, it is suspected that in cucumber C-6989 is taken up equally by roots and hypocotyl and/ or cotyledons. The results for cucumber uptake of C-6989 are presented in Table 7.

Table 7. The Effect of C-6989 at Four Rates and at Two Soil Placement Levels on the Average Dry Weight of Cucumber Seedlings. a/

Rate lb/A	Herbicide placement: Surface	Herbicide placement: Below seed
0	.768	.768
4	.435	.426
8	.553	.423
12	.519	.495
16	.521	.462

a/ Average dry weight (gm) of plants from 4 replications.

$LSD_{05} = .156$ $LSD_{01} = .210$

EXPERIMENT II. THE RESPONSE OF BEAN SEEDLINGS TO C-6989 WHEN APPLIED TO CRUSTED AND NON-CRUSTED SOIL SURFACE CONDITIONS

There have been examples of injury to the first true leaves of large-seeded legumes with relatively low rates of C-6989. This injury has been characterized by a shortening or crinkling of the mid rib, as was noted in field trials. There is reason to suspect that this injury occurs as a result of contact of the cotyledonary tissue with the herbicide at the time of emergence. It is possible that the surface moisture conditions at the time of emergence may be responsible for this. If the soil surface is dry and crusted at the time of emergence the herbicide may not come in direct contact with the emerging plant tissue, because the cotyledons could move the treated soil out of the way. If the soil surface is non-crusted at the time of emergence, the cotyledons and hypocotyl could come in contact with the surface layer of C-6989, and be subject to herbicidal injury.

Materials and Methods

Bean, variety Burpee Stringless, was the plant used in this study. Five rates of C-6989 were used: 0, 8, 12, 16 and 20 lb/A. Each treatment was replicated 4 times.

This experiment was conducted in such a way that in one treatment the soil surface was kept smooth by mechanical means until the emergence of the seedlings. The other treatment was left intact in order to obtain a crust formation.

The third day after the appearance of the hypocotyls at the soil surface measurements of the length of mid-rib were taken. Immediately before harvesting, mid-rib lengths were again recorded.

Three plants per pot were clipped at the soil level, 10 days after seeding, and dry weights recorded.

Results

In both treatments, the one with the crusted soil surface and the one with smooth soil surface herbicide injury was observed. Weights of plants are presented in Table 8.

Table 8. The Effect of C-6989 at Five Rates on the Average Dry Weight of Bean Seedlings, as Affected by Two Soil Surface Conditions. a/

Rate	Soil surface	
lb/A	Smooth	Crusted
0	3.040	2.866
8	2.432	1.946
12	2.446	1.927
16	2.071	2.211
20	2.194	2.082

a/ Average dry weight (gm) of plants from 4 replications.

LSD_{05} = .584 LSD_{01} = .786

Results from measurements of the mid-rib length indicated that there was a small but progressive decrease as the rates were increased. The data taken under smooth surface of soil are more consistent, as can be seen in Figure 2, than the data from soil in which a crust was allowed to form.

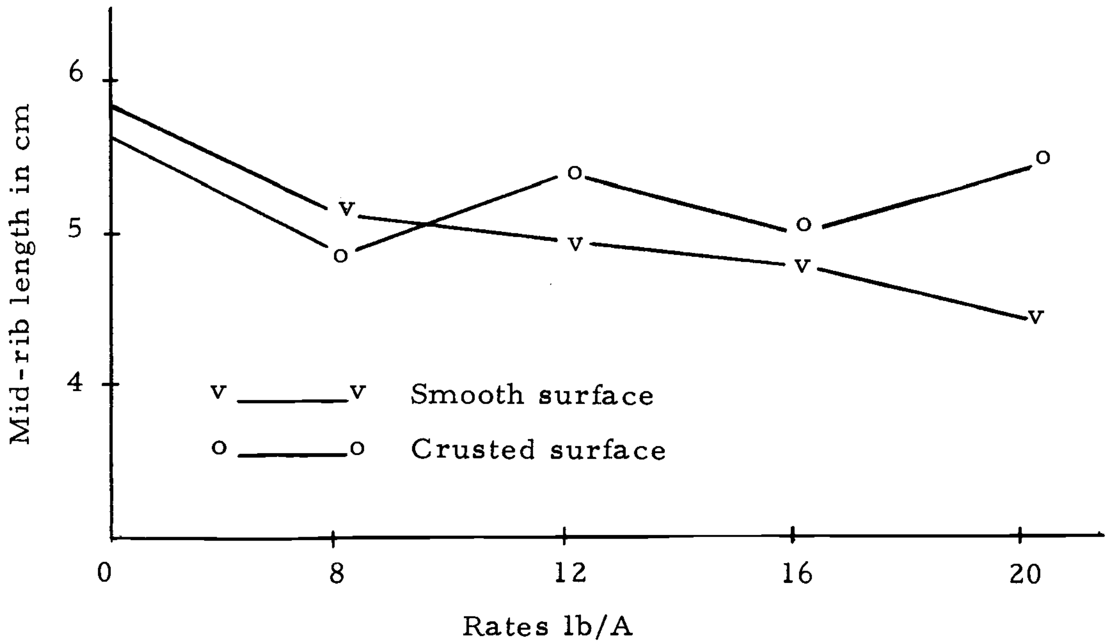


Figure 2. Effect of five rates of C-6989 on the mid-rib length of the first two true leaves of bean under two soil surface conditions.

EXPERIMENT III. RESPONSE OF BEAN SEEDLINGS TO DIRECT APPLICATIONS OF C-6989.

In the previous experiment bean seedlings showed some response to C-6989 especially in smooth soil surface conditions, when measuring toxicity by mid-rib length.

The objective of this experiment was to gain a better understanding on the response of emerging bean seedlings to direct applications of C-6989 and to observe the capacity of the plants to outgrow and overcome the effects of the herbicide.

Materials and Methods

The same variety of bean as described in Experiment II was used. C-6989 was applied at concentrations of: 10, 100, 1, 000, 10, 000 and 100, 000 ppmv plus the untreated check. For each concentration, 30 microliters were applied using a microsyringe. This was accomplished by distributing the herbicide on the cotyledons and hypocotyl area at the time the plants were emerging. Each treated plant was labeled for further identity. When no more plants were emerging the stand was thinned to 2 plants per pot. Mid-ribs were measured 3 days after the herbicide applications. Plants were clipped at the soil surface 7 days later and dry weights were recorded. Since treatments were made to individual plants, each plant was considered a replication resulting in 12 replications per treatment.

Results

The results of this experiment are presented in Table 9 and Figure 3. From that data, it can be seen, that there was a slow and progressive decrease in dry weight from 10 to 1, 000 ppmv of herbicide. Although some injury was noted at levels of 10 and 100 ppmv, this was not statistically significant when compared to the untreated check. From 1, 000 to 100, 000 ppmv the decrease in plant weight was markedly greater and was statistically significant. This substantiated the visual observations that up to 1, 000 ppmv plants show considerable injury due to contact action; however, they slowly recover from these herbicidal effects. At the highest rates the herbicide probably affected the growing centers, causing death to the plants.

The same situation can be observed for mid-rib length as presented in Figure 3. Leaves showed a progressive shortening of the mid-rib up to 1, 000 ppmv rate. In some instances the first two true leaves did not recover from the injury and the first trifoliate leaf emerged intact. Above 1, 000 ppmv no true leaves were formed and the plant died about four days later.

Table 9. The Effect of C-6989 at Six Concentrations on the Average Dry Weight of Bean Seedlings When Applied Directly to Emerging Plants.

Rates ppmv	Dry weight in gm of 2 bean plants. Avg. of 12 reps.
0	.436
10	.445
100	.390
1, 000	.293
10, 000	.186
100, 000	.138

LSD_{05} = .095 LSD_{01} = .123

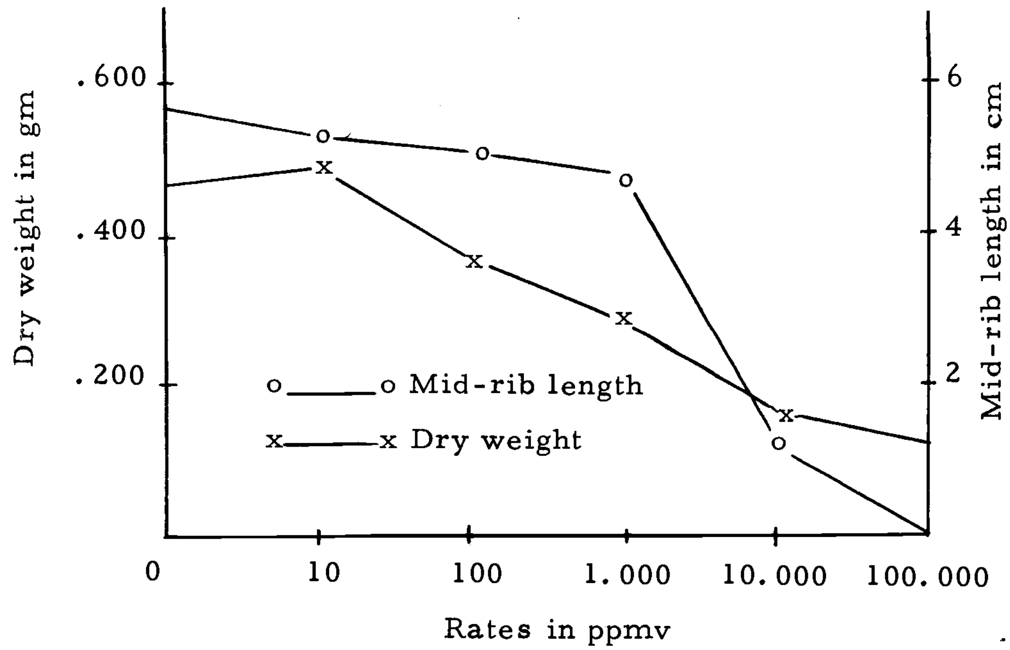


Figure 3. Mid-rib length and total dry weight of bean seedlings under several levels of C-6989.

EXPERIMENT IV. THE EFFECT OF LIGHT ON C-6989 TOXICITY TO BEAN PLANTS.

Some research workers have indicated that the diphenylether herbicides having ortho substituents on one of the benzene rings require light energy to become phytotoxic. These herbicides generally are inactive in the dark.

The following experiment was designed to ascertain whether light is critical to bean seedlings treated with C-6989, since this is an herbicide with the above named molecular characteristic.

Materials and Methods

Two 15 x 50 inch trays were used and wooden frames 15 inches wide by 19 inches high and 50 inches long were devised to cover one tray completely with black polyethylene to provide complete darkness. With the other tray, only lateral walls were made with black polyethylene to avoid the influence of light, other than that provided from the top.

The source of light was a mixture of fluorescent and incandescent lamps which provided a total of 900 fc at the surface of the pots. Light was applied continuously for 24 hours.

Beans were seeded in the pots under normal greenhouse conditions. After emergence when the first two true leaves were fully expanded (approximately 6 days after planting), a thinning was made in which just two plants per pot were left. At this time all plants were sprayed with the herbicide. Five rates of C-6989 were used: 0, 2, 4, 6, and 12 lb/A. The applications were made at night to minimize the influence of light and the plants were taken promptly to the dark and illuminated chambers.

Each pot with two plants denoted one observation. There were 4 observations or replications per treatment.

The plants were harvested four days after treatment, by clipping them at the soil surface.

Results

Very definite symptoms of injury were observed for all rates 10 hours after applications, on the plants exposed to the light. Treated plants appeared wilted and this clearly indicated the acute effects of a potent contact herbicide.

Although a measure of the mid-rib was planned in advance, this was not possible to accomplish because leaves remained curled and slowly died without further formative effects.

When compared to their respective untreated checks, dry weights of plants exposed to the light were less than those plants in the dark. Plants under light weighed 43% of the untreated check while plants under dark conditions weighed 78% of the untreated check (Table 10).

Table 10. The Effect of C-6989 at Five Rates on Dry Weight of Bean Seedlings Under Light and Dark Conditions. a/

Rates lb/A	Light	Dark
0	1.060	.506
2	.516	.464
4	.480	.387
8	.449	.376
12	.368	.358

a/ Average dry weight (gm) of plants from 4 replications.

LSD_{05} = .115 LSD_{01} = .318

EXPERIMENT V. STUDY TO DETERMINE IF C-6989 IS ACTIVATED BY LIGHT WITHIN CUCUMBER PLANTS.

As was noted in Experiment IV, light was necessary for the full expression of herbicide toxicity in bean seedlings following foliar applications of C-6989. Further, the herbicide was activated soon after coming in contact with the leaf surface.

It was desired to know if light has any influence in the activation of the herbicide when its presence within the plant is due to other than direct applications to the leaves.

Another objective of the experiment was to investigate if there was any photodecomposition of C-6989 in the soil surface.

Materials and Methods

Cucumber was used as the indicator plant.

Five rates of C-6989 were used: 0, 2, 4, 8 and 12 lb/A. The herbicide was placed on the surface of the soil and below the surface but between the surface and the seeds, as presented in Figure 4.

When the herbicide was placed on the surface, light was provided (900 fc), the same as described in Experiment IV.

By placing the herbicide on the surface and exposing the pots to light and by placing the herbicide just under the surface,

xxxxx Herbicide

o o o Seeds

Figure 4. Cross section of pots showing position of seeds and placement of herbicide.

therefore in the dark, a measure of comparison was obtained and the second objective of this experiment achieved.

Five plants per pot were harvested 15 days after planting. Each pot comprised one observation and there were four observations per treatment.

Results

It was observed that cucumber plants emerged intact but within a limit of 12 hours the leaves curled downward the effect being greater at the higher rates. This was more noticeable in the subsurface treatment. It was observed that C-6989 caused chlorosis and stunting and therefore was activated within the plant (probably within the green tissue) regardless of the point of entry.

When placement positions of C-6989 were evaluated, it was noted that the surface treatment produced less cucumber injury at the same rates, than the below surface placement (Table 11). The average weight of plants from the surface treatment was 63% of the check as compared to those grown in the subsurface treatment which weighed 59% of the check. This may be an indication that C-6989 is being subjected to inactivation on the surface of the soil probably by the effect of light.

Table 11. The Effect of C-6989 at Five Rates on the Average Dry Weight of Cucumber Seedlings Under Two Light Regimes.[a/]

Rate	Herbicide placement	
lb/A	Surface	Subsurface
0	.668	.617
2	.496	.473
4	.377	.457
8	.452	.333
12	.345	.225

a/ Average dry weight (gm) from plants of 4 replications.

$LSD_{05} = .170$ $LSD_{01} = .239$

EXPERIMENT VI. THE EFFECT OF SPACING TIME OF APPLICATION, VARIETIES, AND OTHER FACTORS, IN THE FIELD PERFORMANCE OF C-6989.

In general, herbicide experiments carried out in the greenhouse are only suggestive of herbicide performance because cultivated plants and weeds are not subjected to the many stresses of nature under field conditions. Three experiments were conducted under field conditions, to further investigate the data taken in the greenhouse. Some aspects which will directly benefit the grower such as time of application of C-6989 and the effect of irrigation were studied.

Materials and Methods

The subject under study in the first experiment was if there is any intervarietal response of beans to C-6989. The following 5 varieties of bean were tested: Field (Red Mexican), Romano, Puregold Wax, Tendercrop (G-50) and Lima (Thorgreen). C-6989 was applied preemergence at 4 lb/A immediately after sowing. The following day the plots were sprinkler irrigated. After emergence, visual notes about crop injury were taken. A randomized block design with 3 blocks was used.

In another experiment the response of beans to C-6989 was studied, with regard to its time of application. The herbicide was applied at time of planting (day 0), three days after planting, and five days after planting. Rates of 3 and 9 lb/A were applied at each date. Irrigation from a sprinkler, in the amount of 2 inches/A was applied on the fifth day after planting. All treatments were evaluated for crop injury every week since emergence, up to the 4th week. Notes about weed control were also taken. A split plot design with 3 replications was used. Time of application was the main plot with rates and irrigation as sub plots.

In the third experiment, the residual effects seven weeks after the last application of the herbicide was traced by planting table beets (Beta vulgaris Linn.), cucumber, corn and tomatoes (Licopersicon esculentum). These crops are likely to follow beans in the rotation and are susceptible to C-6989. These four crops were planted in the same plots in which the above described experiments took place.

Results

None of the 5 varieties of bean demonstrated a special sensitivity to C-6989 at 4 lb/A. Notes on crop injury are presented in Table 12. With regard to the study of timing of herbicide application, it was noted that the greatest injury occurred within the first week of emergence. The amount of injury from application made

Table 12. The Effect of C-6989 at 4 lb/A on Five Varieties of Bean. a, b/

Variety	Crop injury at weeks after emergence			
	0	1	2	3
Field bean	0	0	0	0
Wax bean	0	10	0	0
Romano bean	0	0	0	0
Tendercrop bean	5	0	5	0
Lima bean	5	0	0	0

a/ Evaluation by visual rating: 0 = no effect, 100 = complete kill, as compared with untreated check.
b/ Average of three applications.

at the time of planting did not differ from the one done five days later. However, plants treated at planting recovered faster than the ones treated the fifth day. In all cases, plants treated with 3 lb/A showed less than 50% injury with respect to those treated with 9 lb/A. This proportion was fairly constant for all treatments and remained steady for the duration of the experiment. Irrigation apparently was not as important as the time of herbicide application, because injury was similar whether the herbicide was applied at planting or delayed for five days. These results can be seen in the first part of Table 13.

In the same test, notes were taken for weed control. The population of weeds was basically pigweed (*Amaranthus retroflexus*) and ryegrass (*Lolium multiflorum* Lam.), which were seeded.

Naturally occurring but in lesser degree were foxtails (Setaria sp.), nightshade (Solanum nigrum L.) and lambsquarters (Chenopodium album L.). The control of broadleaves was nearly 100% with all rates regardless of time of application. C-6989 was somewhat weaker on grasses; however, the herbicide potential was still acceptable when compared to the untreated check. In general, grasses were controlled better at the higher rate, regardless of the time of application. The results for pigweed and ryegrass control can be seen in the last portion of Table 13.

The results of the residual effect of C-6989 on rotation crops shows that C-6989 up to the rate of 3 lb/A had little or no residual action on the species tested seven weeks after the treatments were applied. The residual effects increased slightly at the higher rate, but no important differences could be observed between treatments. The emergence of tomatoes was poor even in the check, thus no observations were made. Results are presented in Table 14.

Table 13. The Effect of Timed Application of C-6989 on Injury to Bean Seedlings and Weed Control.

Days after planting	Rate lb/A	Crop injury a, b, c/				Weed control b, c/	
		0	1	2	3	Ryegrass	Pigweed
Zero	3	15	0	5	0	70	100
Zero	9	30	20	10	5	80	100
Three	3	10	5	5	5	50	100
Three	9	25	15	10	10	70	100
Five	3	15	5	5	5	65	95
Five	9	30	20	5	5	90	100

a/ Weeks from emergence.
b/ Evaluated visually, 0 = no effect, 100 = total kill as compared with untreated check.
c/ Average from 3 replications.

Table 14. Residual Effect of C-6989 after Seven Weeks on Three Susceptible Crops. a, b/

Days after planting	Rates lb/A	Corn	Cucumber	Table beets
Zero	3	0	0	0
Zero	9	0	10	5
Three	3	0	0	0
Three	9	0	0	5
Five	3	0	5	0
Five	9	0	10	0

a/ 0 = no effect, 100 = complete kill, as compared with untreated check.

b/ Measures are average of three replications taken at time of emergence.

DISCUSSION AND CONCLUSIONS

Results obtained from experiments involving herbicide placement in the shoot or root regions of soils, indicate that there is a very marked and different response for each species tested. Beans which are fairly tolerant to C-6989 do not seem to have a preferential site of uptake. Cucumbers which are sensitive, absorb C-6989 by roots as well as by emerging shoot tissue, the root pathway resulting in somewhat more phytotoxicity. Rice and barnyardgrass, although they belong to the same family, take up C-6989 through markedly different parts. Rice absorb C-6989 almost entirely through the roots, while barnyardgrass mainly through the shoot. An important practical implication of these observations is that site of uptake could be a means of affecting selectivity between crop plants and undesirable plants.

In analyzing each species separately in an attempt to find an explanation for the results obtained with beans, it was assumed that the favorable conditions for growth in the greenhouse (moisture, fertility, etc.) facilitated the recovery of plants from the biochemical pressure imposed by the herbicide. Therefore no marked responses were observed. On the other hand, Rogers (29) reported that in soybeans C-6989 tends to be absorbed by roots, and mentioned the existence of a strong detoxifying mechanism. The possibility must not be excluded that the fate of C-6989 in beans follows a similar pathway. The explanation for crop injury under field conditions may be that the environmental stresses at the early seedling stage, may have lowered the tolerance of the crop to the herbicide thus causing severe injury to the crop.

The explanation for the herbicidal activity of C-6989 on cucumbers is difficult. Despite the existence of a degradation pathway in cucumber (14), this degradation may not be rapid enough. Furthermore, the metabolites of the degradation mechanism may still be phytotoxic. It may be that the slow mobility through xylem as will be discussed later may cause local necrosis thus blocking the normal flow of nutrients.

From the results of rice and C-6989 placement study it was noted that green weights did not reflect large difference between treatments and controls; however, when dry weights were analyzed highly significant differences were found. It was concluded that the main pathway of uptake was through the roots. A possible explanation is that the large differences between green and dry weights are due to the fact that C-6989 destroys some photosynthetic pigments, therefore reducing the formation of new carbohydrates, but the plant still remains turgid for a considerable time. The possibility must be accepted here also, that C-6989 acted as a root inhibitor. As it has been reported, this family of herbicides inhibits the germination of certain grass seeds. It would be desirable to explore this possibility further, by taking dry weights of roots. Under field conditions rice is tolerant to C-6989 and this may be explained as follows: a) although the herbicide is absorbed through the roots, the chemical is placed nearly always on the surface of the soil, and b) the slow mobility of the herbicide within the plant.

The relative difference between shoot and root uptake in barnyardgrass was greater than in rice. In the former, the uptake of C-6989 was mainly through the shoots.

When an herbicide is placed on the surface of the soil there is a prolonged and intimate contact with the sensitive meristematic tissues of the rapidly-growing shoot. The herbicide penetration into the emerging shoot is at an optimum since the emerging shoot has very little or no cuticle. On the other hand, the point can be made that the absorptive surface is comparatively larger through the roots and eventually there is less decomposition or loss of the herbicide.

The literature cited indicated that different types of soils, especially with different organic matter content, may alter the responses of several types of herbicides. A preliminary test not reported in this thesis was conducted to observe the behavior of C-6989 on bean plants using the same rates but two soil types.

For example, one from the Lake Labish series with an organic matter content of nearly 60%, and the other from the Newberg series with an organic matter content of 1.3%. Plants responded the same to the herbicide in both soils, therefore no further tests were made. A similar trial indicated that soil temperature does not appear to be an important factor, as demonstrated using water temperature tanks at 5 and 30^{o}C.

The formation of a crust on the surface of the soil does not appear to prevent completely the herbicidal injury of C-6989 to emerging seedlings. There was somewhat less noticeable injury, under crusting conditions of the surface as compared with non-crusting conditions, although the data do not provide strong evidence. On the other hand, crusted soil surfaces are created by overhead irrigation, and it is difficult to determine in advance the exact amount of water needed for that purpose.

Bean seedlings do respond to direct applications of C-6989. The injury produced at levels about 1, 000 ppmv is of a burning type and suggests immediately a contact effect. It seems that when growing centers are not affected, plants recover readily. This may be an indication that C-6989 has very little mobility within bean plants and to a certain extent, may suggest a degradation pathway exists. A possible explanation of this may be that at lower rates the injured tissue does not dry off and later resumes a normal appearance. Further evidence for this explanation is that when droplets of C-6989 were placed on the surface of fully developed leaves, necrotic spots resulted at rates higher than 1, 000 ppmv. At lower rates only a browning of the tissue was observed and this soon disappeared.

The importance of light on toxicity to bean seedlings treated with C-6989 was evident. Plants kept in the dark for four days after being treated did not show signs of herbicidal action. Those exposed to the light developed injury symptoms soon after the application. It is not possible to speculate, based on this experiment, at what level of the photosynthetic process C-6989 is acting but it may be worth mentioning that when etiolated bean seedlings were sprayed, and placed under the light, the herbicidal effects were slow (the first ones appeared after 36 hours), and were never as intense as those symptoms on non-etiloted or green plants. One explanation for this could be that C-6989 destroys some photosynthetic pigments. In any case, any observations at this stage would be misleading if not complemented with further studies.

In working with cucumber plants, it was found that C-6989 was photoactivated within the plant, no matter whether it was absorbed through the roots or shoots. Another interesting point illustrated by this experiment was, that in beans, the amount of injury observed visually did not increase as the rates increased.

When C-6989 was placed on the surface of the soil and exposed to light of high intensity, the herbicidal properties decreased markedly, although injury symptoms were still visible for all rates, mainly in the form of chlorosis of the central veins. This could be an indication that the chemical (or some toxic metabolites) are being translocated with the upward movement of nutrients, through the xylem. Eastin (14) reported that in cucumbers, some upward translocation of C-6989 does occur.

Under field conditions, 4 lb/A of C-6989 will control a wide range of common weeds of Oregon, such as pigweed, common lambsquarters, nightshade, annual bluegrass, ryegrass and barnyardgrass. The assumption can be made that C-6989 is safe at that rate, for use on green beans under normal conditions since no adverse effects were noted with the five varieties tested. However, this does not completely eliminate the possibility that under certain sprinkler irrigation practices crop injury may result at normal use rates.

By examining the data for timed applications of C-6989 to beans, it was noted that the greater the delay in the applications, the greater is the injury produced to the crop, specially if sprinkler irrigation is provided five days after planting. The situation just described is valid also for weed control. The explanation for this fact may be in a greater contact of the herbicide with the emerging seedlings, or it is possible too that by increasing the moisture of the soil surface, the absorption process of the plant will become more active.

From the experiment on the residual action of C-6989 it was noted that no potential hazards exist seven weeks after the application under summer conditions. The herbicide dissipated to levels in which it did not affect the emergence of any of the vegetables tested.

SUMMARY

Greenhouse and field experiments were conducted to determine the tolerance of beans to 2, 4'-dinitro-4-trifluoromethyl-diphenylether (C-6989). Other variables were also studied; the site of uptake by four plant specie, the effect of light in the action of the herbicide, the preemergence activity on weeks and its residual action in rotation crops.

Apparently, beans do not have a preferential site of uptake for C-6989 and are fairly resistant to the herbicide when applied pre-emergence. Other plants like cucumber, rice and barnyardgrass take up the herbicide very readily but little or no translocation occurs.

The site of action appears to be at the photosynthetic level. The activity of the herbicide was marked under the light. No activity was observed in plants treated and kept in the dark for four days.

The formation of a soil crust tends to diminish the injury that C-6989 causes to bean seedlings. As they emerge, they push the herbidice out of the way by breaking the crust of the soil surface.

The preemergence activity of C-6989 on weeds such as pigweed, lambsquarter, barnyardgrass and ryegrass was good at 4 lb/A under field conditions.

The residues of C-6989 from rates which were effective in controlling susceptible weeds, caused no injury on rotation crops such as corn, cucumbers and table beets, when tested 7 weeks after applications under summer conditions.

BIBLIOGRAPHY

1. Appleby, A. P. and W. R. Furtick. A technique for controlled exposure of emerging grass seedlings to soil-active herbicides. Weeds 13:172-173. 1965.

2. Appleby, A. P. Advanced weed science. Class notes. Oregon State University. 120 p. 1969.

3. Arai, M., H. Chisaka and T. Kataoka. Studies on the chemicals effective for breaking seed dormancy and for killing dormant seed of barnyardgrass (Echinochloa crus-galli B. var oryzicola). 1. Screening of effective chemicals. Proceedings of the Crop Science Society of Japan 36(3): 321-325. 1967.

4. Butler, W. L. and R. J. Dows. Light and plant development. Scientific American 203:56-63. 1960.

5. Chandra, P. and R. Grover. 1967. Residual effect of numerous herbicides on the soil microorganisms and their activities in a prairie soil of Canada. Abstracted in 6th International Congress of Plant Protection. Vienna. 604-605. 1967.

6. Ciba Agrochemical Company. C-6989, a selective herbicide. Research Bulletin No. 36, February 1968. Vero Beach, Florida. U.S.A. 1968.

7. __________________. Residue analysis of Preforan as C-6989 by spectrophotometry. Research Bulletin No. 47. 1968. (Unpublished)

8. __________________. Gas chromatographic analysis of Preforan as C-6989 in soil by electron capture detection. Research Bulletin No. 57. 1968. (Unpublished)

9. Crabtree, G. Weed control research in horticultural crops. O.S.U. Horticulture Department. 1968. (Unpublished)

10. Duncan, A. and G. Crabtree. Horticultural weed control. In: Oregon Weed Control Handbook. O.S.U. Cooperative Extension Service. p. 109-112. April 1970.

11. Duncan A. A. and R. W. Every. Commercail production of pole snap beans in Oregon. Extension Bulletin 783. Cooperative Extension Service. O. S. U., Corvallis, Oregon. 1960.

12. ________________ Commercial production of bush snap beans in Oregon. Extension Bulletin 787. Federal Cooperative Extension Service. O. S. U., Corvallis, Oregon. 1960.

13. Eastin, E. F. Movement and fate of Preforan in peanut seedlings. Plant Physiology 44:1397-1401. 1969.

14. ________________ Movement and fate of Preforan in cucumber seedlings. Weed Science Society of America Abstract No. 105. Montreal, Canada. 1970.

15. Ebner, L., D. H. Green and P. Pande. C-6989 a new selective herbicide. Proceedings of the 9th British Weed Control Conference. p. 126-1032. 1968.

16. Eshel, Y. and G. N. Prendeville. A technique for studying root v/s shoot uptake of soil applied herbicides. Weed Research 7:242-245. 1967.

17. Hayasaka, T. Sutides on a new diphenylether herbicide, HE-314. Part 1. Mode of action and selective herbicidal effect on genus of Gramineae. Weed Research of Japan 6:50-58. 1967.

18. Hendricks, S. B. How light interacts with living matter. Scientific American 219:174-186. 1968.

19. Matsunaka, S. Mode of action of diphenylether herbicides and synergistic action of insecticides with Propanyl. Abstracts of the 115th National Meeting of the American Chemical Society, San Francisco. No. 13. 1968.

20. ________________ Activation and inactivation of herbicides by higher plants. Research Review 25:45-57. 1969.

21. ________________ Acceptor of light energy in photoactivation of diphenylether herbicides. Jounral of Agriculture and Food Chemistry, Vol. 17, No. 2, March-April. p. 171-175. 1969.

22. Matsunaka, S. and K. Inada. Mode of action of diphenylether herbicides. I. Light for herbicidal activity. Abstracts of the Annual Meeting of the Agriculture Chemical Society of Japan. p. 47. 1967.

23. Oregon State University. Summer new herbicide evaluation trial. In: Annual Report 1967-1968. Weed Control Research. Farm Crops Department, Oregon State University. p. 140-141. 1968.

24. O. S. U. / A. I. D. New herbicide evaluations, Hawaii trials 1969-1970. OSU/AID Pub. 70-3. International Plant Protection Center. Corvallis, Oregon. p. 44-49. 1970.

25. ______________. Experimental herbicides, status report by crop OSU/AID Report 70-5. International Plant Protection Center. Corvallis, Oregon. 1970.

26. Parker, C. The importance of shoot entry in the action of herbicides applied to the soil. Weeds 14:117-121. 1966.

27. Prendeville, G. N. et al. Site of uptake of soil applied herbicides. Weed Research 7:316-322. 1967.

28. Rogers, R. L. Behavior and fate of Preforan in soybean and cucumber. Abstract of Weed Science Society of America. No. 171. 1969.

29. ______________ Absorption translocation and metabolism of Preforan by soybeans. CIBA Research report CF-7051. 1969. (Unpublished)

30. Rohm and Haas Company. Tok herbicide for selective pre and post emergence uses. Technical Bulletin AG-269. Philadelphia, U. S. A. 1968.

31. ______________ TOK E-25. A selective agricultural herbicide. Technical Bulletin AG-280b. Philadelphia, U. S. A. 1970.

32. ______________ TOK WP-50. A selective agricultural herbicide. Technical Bulletin AG-281b. Philadelphia, U. S. A. 1970.

33. Ueki, K. and N. Shimizu. Studies on the termination of dormancy in the seeds of cleavers (Galium aparine). 2. The effect of some chemicals on the breaking of dormancy. Weed Research, Japan 6:30-33. 1967.

34. U. S. D. A. Losses in agriculture. Agricultural Research Service. Agricultural handbook 291. 1965.

35. ________________ Agricultural statistics. Vegetables for processing 1969 annual summary. Agricultural Research Service Vg 3-2 (69). p. 12-13. 1969.

36. Walter, J. P., E. F. Eastin and M. G. Merkle. Persistence and movement of C-6989 in soils and plants. Proceedings of the Southern Weed Science Society 22:2. 1969.

APPENDIX

ANALYSES OF VARIANCE AND COEFFICIENTS OF VARIATIONS

Appendix Table 1. C-6989 Placement on Soil 1. Analysis of Variance of Beans.

Source	df	SS	MS	F
Placement	1	.340	.340	3.381*
Rates	4	1.226	.306	3.042
Placement and rates	4	.381	.095	.947
Error	30	3.021	.100	
Total	39	4.970		

* Significant. 5% probability level.
C. V. = 23%.

Appendix Table 2. C-6989 Placement on Soil 2. Analysis of Variance of Rice.

Source	df	SS	MS	F
Placement	1	1.700	1.700	356.774**
Rates	4	.796	.199	41.779**
Placement and rates	4	.434	.108	22.768**
Error	30	.142	.004	
Total	39	3.073		

** Highly significant. 1% probability level.
C. V. = 9%.

Appendix Table 3. C-6989 Placement on Soil 3. Analysis of Variance of Cucumber.

Source	df	SS	MS	F
Placement	1	.019	.019	1.663
Rates	4	.560	.140	11.977**
Placement and rates	4	.022	.005	.476
Error	30	.357	.011	
Total	39	.952		

** Highly significant. 1% probability level.
C. V. = 20%.

Appendix Table 4. C-6989 Placement on Soil 4. Analysis of Variance of Barnyardgrass.

Source	df	SS	MS	F
Placement	1	.801	.801	38.183**
Rates	4	2.655	.663	31.625**
Placement and rates	4	.250	.062	2.978**
Error	30	.629	.029	
Total	39	4.336		

** Highly significant. 1% probability level.
C. V. = 27%.

Appendix Table 5. The Response of Bean Seedlings to C-6989 When Applied to Crusted and Non-crusted Conditions of Soil Surface. Analysis of Variance.

Source	df	SS	MS	F
Surface	1	.277	.277	1.694
Rates	4	3.991	.997	6.098**
Surface and rates	4	.408	.121	.642
Error	30	4.908	.163	
Total	39	9.585		

** Highly significant. 1% probability level.
C. V. = 17%.

Appendix Table 6. The Response of Bean Seedlings to Direct Applications of C-6989. Analysis of Variance.

Source	df	SS	MS	F
Rates	5	1.029	.205	15.4463**
Error	66	.879	.013	
Total	71	1.909		

** Highly significant. 1% probability level.
C. V. = 36%.

Appendix Table 7. The Effect of Light in C-6989 Toxicity to Bean Plants. Analysis of Variance.

Source	df	SS	MS	F
Illumination	1	.243	.243	36.675**
Rates	4	.887	.221	33.385**
Illum. and rates	4	.401	.100	15.124**
Error	30	.199	.006	
Total	39	1.732		

** Highly significant. 1% probability level.
C. V. = 16%.

Appendix Table 8. Study to Determine if C-6989 is Activated by Light Within Cucumber Plants.

Source	df	SS	MS	F
Placement	1	.025	.025	1.686*
Rates	4	.561	.140	9.233**
Placement and rates	4	.045	.011	.741
Error	30	.456	.015	
Total	39	1.089		

** Highly significant. 1% probability level.
* Significant. 5% probability level.

C. V. = 27%.