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**Item ID Number** 03927

**Not Scanned**

**Author**

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**Report/Article Title** Typescript and Photographs: TCDD Uptake in Plants Study

**Journal/Book Title**

**Year** 0000

**Month/Day**

**Color**

**Number of Images** 312

**Description Notes** Documents were filed together in a container labeled "Data, Photographs, and Manuscript - TCDD Uptake in Plants." Only folders 1 and 2 of 11 folders have been scanned. Folder 1 contains manuscript revisions for the article, "A Method for Simulating Subsurface Injection of Herbicides," by J. M. Cupello and A. L. Young. Folder 2 contains black and white photographs from the study. Folders 3-11 contain additional proof sheets, photographs, and negatives taken at various dates during the study.

1  
2 A Method for Simulating Subsurface Injection of Herbicides<sup>1</sup>

3 J.M. Cupello and A.L. Young<sup>2</sup>

4 Abstract. Specially designed growth boxes were used to simulate field  
5 subsurface injection of phenoxy herbicides. Sorghum (Sorghum vulgare  
6 L.) seedlings were grown in stainless steel containers (inserts) which  
7 were placed in plexiglass boxes containing a soil layer that had  
8 received 2,240 kg active ingredient/ha (kg ai/ha) of a 50:50 mixture of  
9 the n-butyl esters of 2,4-D [(2,4-dichlorophenoxy)-acetic acid] and  
10 2,4,5-T [(2,4,5-trichlorophenoxy)-acetic acid]. Leaf-blade length data  
11 were collected periodically for all treatments. ~~Within uncut treatments,~~  
12 ~~Subsurface herbicide application~~ <sup>to intact plants</sup> retarded plant growth, <sup>but</sup> ~~relative to non-~~  
13 ~~herbicide treated controls.~~ <sup>grow</sup> No differences in plant height were  
14 observed between plants having cut roots and exposure to herbicide, and  
15 plants having only cut roots.

16 INTRODUCTION

17 Interest in disposal methodology for selected herbicides or  
18 herbicide manufacturing wastes has prompted field studies where  
19 herbicides have been subsurface injected at massive concentration rates  
20 (2, 9). The premise for such studies is that high concentrations of  
21

22 <sup>1</sup>Received for publication \_\_\_\_\_, 1976. Work was supported by  
23 Frank J. Seiler Research Laboratory (Air Force Systems Command) and Air  
24 Force Logistics Command.

25 <sup>2</sup>Assoc. Professors, Dep. Chem. Biol. Sci., (DFCBS), United States Air  
26 Force Academy, CO 80840.  
27

1 herbicides or their manufacturing wastes will be degraded to innocuous  
2 products by the combined action of soil microorganisms and soil  
3 hydrolysis (15). Numerous field methods and incorporation equipment  
4 have been described for the subsurface placement of herbicides (4, 5,  
5 14). Laboratory simulation of these field techniques has been confined  
6 to "normal" rates for herbicide application and to studies of root versus  
7 versus shoot uptake. A double pot technique first described by Eshel  
8 and Prendeville (7) has been most frequently used (3, 12); however, the  
9 layering of treated soil in pots or cans has also been popular (8, 10).  
10 A few other techniques have also been described. Parker (13), for  
11 example, used a double-dish technique using petri dishes to separately  
12 expose roots and shoots to sand or soil containing herbicides, while  
13 Appleby and Furtick (1) developed a plastic envelope device for allowing  
14 separate exposure of seeds, roots, and coleoptiles of emerging grass  
15 seedlings to soil-incorporated herbicides. Techniques to observe the  
16 growth of roots and the effects of root-active chemicals have been  
17 described by Muzik and Whitworth (11) and Duffy (6). The latter study  
18 involved chemical treatment of isolated portions of root systems without  
19 disturbance or injury to the untreated root mass.

20 All of the above techniques have been limited to the study of  
21 intact (uncut) root or shoot systems. In a field situation where an  
22 agricultural subsoiler would be used, many roots and stems would be  
23 severed by the shank or blade. Goulding (9) undercut a 4.05 ha plot of  
24 sparse to moderately dense greasewood [Sarcobatus vermiculatus (Hook.)  
25 Torr.] and injected a total of 62,457 liters of liquid waste from the  
26 manufacture of 2,4-D. Slow recovery of the shrubs was observed,  
27 principally between the injection points. In a unique experiment in

1 Southwest Kansas, growing grain sorghum was undercut with Noble blade  
2 equipment and simultaneously treated with 2,240 kg active ingredient/ha  
3 (kg ai/ha) of a 50:50 mixture of 2,4-D and 2,4,5-T<sup>3</sup>. Plant height at  
4 harvest for control plots was visibly different (greater) than plots  
5 receiving either undercutting with herbicide or undercutting without  
6 herbicide.

7 The present study was initiated to develop a laboratory method that  
8 would simulate field disposal studies of phenoxy herbicides using sub-  
9 surface injection and ~~to~~ quantify the effects of undercutting sorghum  
10 with and without the addition of massive quantities of phenoxy  
11 herbicides.

#### 12 MATERIALS AND METHODS

13 Special growth boxes (Figure 1) were designed to permit simultan-  
14 eous cutting and exposure of plant root systems to herbicides. The  
15 boxes had dimensions of 30.5 cm x 30.5 cm x 17.8 cm (inside diameter),  
16 constructed of 0.64 cm plexiglass. A 29.8 cm x 29.8 cm x 12.7 cm  
17 (outside diameter) insert constructed of 0.16 cm stainless steel was  
18 made to fit loosely inside the plexiglass boxes. The insert had three  
19 0.16 cm diameter stainless steel rods welded across its bottom as  
20 support for a 30.5 cm x 30.5 cm sheet of 10 mesh (1680 $\mu$  aperture width)  
21 stainless steel screen. The screen retained the soil inside the stain-  
22 less steel insert, while permitting passage of the roots into the  
23

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24 <sup>3</sup>Condray, J.L. 1972. Annual report of the weeds research project.  
25 Garden City Branch Experiment Station, Kansas State University, Garden  
26 City, Kansas 67846.

1 plexiglass container below. Four plexiglass legs, each 1.3 cm x 1.3 cm  
2 x 5.1 cm were placed in the four corners of the plexiglass box to  
3 support the stainless steel insert at a fixed distance from the bottom  
4 of the plexiglass box. This design permitted easy access to the root  
5 systems for cutting, provided that the space (a layer 5.1 cm deep)  
6 beneath the stainless steel screen was loosely packed with vermiculite  
7 or a similar growth medium which permitted removal of the insert from  
8 the plexiglass box without damaging the root systems. Chemical treat-  
9 ment and, hence exposure of the cut roots, was accomplished by removing  
10 the vermiculite layer and replacing it with treated soil.

11  
12 Moisture studies. To determine the sensitivity of this new technique  
13 to variations in the amount of water contained in the vermiculite layer,  
14 a preliminary experiment was conducted. Two plexiglass containers were  
15 filled with a 5.1 cm layer of dry vermiculite. The first, referred to  
16 as the damp vermiculite container, was provided sufficient tap water to  
17 moisten the vermiculite layer without allowing free standing water. A  
18 second container, the saturated vermiculite container, was watered  
19 until free standing water was 5.1 cm deep. A third plexiglass container  
20 was filled with 5.1 cm of moist Ulysses silt loam soil (pH 7, 1.3%  
21 organic matter, and 33, 44, and 23% sand, silt and clay, respectively)  
22 as a control, and was used to indicate whether plants grown in vermicu-  
23 lite had different growth rates than those grown in soil only. The  
24 stainless steel inserts for the three plexiglass boxes were filled with  
25 10.2 cm of the Ulysses silt loam soil and placed in their respective  
26 plexiglass boxes. A cardboard template with the same surface area as  
27 the stainless steel inserts was pre-punched with 100 holes in a 10 x 10

1 square matrix, each hole separated from adjacent holes by a distance of  
2 2.54 cm. The template was placed over the soil in the stainless steel  
3 inserts. One hundred seeds of sorghum (var. Pioneer 846), selected at  
4 random, were placed in the 100 holes and the seeds were pushed into the  
5 soil to a depth of 2.54 cm using a wooden dowel rod. The cardboard was  
6 then removed, <sup>and</sup> the soil lightly raked <sup>and packed</sup> ~~to fill up~~ the holes created by the  
7 ~~dowel rod, and the soil lightly packed.~~ The growth boxes were placed in  
8 an environmental chamber for 46 days. The chamber was maintained at a  
9 14-hour photoperiod, diurnal temperature of  $35 \pm 2^\circ\text{C}$  and  $15 \pm 1^\circ\text{C}$ , and a  
10 relative humidity of 60 and 85 percent, day and night, respectively.

11  
12 Chemical treatment study. Based upon the results of the moisture study,  
13 further experiments were designed to study the effects of chemical  
14 treatment on cut versus uncut root systems. The experiment was of a  
15 2 x 2 design utilizing four growth boxes: (1) cut control; (2) uncut  
16 control; (3) cut treated; and (4) uncut treated. All four stainless  
17 steel inserts were filled with a 10.2 cm layer of the Ulysses silt loam  
18 soil. The four plexiglass containers were handled somewhat differently  
19 depending on whether the root systems were to be cut or uncut.  
20 Initially, however, all four plexiglass containers were filled with  
21 5.1 cm of damp vermiculite, their stainless steel inserts carefully  
22 positioned inside the plexiglass containers and 100 seeds planted in  
23 each of the four inserts. Those growth boxes containing plants whose  
24 root systems were to remain uncut were allowed to grow for 3 days, at  
25 which time their stainless steel inserts were removed, the vermiculite  
26 layer replaced with moist "treated" or "untreated" soils, and the  
27 stainless steel insert replaced. Prior work indicated that by the

1 third day after planting, the roots were just approaching the  
2 stainless steel screen.

3 <sup>plants</sup> The ~~growth boxes~~ which were to be cut were allowed to grow for 22  
4 days after the initial planting, at which time the stainless steel  
5 inserts were removed, the vermiculite replaced with either treated or  
6 untreated soil, the root systems <sup>parallel to the base of the insert</sup> cut, and the stainless steel inserts  
7 replaced. All boxes were maintained in the environmental chamber under  
8 the conditions previously described. The boxes were periodically re-  
9 moved for watering and plant height measurements. The arrangement of  
10 growth boxes inside the chamber was alternated at the time of watering  
11 on a random basis in order to minimize any effects due to nonhomogeneous  
12 environmental factors within the chamber. ~~On those days on which the~~  
13 ~~plant heights were measured,~~ a minimum of ten plants per box were  
14 randomly selected and the heights of the plants, from the soil surface  
15 to the tip of the longest leaf, were recorded <sup>at approx 1 wk intervals</sup>  
16 ( 7 5 )

17 Chemical formulations and application. Those plexiglass containers that  
18 were to receive chemically treated soil at the appropriate point in the  
19 experiment were handled in the following manner. At the time of  
20 chemical treatment for both cut (day 22) and uncut (day 3) root systems,  
21 the 5.1 cm of vermiculite was removed and replaced by 4.1 cm of un-  
22 contaminated soil. The remaining 1.0 cm space was filled with soil  
23 which had been previously mixed with sufficient herbicide formulation  
24 (a 50:50 mixture of the n-butyl esters of 2,4-D and 2,4,5-T) to be  
25 equivalent to 2,240 kg ai/ha. To insure a uniform layering of this  
26 1 cm of contaminated soil, a plastic grid containing a matrix of 1 cm  
27 x 1 cm square holes was first spaced in the plexiglass container (over

Can you state exactly when



1 the 4.1 cm of untreated soil), the contaminated soil placed on this  
2 grid and spread to fill all of the grid squares equally, and the grid  
3 carefully removed. This soil was lightly packed, wetted with 500 ml of  
4 tap water, and the stainless steel insert <sup>re</sup> placed ~~back in position.~~

5 The 1 cm layer of soil ~~was predetermined to weigh~~ 1,500 grams, and  
6 ~~to this weight of soil was added~~ 20.1 ml of the herbicide <sup>was added</sup> formulation.

7 To guarantee quantitative transfer, two 5 ml acetone rinses of the  
8 glassware were also added to the treated soils. The acetone was  
9 allowed to evaporate <sup>for a few minutes</sup> prior to the time the root systems were placed in  
10 contact with the chemically treated soils. The control soils received  
11 no herbicide, but did receive the 10 ml of acetone.

#### 12 RESULTS AND DISCUSSION

13 Moisture study. The data points in Figure 2 represent average sorghum  
14 plant height as a function of time after initial seed planting (time  
15 zero). Two of the curves represent growth rate variations due to  
16 differences in the moisture content of the vermiculite layer placed  
17 beneath the 10.2 cm of seed bearing soil. The data obtained from  
18 the box containing soil rather than vermiculite were considered as  
19 control data and were used as the baseline against which the damp and  
20 saturated vermiculite data were compared. ~~It was felt that the growth~~  
21 ~~rate in soil most truly represented normal environmental growth~~  
22 ~~response.~~

23 Because the data were time variant, they were analyzed by fitting  
24 a curve to the data points using the method of least squares  
25 linear regression. A number of equations relating plant height (Y) and  
26  
27

1 time (X) were tested to determine which mathematical form of a growth  
2 model best fit the experimental data. The best fit was defined as the  
3 equation which resulted in the smallest mean squared error (MSE) between  
4 empirical data and that predicted by the growth model.

5 For the control data in Figure 2 (soil rather than vermiculite),  
6 an equation of the form

$$7 \quad Y = \alpha + \beta_1 X + \beta_2 X^2 + \beta_3 X^3 \quad [1]$$

8 gave the smallest MSE, and was selected as the appropriate growth model  
9 against which to compare the data resulting from root growth in vermicu-  
10 lite. Additional statistical testing of this equation indicated that  
11 no additional terms needed to be included in equation [1]. Utilizing a  
12 matrix inversion technique, the coefficients for [1] were determined as  
13 shown below:

$$14 \quad Y = -11.1 + 2.71X - 0.0642X^2 + 0.000615X^3 \quad [2]$$

15 The experimental data obtained from the damp and saturated vermicu-  
16 lite treatments (Figure 2) were also fitted to the general form of  
17 equation [1] and their coefficients determined, so as to provide the  
18 minimal MSE.

19 Linear regression analysis at the 95 percent confidence level  
20 (used for all statistical tests in this study) indicated that both the  
21 damp and saturated vermiculite growth models were different than the  
22 soil growth model. Likewise, the damp vermiculite growth model was  
23 shown to be statistically different from the saturated vermiculite  
24 growth model.

25 It could be argued that the damp and saturated vermiculite data  
26 should be fitted to a growth model different from the general form of  
27 equation [1]. Even if this were true it would not alter the

1 conclusions; it would only reduce the MSE of the curve fits.

2 It is concluded from these analyses that the laboratory method  
3 described is sensitive to the replacement of soil by vermiculite, and  
4 the water content of the vermiculite layer. Thus, care must be taken to  
5 ensure that all growth boxes used in a given experiment or set of sub-  
6 sequent experiments are alike with respect to the quantity and water  
7 content of the vermiculite layer; at the beginning, and for the  
8 duration, of the experiment(s).

9 The effect of cutting of root systems was studied in the  
10 presence and absence of herbicide. The experimental design required  
11 those treatments that were to have "uncut" root systems to have  
12 their vermiculite layer replaced by soil approximately three weeks  
13 prior to the time when the "cut" treatments had their vermiculite layers  
14 replaced. If the uncut treatments were not so modified on day 3, the  
15 roots would already have penetrated into the vermiculite layers, and  
16 would be damaged when the vermiculite layer was replaced by soil,  
17 and the stainless steel insert placed on top of this soil layer.  
18 The previous results with the vermiculite suggested that a comparison  
19 of "cut" and "uncut" treatment data might be invalid. The fact that  
20 the environmental growth conditions are different for a period of up to  
21 three weeks could, in itself, cause significant differences in plant  
22 growth rates. Thus we would <sup>are?</sup> be unable to ascribe any observed growth  
23 rate differences to chemical treatment or cutting, alone.

24  
25 Chemical treatment study. Figure 3 illustrates the data and best curve  
26 fits for the cut and uncut control treatments (no herbicide). Both  
27 curve-fits in Figure 3 were independently determined using the

1 procedures described in the moisture studies.

2 The growth models which provided the best fit to the data for the  
3 uncut control and cut control treatments were, respectively:

4 
$$Y = 30.42 - .01817X^2 + .0002215X^3 + 18.63 \ln(X) \quad [3]$$

5 
$$Y = 30.52 - 1.348X + .01050X^2 + 23.39 \ln(X) \quad [4]$$

6 Statistical analysis confirmed that the growth models for cut and  
7 uncut controls were significantly different. However, whether this  
8 observed difference was due to the physical act of root cutting,  
9 growth rate alterations induced by handling the uncut and cut treatments  
10 differently (changing vermiculite on day 3 versus day 22, respectively),  
11 other experimental variables, or a combination of all of these could  
12 not be determined. Therefore, further discussion is limited to  
13 comparisons within the categories of cut and uncut treatments.

14 Figure 4 shows the comparison of uncut control and the uncut  
15 treated growth models. The general form of equation [3] was used to  
16 determine the coefficients (for the uncut treated growth model). Analysis  
17 ~~of the coefficients indicated~~ <sup>showed</sup> that treatment with herbicide signifi-  
18 ~~cantly reduced the rate of growth relative to uncut controls.~~ <sup>of the plants with</sup> ~~intact root systems.~~ <sup>plants with</sup>

19 Figure 5 illustrates the comparison between cut control and the  
20 cut treated growth models. The same procedures were used to make sta-  
21 tistical inferences as were used to analyze the data in Figure 4; the  
22 only difference being that the general form of the growth model being  
23 tested was of the form shown in equation [4]. The results of these  
24 analyses indicated no difference in growth rate between control and  
25 treated plots, <sup>plants</sup> ~~both~~ which had their root systems severed: <sup>i.e.,</sup> cutting  
26 the root systems caused the plants to grow as if the herbicide were  
27 not present.

1 Technique evaluation. The development of this laboratory method for  
2 simulating massive subsurface field disposal of herbicides was prompted  
3 by our need for a subsurface application method that mimicked actual  
4 field methodology; namely, cutting of root systems during application.  
5 The addition of this refinement over existing methods is the principal  
6 justification for preferential use of this technique. Frequent employ-  
7 ment of subsurface herbicide placement techniques in the field, to  
8 enhance chemical persistence and to place the agent in the zone of plant  
9 uptake, seems to warrant continued improvement of laboratory simulation  
10 methods. When root systems are not cut, herbicide application causes a  
11 significant decrease in plant growth, but we find no herbicide effect  
12 on growth when root systems are cut. The introduction of this one  
13 additional experimental variable, root cutting, may completely alter  
14 the conclusions drawn from such studies; it is too important a  
15 consideration to ignore.

16 Our use of the technique for massive quantities of phenoxy  
17 herbicide would not preclude its use at rates commonly found in  
18 commercial applications. It is questionable, however, whether such  
19 low rates of application of these chemicals would appreciably affect  
20 sorghum growth rate.

21 During the course of these experiments a number of interesting  
22 physiological phenomena were observed. Those plexiglass growth boxes  
23 treated with subsurface herbicide showed little, if any, root penetra-  
24 tion into or beyond the chemically treated soil layer. Boxes with  
25 untreated soil showed significant root mass penetration throughout the  
26 soil. If similar results can be verified in field studies, one could  
27 argue that the presence of this chemical barrier to root penetration

1 would limit or prevent plant uptake of herbicide. Were this so, massive  
2 subsurface placement of herbicides for biodegradation purposes could be  
3 accomplished without fear of animal food chain contamination.

4 As anticipated, rates of seed germination were similar for all  
5 treatments. Since the seeds were placed beneath the soil surface, and  
6 7.6 cm above the site of herbicide placement, no germination effects  
7 were expected.

8 Those plants directly adjacent to the walls of the stainless steel  
9 inserts, and especially those plants located in the corners of the  
10 inserts, were visibly taller than those more centrally located. We  
11 attribute this growth advantage to the fact that the outer perimeter  
12 of plants had less root competition for nutrients due to the lack of  
13 an adjacent row of plants on one of their two sides. The plants located  
14 in the corners would have such an advantage from two sides, rather than  
15 just one. A random selection of plants for height determinations  
16 ~~tended to~~ <sup>the effect is</sup> minimize this bimodal distribution of plant heights. (Another  
17 way to reduce this artifact would be to confine plant measurements to  
18 plants other than those located adjacent to the four stainless steel )  
19 walls.

20 The present studies were of a 2 x 2 statistical design, and were  
21 not replicated over time under exact environmental conditions. However,  
22 a number of other studies using the identical growth boxes were con-  
23 ducted to study the effects of temperature and relative humidity,  
24 alternative methods of cutting the roots, subsurface watering, etc. In  
25 all cases, the technique was reliable. Although the mathematical plant  
26 growth models will obviously change as the technique is modified  
27 (altering soil composition, type of plants, etc.) we are confident that

1 results obtained within a given set of experimental conditions will  
2 permit quantitative comparisons between treatments.

3 ACKNOWLEDGEMENTS

4 The authors are grateful to Mr Joseph C.H. Smith, Department of  
5 Mathematical Sciences, United States Air Force Academy, for his  
6 assistance in analyzing data, and to Mr Jerry L. Condray, Kansas  
7 Agricultural Experiment Station, Garden City, Kansas, for furnishing  
8 the seed and soil used in this study.

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Captions for Figures

1  
2 Figure 1. Plexiglass growth boxes with stainless steel inserts.

3 Figure 2. Average height of sorghum plants following root exposure to  
4 damp or saturated vermiculite, and moist Ulysses silt loam  
5 soil. Data points are averages of ten or more plant heights.  
6 Curve fits determined by least squares linear regression.  
7 The curves were significantly different at the 0.95 confi-  
8 dence level for all treatment comparisons.

9 Figure 3. The effect of subsurface root cutting on the growth rate of  
10 non-herbicide treated sorghum plants. Data points are  
11 averages of ten or more plant heights. Curve fits deter-  
12 mined by least squares linear regression. The curves  
13 significantly different at the 0.95 confidence level.

14 Figure 4. The effect of subsurface herbicide application on the growth  
15 rate of sorghum plants having uncut root systems. Data  
16 points are averages of ten or more plant heights. Curve fits  
17 determined by least squares linear regression. The curves  
18 significantly different at the 0.95 confidence level.

19 Figure 5. The effect of subsurface herbicide application on the growth  
20 rate of sorghum plants having cut root systems. Data points  
21 are averages of ten or more plant heights. Curve fits deter-  
22 mined by least squares linear regression. The curves were  
23 not significantly different at the 0.95 confidence level.  
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A Method for Simulating Subsurface Injection of Herbicides<sup>1</sup>

J.M. Cupello and A.L. Young<sup>2</sup>

Abstract. Specially designed growth boxes were used to simulate field subsurface injection of phenoxy herbicides. Sorghum (Sorghum vulgare L.) seedlings were grown in stainless steel containers (inserts) which were placed in plexiglass boxes containing a soil layer that had received 2,240 kg active ingredient/ha (kg ai/ha) of a 50:50 mixture of the n-butyl esters of 2,4-D [(2,4-dichlorophenoxy)-acetic acid] and 2,4,5-T [(2,4,5-trichlorophenoxy)-acetic acid]. Leaf-blade length data were collected periodically for all treatments. Subsurface herbicide application to intact root systems retarded plant growth. No differences in growth were observed between plants whose root systems were cut and exposed to herbicide, and those plants whose root systems were cut but not exposed to herbicide.

INTRODUCTION

Interest in disposal methodology for selected herbicides or herbicide manufacturing wastes has prompted field studies where herbicides have been subsurface injected at massive concentration rates (2, 9). The premise for such studies is that high concentrations of

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<sup>1</sup>Received for publication \_\_\_\_\_, 1976. Work was supported by Frank J. Seiler Research Laboratory (Air Force Systems Command) and Air Force Logistics Command.

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1 herbicides or their manufacturing wastes will be degraded to innocuous  
2 products by the combined action of soil microorganisms and soil  
3 hydrolysis (15). Numerous field methods and incorporation equipment  
4 have been described for the subsurface placement of herbicides (4, 5,  
5 14). Laboratory simulation of these field techniques has been confined  
6 to "normal" rates for herbicide application and to studies of root  
7 versus shoot uptake. A double pot technique first described by Eshel  
8 and Prendeville (7) has been most frequently used (3, 12); however, the  
9 layering of treated soil in pots or cans has also been popular (8, 10).  
10 A few other techniques have also been described. Parker (13), for  
11 example, used a double-dish technique using petri dishes to separately  
12 expose roots and shoots to sand or soil containing herbicides, while  
13 Appleby and Furtick (1) developed a plastic envelope device for allowing  
14 separate exposure of seeds, roots, and coleoptiles of emerging grass  
15 seedlings to soil-incorporated herbicides. Techniques to observe the  
16 growth of roots and the effects of root-active chemicals have been  
17 described by Muzik and Whitworth (11) and Duffy (6). The latter study  
18 involved chemical treatment of isolated portions of root systems without  
19 disturbance or injury to the untreated root mass.

20 All of the above techniques have been limited to the study of  
21 intact (uncut) root or shoot systems. In a field situation where an  
22 agricultural subsoiler would be used, many roots and stems would be  
23 severed by the shank or blade. Goulding (9) undercut a 4.05 ha plot of  
24 sparse to moderately dense greasewood [Sarcobatus vermiculatus (Hook.)  
25 Torr.] and injected a total of 62,457 liters of liquid waste from the  
26 manufacture of 2,4-D. Slow recovery of the shrubs was observed,  
27 principally between the injection points. In a unique experiment in

1 Southwest Kansas, growing grain sorghum was undercut with Noble blade  
2 equipment and simultaneously treated with 2,240 kg ai/ha of a 50:50  
3 mixture of 2,4-D and 2,4,5-T<sup>3</sup>. Plant height at harvest for control  
4 plots was visibly different (greater) than plots receiving either  
5 undercutting with herbicide or undercutting without herbicide.

6 The present study was initiated to develop a laboratory method that  
7 would (1) simulate field disposal studies of phenoxy herbicides using  
8 subsurface injection and (2) quantify the effects of undercutting  
9 sorghum with and without the addition of massive quantities of phenoxy  
10 herbicides.

#### 11 MATERIALS AND METHODS

12 Special growth boxes (Figure 1) were designed to permit simultan-  
13 eous cutting and exposure of plant root systems to herbicides. The  
14 boxes had dimensions of 30.5 cm x 30.5 cm x 17.8 cm (inside diameter),  
15 constructed of 0.64 cm plexiglass. A 29.8 cm x 29.8 cm x 12.7 cm  
16 (outside diameter) insert constructed of 0.16 cm stainless steel was  
17 made to fit loosely inside the plexiglass boxes. The insert had three  
18 0.16 cm diameter stainless steel rods welded across its bottom as  
19 support for a 30.5 cm x 30.5 cm sheet of 10 mesh (1680 $\mu$  aperture width)  
20 stainless steel screen. The screen retained the soil inside the stain-  
21 less steel insert, while permitting passage of the roots into the

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22  
23 <sup>3</sup>Condray, J.L. 1972. Annual report of the weeds research project.

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25 City, Kansas 67846.

1 plexiglass container below. Four plexiglass legs, each 1.3 cm x 1.3 cm  
2 x 5.1 cm were placed in the four corners of the plexiglass box to  
3 support the stainless steel insert at a fixed distance from the bottom  
4 of the plexiglass box. This design permitted easy access to the root  
5 systems for cutting, provided that the space (a layer 5.1 cm deep)  
6 beneath the stainless steel screen was loosely packed with vermiculite  
7 or a similar growth medium which permitted removal of the insert from  
8 the plexiglass box without damaging the root systems. Chemical treat-  
9 ment and, hence exposure of the cut roots, was accomplished by removing  
10 the vermiculite layer and replacing it with treated soil.

11  
12 Moisture studies. To determine the sensitivity of this new technique  
13 to variations in the amount of water contained in the vermiculite layer,  
14 a preliminary experiment was conducted. Two plexiglass containers were  
15 filled with a 5.1 cm layer of dry vermiculite. The first, referred to  
16 as the damp vermiculite container, was provided sufficient tap water to  
17 moisten the vermiculite layer without allowing free standing water. A  
18 second container, the saturated vermiculite container, was watered  
19 until free standing water was 5.1 cm deep. A third plexiglass container  
20 was filled with 5.1 cm of moist Ulysses silt loam soil (pH 7, 1.3%  
21 organic matter, and 33, 44, and 23% sand, silt and clay, respectively)  
22 as a control, and was used to indicate whether plants grown in vermicu-  
23 lite had different growth rates than those grown in soil only. The  
24 stainless steel inserts for the three plexiglass boxes were filled with  
25 10.2 cm of the Ulysses silt loam soil and placed in their respective  
26 plexiglass boxes. A cardboard template with the same surface area as  
27 the stainless steel inserts was pre-punched with 100 holes in a 10 x 10

1 square matrix, each hole separated from adjacent holes by a distance of  
2 2.54 cm. The template was placed over the soil in the stainless steel  
3 inserts. One hundred seeds of sorghum (var. Pioneer 846), selected at  
4 random, were placed in the 100 holes and the seeds were pushed into the  
5 soil to a depth of 2.54 cm using a wooden dowel rod. The cardboard was  
6 then removed and the soil lightly raked and packed. The growth boxes  
7 were placed in an environmental chamber for 46 days. The chamber was  
8 maintained at a 14-hour photoperiod, diurnal temperature of  $35\pm 2^{\circ}\text{C}$  and  
9  $15\pm 1^{\circ}\text{C}$ , and a relative humidity of 60 and 85 percent, day and night,  
10 respectively.

11  
12 Chemical treatment study. Based upon the results of the moisture study,  
13 further experiments were designed to study the effects of chemical  
14 treatment on cut versus uncut root systems. The experiment was of a  
15 2 x 2 statistical design utilizing four growth boxes: (1) cut control;  
16 (2) uncut control; (3) cut treatment; and (4) uncut treatment. All four  
17 stainless steel inserts were filled with a 10.2 cm layer of the Ulysses  
18 silt loam soil. The four plexiglass containers were handled somewhat  
19 differently depending on whether the root systems were to be cut or un-  
20 cut. Initially, however, all four plexiglass containers were filled  
21 with 5.1 cm of damp vermiculite, their stainless steel inserts carefully  
22 positioned inside the plexiglass containers and 100 seeds planted in  
23 each of the four inserts. Those growth boxes containing plants whose  
24 root systems were to remain uncut were allowed to grow for 3 days, at  
25 which time their stainless steel inserts were removed, the vermiculite  
26 layer replaced with moist "treated" or "untreated" soils, and the  
27 stainless steel insert replaced. Prior work indicated that by the



1 third day after planting, the roots were just approaching the  
2 stainless steel screen.

3 The plants which were to be cut were allowed to grow for 22  
4 days after the initial planting, at which time the stainless steel  
5 inserts were removed, the vermiculite replaced with either treated or  
6 untreated soil, the root systems cut flush against the stainless steel  
7 screen, and the stainless steel inserts replaced. All boxes were  
8 maintained in the environmental chamber under the conditions previously  
9 described. The boxes were periodically removed for watering and plant  
10 height measurements. The arrangement of growth boxes inside the chamber  
11 was alternated at the time of watering on a random basis in order to  
12 minimize any effects due to nonhomogeneous environmental factors within  
13 the chamber. At approximately one week intervals, a minimum of ten  
14 plants per box were randomly selected, and the heights of the plants  
15 recorded.

16  
17 Chemical formulations and application. Those plexiglass containers that  
18 were to receive chemically treated soil at the appropriate point in the  
19 experiment were handled in the following manner. At the time of  
20 chemical treatment for both cut (day 22) and uncut (day 3) root systems,  
21 the 5.1 cm of vermiculite was removed and replaced by 4.1 cm of un-  
22 contaminated soil. The remaining 1.0 cm space was filled with soil  
23 which had been previously mixed with sufficient herbicide formulation  
24 (20.1 ml of a 50:50 mixture of the n-butyl esters of 2,4-D and 2,4,5-T)  
25 to be equivalent to 2,240 kg ai/ha. To guarantee quantitative transfer,

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1 two 5 ml acetone rinses of the glassware were also added to the treated  
2 soils. The acetone was allowed to evaporate prior to the time the root  
3 systems were placed in contact with the chemically treated soils. The  
4 control soils received no herbicide, but did receive the 10 ml of  
5 acetone.

6 To insure a uniform layering of this 1 cm of contaminated soil, a  
7 plastic grid containing a matrix of 1 cm x 1 cm square holes was first  
8 placed in the plexiglass container (over the 4.1 cm of untreated soil),  
9 the contaminated soil placed on this grid and spread to fill all of  
10 the grid squares equally, and the grid carefully removed. This soil  
11 was lightly packed, wetted with 500 ml of tap water, and the stainless  
12 steel insert replaced.

#### 13 RESULTS AND DISCUSSION

14 Moisture study. The data points in Figure 2 represent average sorghum  
15 plant height as a function of time after initial seed planting (time  
16 zero). Two of the curves represent growth rate variations due to  
17 differences in the moisture content of the vermiculite layer placed  
18 beneath the 10.2 cm of seed bearing soil. The data obtained from  
19 the box containing soil rather than vermiculite were considered as  
20 control data and were used as the baseline against which the damp and  
21 saturated vermiculite data were compared.

22 Because the data were time variant, they were analyzed by fitting  
23 a curve to the data points using the method of least squares  
24 linear regression. A number of equations relating plant height (Y) and  
25  
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1 time (X) were tested to determine which mathematical form of a growth  
2 model best fit the experimental data. The best fit was defined as the  
3 equation which resulted in the smallest mean squared error (MSE) between  
4 empirical data and that predicted by the growth model.

5 For the control data in Figure 2 (soil rather than vermiculite),  
6 an equation of the form

$$7 \quad Y = \alpha + \beta_1 X + \beta_2 X^2 + \beta_3 X^3 \quad [1]$$

8 gave the smallest MSE, and was selected as the appropriate growth model  
9 against which to compare the data resulting from root growth in vermicu-  
10 lite. Additional statistical testing of this equation indicated that  
11 no additional terms needed to be included in equation [1]. Utilizing a  
12 matrix inversion technique, the coefficients for [1] were determined as  
13 shown below:

$$14 \quad Y = -11.1 + 2.71X - 0.0642X^2 + 0.000615X^3 \quad [2]$$

15 The experimental data obtained from the damp and saturated vermicu-  
16 lite treatments (Figure 2) were also fitted to the general form of  
17 equation [1] and their coefficients determined, so as to provide the  
18 minimal MSE.

19 Linear regression analysis at the 95 percent confidence level  
20 (used for all statistical tests in this study) indicated that both the  
21 damp and saturated vermiculite growth models were different than the  
22 soil growth model. Likewise, the damp vermiculite growth model was  
23 shown to be statistically different from the saturated vermiculite  
24 growth model.

25 It could be argued that the damp and saturated vermiculite data  
26 should be fitted to a growth model different from the general form of  
27 equation [1]. Even if this were true it would not alter the

1 conclusions; it would only reduce the MSE of the curve fits.

2       It is concluded from these analyses that the laboratory method  
3 described is sensitive to the replacement of soil by vermiculite, and  
4 the water content of the vermiculite layer. Thus, care must be taken to  
5 ensure that all growth boxes used in a given experiment or set of sub-  
6 sequent experiments are alike with respect to the quantity and water  
7 content of the vermiculite layer; at the beginning, and for the  
8 duration, of the experiment(s).

9       The effect of cutting of root systems was studied in the  
10 presence and absence of herbicide. The experimental design required  
11 those treatments that were to have "uncut" root systems to have  
12 their vermiculite layer replaced by soil approximately three weeks  
13 prior to the time when the "cut" treatments had their vermiculite layers  
14 replaced. If the uncut treatments were not so modified on day 3, the  
15 roots would already have penetrated into the vermiculite layers, and  
16 would be damaged when the vermiculite layer was replaced by soil,  
17 and the stainless steel insert placed on top of this soil layer.  
18 The previous results with vermiculite suggested that a comparison  
19 of "cut" and "uncut" treatment data might be invalid. The fact that  
20 the environmental growth conditions are different for a period of up to  
21 three weeks could, in itself, cause significant differences in plant  
22 growth rates. Thus we are unable to ascribe any observed growth  
23 rate differences to chemical treatment or cutting, alone.

24

25 Chemical treatment study. Figure 3 illustrates the data and best curve  
26 fits for the cut and uncut control treatments (no herbicide). Both  
27 curve-fits in Figure 3 were independently determined using the

1 procedures described in the moisture studies.

2 The growth models which provided the best fit to the data for the  
3 uncut control and cut control treatments were, respectively:

$$4 \quad Y = -30.42 - .01817X^2 + .0002215X^3 + 18.63 \ln(X) \quad [3]$$

$$5 \quad Y = -30.52 - 1.348X + .01050X^2 + 23.39 \ln(X) \quad [4]$$

6 Statistical analysis confirmed that the growth models for cut and  
7 uncut controls were significantly different. However, whether this  
8 observed difference was due to the physical act of root cutting,  
9 growth rate alterations induced by handling the uncut and cut treatments  
10 differently (changing vermiculite on day 3 versus day 22, respectively),  
11 other experimental variables, or a combination of all of these could  
12 not be determined. Therefore, further discussion is limited to  
13 comparisons within the categories of cut and uncut treatments.

14 Figure 4 shows the comparison of uncut control and the uncut  
15 treated growth models. The general form of equation [3] was used to  
16 determine the coefficients for the growth model. Analysis showed that  
17 subsurface herbicide treatment of intact root systems significantly  
18 reduced the rate of plant growth.

19 Figure 5 illustrates the comparison between cut control and the  
20 cut treated growth models. The same procedures were used to make sta-  
21 tistical inferences as were used to analyze the data in Figure 4; the  
22 only difference being that the general form of the growth model being  
23 tested was of the form shown in equation [4]. The results of these  
24 analyses indicated no difference in growth rate between control and  
25 treated plots which had their root systems severed, i.e., cutting  
26 the root systems caused the plants to grow as if the herbicide were  
27 not present.

1 Technique evaluation. The development of this laboratory method for  
2 simulating massive subsurface field disposal of herbicides was prompted  
3 by our need for a subsurface application method that mimicked actual  
4 field methodology; namely, cutting of root systems during application.  
5 The addition of this refinement over existing methods is the principal  
6 justification for preferential use of this technique. Frequent employ-  
7 ment of subsurface herbicide placement techniques in the field, to  
8 enhance chemical persistence and to place the agent in the zone of plant  
9 uptake, seems to warrant continued improvement of laboratory simulation  
10 methods. When root systems are not cut, herbicide application causes a  
11 significant decrease in plant growth, but we find no herbicide effect  
12 on growth when root systems are cut. The introduction of this one  
13 additional experimental variable, root cutting, may completely alter  
14 the conclusions drawn from such studies; it is too important a  
15 consideration to ignore.

16 Our use of the technique for massive quantities of phenoxy  
17 herbicide would not preclude its use at rates commonly found in  
18 commercial applications. It is questionable, however, whether such  
19 low rates of application of these chemicals would appreciably affect  
20 sorghum growth rate.

21 During the course of these experiments a number of interesting  
22 physiological phenomena were observed. Those plexiglass growth boxes  
23 treated with subsurface herbicide showed little, if any, root penetra-  
24 tion into or beyond the chemically treated soil layer. Boxes with  
25 untreated soil showed significant root penetration throughout the  
26 soil. If similar results can be verified in field studies, one could  
27 argue that the presence of this chemical barrier to root penetration

1 would limit or prevent plant uptake of herbicide. Were this so, massive  
2 subsurface placement of herbicides for biodegradation purposes could be  
3 accomplished without fear of animal food chain contamination.

4 As anticipated, rates of seed germination were similar for all  
5 treatments. Since the seeds were placed beneath the soil surface, and  
6 7.6 cm above the site of herbicide placement, no germination effects  
7 were expected.

8 Those plants directly adjacent to the walls of the stainless steel  
9 inserts, and especially those plants located in the corners of the  
10 inserts, were visibly taller than those more centrally located. We  
11 attribute this growth advantage to the fact that the outer perimeter  
12 of plants had less root competition for nutrients due to the lack of  
13 an adjacent row of plants on one of their two sides. The plants located  
14 in the corners would have such an advantage from two sides, rather than  
15 just one. A random selection of plants for height determinations  
16 minimized the effect of this bimodal distribution of plant heights.

17 The present studies were of a 2 x 2 statistical design, and were  
18 not replicated over time under exact environmental conditions. However,  
19 a number of other studies using the identical growth boxes were con-  
20 ducted to study the effects of temperature and relative humidity,  
21 alternative methods of cutting the roots, subsurface watering, etc. In  
22 all cases, the technique was reliable. Although the mathematical plant  
23 growth models will obviously change as the technique is modified  
24 (altering soil composition, type of plants, etc.) we are confident that  
25 results obtained within a given set of experimental conditions will  
26 permit quantitative comparisons between treatments.

27

1 ACKNOWLEDGEMENTS

2 The authors are grateful to Mr Joseph C.H. Smith, Department of  
3 Mathematical Sciences, United States Air Force Academy, for his  
4 assistance in analyzing data, and to Mr Jerry L. Condray, Kansas  
5 Agricultural Experiment Station, Garden City, Kansas, for furnishing  
6 the seed and soil used in this study.

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Captions for Figures

1  
2 Figure 1. Plexiglass growth boxes with stainless steel inserts.

3 Figure 2. Average height of sorghum plants following root exposure to  
4 damp or saturated vermiculite, and moist Ulysses silt loam  
5 soil. Data points are averages of ten or more plant heights.  
6 Curve fits determined by least squares linear regression.  
7 The curves are significantly different at the 0.95 confi-  
8 dence level for all treatment comparisons.

9 Figure 3. The effect of subsurface root cutting on the growth rate of  
10 non-herbicide treated sorghum plants. Data points are  
11 averages of ten or more plant heights. Curve fits deter-  
12 mined by least squares linear regression. The curves are  
13 significantly different at the 0.95 confidence level.

14 Figure 4. The effect of subsurface herbicide application on the growth  
15 rate of sorghum plants having uncut root systems. Data  
16 points are averages of ten or more plant heights. Curve fits  
17 determined by least squares linear regression. The curves  
18 are significantly different at the 0.95 confidence level.

19 Figure 5. The effect of subsurface herbicide application on the growth  
20 rate of sorghum plants having cut root systems. Data points  
21 are averages of ten or more plant heights. Curve fits deter-  
22 mined by least squares linear regression. The curves are  
23 not significantly different at the 0.95 confidence level.

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DFCBS (303/247-2720)

26 Jan 1977

Submission of Manuscript

Dr. D.E. Davis, Editor  
WEED SCIENCE  
Dept of Botany and Microbiology  
Auburn University  
Auburn, Alabama 36830

Dear Dr. Davis

Attached please find three (3) copies of the revised manuscript "A Method for Simulating Subsurface Disposal of Herbicides." The comments by the reviewers of the earlier manuscript were excellent and were incorporated in the revision. We have presented data on replication of the technique and have re-drawn Figures 3, 4, and 5 to include error bars for statistical significance. We have attached three copies of the new figures, but only one copy of Figures 1 and 2. Correspondence should be addressed to:

Capt Alvin L. Young, PhD  
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Sincerely

ALVIN L. YOUNG, Capt, USAF, PhD  
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1 Atch  
Manuscript (3)

JAMES M. CUPELLO, Capt, USAF, PhD  
Associate Professor of Biological Science  
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1  
2 A Method for Simulating Subsurface Disposal of Herbicides<sup>1</sup>

3 J.M. CUPELLO, A.L. YOUNG AND J.C.H. SMITH<sup>2</sup>

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10 acetic acid]. Plant height data were collected periodically for all  
11 treatments. Subsurface herbicide application to both intact and cut  
12 root systems significantly altered root growth. Plants with treated,  
13 intact root systems showed retarded growth which became more pronounced  
14 with time. Plants whose root systems were treated, and cut on day 22,  
15 showed an initial acceleration of growth; a trend which eventually  
16 reversed itself and resulted in control plant height exceeding that of  
17 treated plants.

18 INTRODUCTION

19 Interest in disposal methodology for selected herbicides or herbicide  
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21 been subsurface injected at massive concentration rates (2,9). The

22  
23 <sup>1</sup>Received for publication September 21, 1976. Work was supported by  
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26 <sup>2</sup>Assoc. Prof. Biol. Sci., Assoc. Prof. Biol. Sci., and Asst. Prof. Math.  
27 Sci., respectively, U.S. Air Force Academy, CO 80840.

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2 Southwest Kansas, growing grain sorghum was undercut with Noble blade  
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5 to produce grain. Plant height at harvest, however, was visibly less  
6 for the treated plots than for control plots receiving neither herbicide  
7 nor undercutting. Unfortunately, no other comparisons were made in this  
8 study.

9 The present study was initiated to develop a laboratory method that  
10 would (a) simulate field disposal studies of phenoxy herbicides using  
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12 sorghum with and without the addition of massive quantities of phenoxy  
13 herbicides.

#### 14 MATERIALS AND METHODS

15 Special growth boxes (Figure 1) were designed to permit simultaneous  
16 cutting and exposure of plant root systems to herbicides. The boxes  
17 have dimensions of 30.5 cm by 30.5 cm by 17.8 cm (inside diameter),  
18 constructed of 0.64 cm plexiglass. A 29.8 cm by 29.8 cm by 12.7 cm  
19 (outside diameter) insert constructed of 0.16 cm stainless steel was  
20 made to fit loosely inside the plexiglass boxes. The insert had three  
21 0.16 cm diameter stainless steel rods welded across its bottom as  
22 support for a 29.8 cm by 29.8 cm sheet of stainless steel screen con-  
23 taining 4 mesh/cm. The screen retained the soil inside the stainless  
24 steel insert, while permitting passage of the roots into the plexiglass  
25

---

26 <sup>3</sup>Condray, J.L. 1972. Annual report of the weeds research project.

27 Garden City Branch Exp. Stn., Kansas State Univ., Garden City, KS 67846.

1 container below. Four plexiglass legs, each 1.3 cm by 1.3 cm by 5.1 cm  
2 were placed in the four corners of the plexiglass box to support the  
3 stainless steel insert at a fixed distance from the bottom of the plexi-  
4 glass box. This design permitted easy access to the root system for  
5 cutting, provided that the space (a layer 5.1 cm deep) beneath the  
6 stainless steel screen was loosely packed with vermiculite or a similar  
7 growth medium which permitted removal of the insert from the plexiglass  
8 box without damaging the root system. Chemical treatment and, hence  
9 exposure of the cut roots, was accomplished by removing the vermiculite  
10 layer and replacing it with treated soil.

11  
12 Moisture studies. To determine the sensitivity of this new technique to  
13 variations in the amount of water contained in the vermiculite layer, a  
14 preliminary experiment was conducted. Two plexiglass containers were  
15 filled with moistened vermiculite. The first, referred to as the damp  
16 vermiculite container, contained 128% water (w/w). A second container,  
17 the saturated vermiculite container, held vermiculite containing 502%  
18 water (w/w). As noted, vermiculite will absorb five times its weight in  
19 water. A third plexiglass container was filled with 5.1 cm of moist  
20 (11% water) Ulysses silt loam soil (pH 7, 1.3% organic matter, and 33,  
21 44, and 23% sand, silt and clay, respectively) as a control, and was  
22 used to indicate whether plants grown in vermiculite had different  
23 growth rates than those grown in soil only. The stainless steel inserts  
24 for the three plexiglass boxes were filled with 10.2 cm of the Ulysses  
25 silt loam soil and placed in their respective plexiglass boxes. A card-  
26 board template with the same surface area as the stainless steel inserts  
27 was pre-punched with 100 holes in a 10 by 10 square matrix, each hole



1 separated from adjacent holes by a distance of 2.54 cm. The template  
2 was placed over the soil in the stainless steel inserts. One hundred  
3 seeds of 'Pioneer 846' grain sorghum, selected at random, were placed in  
4 the 100 holes and the seeds were pushed into the soil to a depth of 2.54  
5 cm using a wooden dowel rod. The cardboard was then removed and the  
6 soil lightly raked and packed. The growth boxes were placed in an  
7 environmental chamber for 46 days. The chamber was maintained at a 14-h  
8 photoperiod, diurnal temperature of  $35 \pm 2$  C and  $15 \pm 1$  C, and a relative  
9 humidity of 60 and 85%, day and night, respectively.

10

11 Chemical treatment study. Based on the results of the moisture study,  
12 further experiments were designed to study the effects of chemical treat-  
13 ment on cut versus uncut root systems. The experiment was of a 2 by 2  
14 statistical design involving four treatment variables: (a) cut control;  
15 (b) uncut control; (c) cut treatment; and (d) uncut treatment. Experi-  
16 mental replication during the course of this study consisted of two rep-  
17 licates of each control growth box and three replicates of each treated  
18 growth box; for a total of 10 growth boxes. Additional replication was  
19 not possible due to space limitations inside the environmental chamber.  
20 All 10 stainless steel inserts were filled with a 10.2-cm layer of the  
21 Ulysses silt loam soil. The 10 plexiglass containers were handled some-  
22 what differently depending on whether the root systems were to be cut or  
23 uncut. Initially, however, all 10 plexiglass containers were filled  
24 with 5.1 cm of damp vermiculite, their stainless steel inserts carefully  
25 positioned inside the plexiglass containers and 100 seeds planted in  
26 each of the 10 inserts. Those plants whose root systems were to remain  
27 uncut were allowed to grow for 3 days, at which time the stainless steel

1 inserts were removed from the growth boxes, the vermiculite layer re-  
2 placed with moist "treated" or "untreated" soils, and the stainless  
3 steel insert replaced. Prior work indicated that by the third day after  
4 planting, the roots were just approaching the stainless steel screen.

5 The plants which were to be cut were allowed to grow for 22 days  
6 after the initial planting, at which time the stainless steel inserts  
7 were removed, the vermiculite replaced with either treated or untreated  
8 soil, the root systems cut flush against the stainless steel screen, and  
9 the stainless steel inserts replaced. All boxes were maintained in the  
10 environmental chamber under the conditions previously described. The  
11 boxes were periodically removed for watering and plant height measure-  
12 ments. The arrangement of growth boxes inside the chamber was alternat-  
13 ed at the time of watering on a random basis in order to minimize any  
14 effects due to nonhomogeneous environmental factors within the chamber.  
15 At approximately 1 week intervals, a minimum of 10 plants per box were  
16 randomly selected, and the heights of the plants recorded.

17  
18 Chemical formulations and application. Those plexiglass containers that  
19 were to receive chemically treated soil at the appropriate point in the  
20 experiment were handled in the following manner. At the time of chemi-  
21 cal treatment for both cut (day 22) and uncut (day 3) root systems, the  
22 5.1 cm of vermiculite was removed and replaced by 4.1 cm of uncontami-  
23 nated soil. The remaining 1.0-cm space was filled with soil which had  
24 been previously mixed with sufficient herbicide formulation (20.1 ml of  
25 a 50:50 mixture of the n-butyl esters of 2,4-D and 2,4,5-T) to be equiv-  
26 alent to 2,240 kg/ha. The herbicide was mixed in a 1-cm thick soil  
27 layer to ensure a homogeneous distribution of the chemical. To

1 guarantee quantitative transfer, two 5-ml acetone rinses of the glass-  
2 ware were also added to the treated soils. The acetone was allowed to  
3 evaporate prior to the time the root systems were placed in contact with  
4 the chemically treated soils. The control soils received no herbicide,  
5 but did receive the 10 ml of acetone.

6 To ensure a uniform layering of this 1 cm of contaminated soil, a  
7 plastic grid containing a matrix of 1 cm by 1 cm square holes was first  
8 placed in the plexiglass container (over the 4.1 cm of untreated soil),  
9 the contaminated soil placed on this grid and spread to fill all of the  
10 grid squares equally, and the grid carefully removed. This soil was  
11 lightly packed, wetted with 500 ml of tap water, and the stainless steel  
12 insert replaced.

#### 13 RESULTS AND DISCUSSION

14 Moisture study. The data points in Figure 2 represent average sorghum  
15 plant height as a function of time after initial seed planting (time  
16 zero). Two of the curves represent growth rate variations due to  
17 differences in the moisture content of the vermiculite layer placed  
18 beneath the 10.2 cm of seed bearing soil. The data obtained from the  
19 box containing soil rather than vermiculite were considered control  
20 data and were used as the baseline against which the damp and saturated  
21 vermiculite data were compared.

22 Because the data were time variant, they were analyzed by fitting a  
23 curve to the data points using the method of least squares linear re-  
24 gression. A number of equations relating plant height and time were  
25 tested to determine which mathematical form of a growth model best fit  
26 the experimental data. The best fit was defined as the equation which  
27 resulted in the smallest mean squared error (MSE) between empirical

1 data and that predicted by the growth model.

2 For the control data in Figure 2 (soil rather than vermiculite), an  
3 equation of the form

$$4 \quad Y = \alpha + \beta_1 x + \beta_2 x^2 + \beta_3 x^3 \quad [\text{Equation No. 1}]$$

5 gave the smallest MSE, and was selected as the appropriate growth model  
6 against which to compare the data resulting from root growth in vermicu-  
7 lite. In the above equation, and those equations which will be intro-  
8 duced later,  $Y$  represents the plant height above the soil surface in cm,  
9 at various times,  $X$ , after planting of the seeds. Various powers of the  
10  $X$  term are included in the equation in order to give a reasonable fit.

11 The constant coefficients such as  $\alpha$ ,  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  are statistically  
12 selected to provide the minimum MSE between the empirical data and the  
13 growth model being fitted to the empirical data. Additional statistical  
14 testing of Equation No. 1 indicated that no additional terms needed to  
15 be included. Utilizing a matrix inversion technique, the coefficients  
16 for Equation No. 1 were determined as shown below:

$$17 \quad Y = -11.1 + 2.71X - 0.0642X^2 + 0.000615X^3 \quad [\text{Equation No. 2}]$$

18 The experimental data obtained from the damp and saturated vermicu-  
19 lite treatments (Figure 2) were also fitted to the general form of  
20 Equation No. 1 and their coefficients determined, so as to provide the  
21 minimal MSE.

22 Linear regression analysis at the 95% confidence level (used for  
23 all statistical tests in this study) indicated that both the damp and  
24 saturated vermiculite growth models were different from the soil growth  
25 model. Likewise, the damp vermiculite growth model was shown to be  
26 statistically different from the saturated vermiculite growth model.

27 It could be argued that the damp and saturated vermiculite data

1 should be fitted to a growth model different from the general form of  
2 Equation No. 1. Even if this were true it would not alter the conclu-  
3 sions; it would only reduce the MSE of the curve fits.

4 It is concluded from these analyses that the laboratory method  
5 described is sensitive to the replacement of soil by vermiculite, and  
6 the water content of the vermiculite layer. Thus, care must be taken to  
7 ensure that all growth boxes used in a given experiment or set of subse-  
8 quent experiments are alike with respect to the quantity of water in the  
9 vermiculite layer; at the beginning, and for the duration, of the  
10 experiment(s).

11  
12 Chemical treatment study. The effect of cutting of root systems was  
13 studied in the presence and absence of herbicide. The experimental  
14 design required those treatments that were to have "uncut" root systems  
15 to have their vermiculite layer replaced by soil approximately 3 weeks  
16 prior to the time when the "cut" treatments had their vermiculite layers  
17 replaced. If the uncut treatments were not so modified on day 3, the  
18 roots would already have penetrated into the vermiculite layers, and  
19 would be crushed when the vermiculite layer was replaced by soil, and  
20 the stainless steel insert placed on top of this soil layer. The  
21 previous results with vermiculite suggested that a comparison of "cut"  
22 and "uncut" treatment data might be invalid. The fact that the environ-  
23 mental growth conditions are different for a period of up to 3 weeks  
24 could, in itself, cause significant differences in plant growth rates.  
25 Thus we are unable to ascribe any observed growth rate differences to  
26 chemical treatment or cutting, alone.

27 Figure 3 illustrates the data and best curve fits for the cut and

1 uncut control treatments (no herbicide). Both curve-fits in Figure 3  
2 were independently determined using the procedures described in the  
3 moisture studies.

4 The growth models which provided the best fit to the data for the  
5 uncut control and cut control treatments were, respectively:

6 
$$Y = -50.01 - 1.983X + 0.01541X^2 + 34.58 \ln X \quad [\text{Equation No. 3}]$$

7 
$$Y = -37.90 - 1.655X + 0.01310X^2 + 27.96 \ln X \quad [\text{Equation No. 4}]$$

8 Statistical analysis confirmed that the growth models for cut and  
9 uncut controls were significantly different. However, whether this  
10 observed difference was due to the physical act of root cutting, growth  
11 rate alterations induced by handling the uncut and cut treatments  
12 differently (changing vermiculite on day 3 vs day 22, respectively),  
13 other experimental variables, or a combination of all of these could not  
14 be determined. Therefore, further discussion is limited to comparisons  
15 within the categories of cut and uncut treatments.

16 Figure 4 shows the comparison of uncut control and the uncut  
17 treated growth models. The general form of Equation No. 3 was used to  
18 determine the coefficients for the growth model. Analysis showed that  
19 subsurface herbicide treatment of intact root systems significantly  
20 reduced the rate of plant growth.

21 Figure 5 illustrates the comparison between cut control and the  
22 cut treated growth models. The same procedures were used to make sta-  
23 tistical inferences as were used to analyze the data in Figure 4; the  
24 only difference being that the general form of the growth model being  
25 tested was of the form shown in Equation No. 4. The results of these  
26 analyses indicated a lesser growth rate effect between control and  
27 treated plots than that shown in Figure 4, but the difference is still

1 statistically significant for those plots which had their root systems  
2 severed; i.e., cut root systems that are chemically treated with this  
3 herbicide grow at a different rate than cut controls. The initial  
4 growth rate of cut, treated plants is greater than cut controls, with a  
5 reversal in this trend occurring somewhere around day 50 of the experi-  
6 ment. There is no apparent explanation for this growth rate reversal  
7 with the data presently available.

8  
9 Technique evaluation. The development of this laboratory method for  
10 simulating massive subsurface field disposal of herbicides was prompted  
11 by our need for a subsurface application method that mimicked actual  
12 field methodology; namely, cutting of root systems during application.  
13 The addition of this refinement over existing methods is the principal  
14 justification for preferential use of this technique. Frequent employ-  
15 ment of subsurface herbicide placement techniques in the field, to en-  
16 hance chemical persistence and to place the agent in the zone of plant  
17 uptake, seems to warrant continued improvement of laboratory simulation  
18 methods. Our work has shown that subsurface application of massive  
19 amounts of herbicide does affect the growth rate of both cut and uncut  
20 root systems. The quantitative effect does differ between cut and uncut  
21 root systems, however,

22 Our use of the technique for massive quantities of phenoxy herbi-  
23 cide would not preclude its use at rates commonly found in commercial  
24 applications.

25 During the course of these experiments a number of interesting  
26 physiological phenomena were observed. The plexiglass growth boxes  
27 treated with subsurface herbicide showed little, if any, root

1 penetration into or beyond the chemically treated soil layer. Boxes  
2 with untreated soil showed significant root penetration throughout the  
3 soil. If similar results can be verified in field studies, one could  
4 argue that the presence of this chemical barrier to root penetration  
5 would limit or prevent plant uptake of herbicide. Were this so, massive  
6 subsurface placement of herbicides for biodegradation purposes could be  
7 accomplished without fear of animal food chain contamination. A reduc-  
8 tion in plant yields due to a shallow root system might also be expected  
9 if the herbicide injection points were not deep enough.

10 As anticipated, rates of seed germination were similar for all  
11 treatments. Since the seeds were placed beneath the soil surface, and  
12 7.6 cm above the site of herbicide placement, no germination effects  
13 were expected.

14 Those plants directly adjacent to the walls of the stainless steel  
15 inserts, and especially those plants located in the corners of the  
16 inserts, were visibly taller than those more centrally located. We  
17 attributed this growth advantage to the fact that the outer perimeter of  
18 plants had less root competition for nutrients due to the lack of an  
19 adjacent row of plants on one of their two sides. The plants located  
20 in the corners would have such an advantage from two sides, rather than  
21 just one. A random selection of plants for height determinations  
22 minimized the effect of this bimodal distribution of plant heights.

23 The present studies were of a 2 by 2 statistical design. A number  
24 of other studies using the identical growth boxes were conducted to  
25 study the effects of temperature and relative humidity, alternative  
26 methods of cutting the roots, subsurface watering, etc. In all cases,  
27 the technique was reliable. Although the mathematical plant growth



1 models will obviously change as the technique is modified (altering  
2 soil composition, type of plants, etc.) we are confident that results  
3 obtained within a given set of experimental conditions will permit  
4 quantitative comparisons between treatments.

5 ACKNOWLEDGMENTS

6 The authors are grateful to Mr Jerry L. Condray, Kansas Agric.  
7 Exp. Stn., Garden City, Kansas, for furnishing the seed and soil used  
8 in this study.

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Captions for Figures

1  
2 Figure 1. Plexiglass growth boxes with stainless steel inserts.

3 Figure 2. Average height of sorghum plants following root exposure to  
4 damp or saturated vermiculite, and moist Ulysses silt loam soil. Data  
5 points are averages of ten or more plant heights. Curves are signifi-  
6 cantly different at the 5% level for all treatment comparisons.

7 Figure 3. The effect of subsurface root cutting on the growth rate of  
8 sorghum. Data points represent the mean of at least 20 plant heights.  
9 Curve fits determined by least squares linear regression. Error bars  
10 represent the 95% confidence limits of the mean plant height. The  
11 curves are significantly different at the 5% level.

12 Figure 4. The effect of subsurface herbicide application on the growth  
13 rate of sorghum plants having uncut root systems. Control and treated  
14 data points represent the mean of at least 20 and 30 plant heights,  
15 respectively. Curve fits determined by least squares linear regression.  
16 Error bars represent the 95% confidence limits of the mean plant height.  
17 The curves are significantly different at the 5% level.

18 Figure 5. The effect of subsurface herbicide application on the growth  
19 rate of sorghum plants having cut root systems. Control and treated  
20 data points represent the mean of at least 20 and 30 plant heights,  
21 respectively. Curve fits determined by least squares linear regression.  
22 Error bars represent the 95% confidence limits of the mean plant height.  
23 The curves are significantly different at the 5% level.

24  
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26  
27

TCDD ANALYSIS, LIQUID ORANGE SAMPLES

Analysis Performed by ARL/LJ, WPAFB, Ohio

Samples submitted: 1 February 1975

Data Received: 11 March 1975

<u>Sample Source</u>	<u>Sample Number</u>	<u>Date Sampled</u>	<u>TCDD PPM</u>	
*Johnston Island	1	1 Aug 74	< 0.25	(a)
" "	2	"	1.3	(a)
" "	3	"	1.3	(a)
" "	4	"	< 0.07	
" "	5	"	< 0.07	
" "	6	"	0.07	
" "	7	"	4.6	
" "	8	"	4.6	
" "	9	"	5.3	
" "	10	"	0.28	
**Eglin AFB	1	1 Jan 70	< 0.04	
***Eglin AFB	2	"	< 0.04	

(a) TCDD peak appeared on top of large interference peak.

\* Samples collected from drums that were to be re-barrelled.

\*\* Sample routinely used at USAFA for laboratory experiments.

\*\*\* Samples used in Biodegradation Plots, Eglin AFB, Florida, April, 1972.

1 2 3 4 5 6 7 8 9 10

TREATMENT	ORANGE + C-14-TCDD		ORANGE + C-14-TCDD		ORANGE + C-12-TCDD		TCDD		CONTROL		OVERALL AVERAGE
	CUT	UNCUT	CUT	UNCUT	CUT	UNCUT	CUT	UNCUT	CUT	UNCUT	
8	7.3 (0.4)	6.9 (0.2)	7.1 (0.3)	6.2 (0.4)	7.2 (0.2)	7.2 (0.2)	7.3 (0.2)	7.2 (0.3)	7.2 (0.1)	7.1 (0.2)	7.1 ± (0.3)
11	14.0 (0.3)	11.9 (0.4)	12.6 (0.8)	12.4 (0.6)	11.1 (0.7)	12.3 (0.7)	13.8 (0.4)	13.1 (0.6)	13.3 (0.3)	12.8 (0.3)	12.7 ± (0.9)
15	17.4 (0.5)	17.0 (0.7)	15.1 (0.6)	16.8 (0.3)	16.2 (0.7)	16.9 (0.5)	17.5 (0.4)	17.7 (0.6)	15.0 (0.2)	16.7 (0.6)	16.8 ± (0.8)
21	18.2 (1.3)	16.2 (1.2)	20.7 (0.6)	17.4 (0.6)	18.6 (1.0)	18.3 (0.8)	17.0 (0.9)	20.8 (1.1)	16.8 (0.9)	20.2 (1.2)	18.4 ± (1.7)
25	20.3 (0.5)	18.9 (1.1)	19.1 (0.7)	20.2 (0.6)	17.8 (0.8)	18.7 (0.6)	21.0 (0.8)	21.5 (1.0)	16.8 (0.7)	21.1 (0.8)	19.5 ± (1.5)
32	21.6 (1.1)	20.8 (0.5)	19.9 (0.8)	18.8 (0.7)	20.5 (0.9)	18.9 (1.1)	21.9 (1.2)	21.4 (0.9)	18.5 (0.5)	23.7 (0.7)	20.6 ± (1.6)
50	23.4 (1.2)	22.7 (1.0)	19.1 (1.0)	19.9 (1.0)	20.4 (1.0)	20.5 (1.1)	22.3 (1.1)	24.9 (1.6)	20.0 (1.0)	23.5 (1.6)	21.7 ± (1.9)
57	23.3 (1.1)	20.9 (1.4)	21.8 (1.2)	23.9 (0.7)	19.6 (1.3)	21.9 (1.0)	25.9 (0.9)	26.0 (1.2)	21.6 (0.9)	28.9 (0.7)	23.4 ± (2.8)
64	22.9 (1.5)	23.7 (1.3)	24.4 (1.6)	25.6 (2.0)	21.8 (1.8)	22.5 (1.2)	29.2 (1.2)	29.7 (1.1)	23.2 (1.2)	30.2 (0.9)	25.4 ± (3.1)

1 vs 2 \*  
 3 vs 4 NS  
 5 vs 6 NS  
 7 vs 8 \*  
 9 vs 10 \*  
 1 vs 3 NS  
 2 vs 4 NS  
 7 vs 9 \*  
 5 vs 9 NS  
 3 vs 9 NS  
 1 vs 9 \*

# Soil Sample Information

**SOIL TESTING LABORATORY**  
Agronomy Department  
Kansas State University  
Manhattan, Kansas 66502

and

**SOIL TESTING LABORATORY**  
Garden City Branch Expt. Sta.  
Kansas State University  
Garden City, Kansas 67846

Name: \_\_\_\_\_  
Address: \_\_\_\_\_  
(Street or Route)  
\_\_\_\_\_  
(City and State) Zip Code  
County: \_\_\_\_\_  
Legal Description: \_\_\_\_\_  
Date: \_\_\_\_\_ 19 \_\_\_\_\_

Sample(s) Submitted by: (If different than name at left)

Name: \_\_\_\_\_  
Address: \_\_\_\_\_  
(Street or Route)  
\_\_\_\_\_  
(City and State) Zip Code

### For Laboratory Use Only

Fee Payment: \_\_\_\_\_  
Date Received: \_\_\_\_\_ 19 \_\_\_\_\_  
Date Results Reported: \_\_\_\_\_ 19 \_\_\_\_\_

Fee Payment: Enclosed \$ \_\_\_\_\_ Account No. \_\_\_\_\_

SAMPLE IDENTIFICATION					SOIL DESCRIPTION			IRRIGATION		PREVIOUS TREATMENTS						TEST DESIRED (✓) Refer to Fee Schedule				
Laboratory Number (Do not write below)	Field Number or Letter	Sample Number or Letter	Depth i.e. 0-6" 0-24" 6-24"	Acres Represented by Sample	Slope L-level G-gentle S-steep I-irregular	Drainage P-poor G-good E-excessive	Soil Type  i.e. Geary Silt Loam	Type		Last Year				Lime		General Fertility	Available Nitrogen	Available Zinc	Organic Matter	Lawn or Garden
								Sprinkler	None Moderate Severe	Nitrogen (N) lbs./A.	Phosphate (P <sub>2</sub> O <sub>5</sub> ) lbs./A.	Potash (K <sub>2</sub> O) lbs./A.	Zinc (Zn) lbs./A.	Manure or Other Nutrients T./A. or lbs./A.	Rate Last Three Years E.C.C. lbs./A.					
<i>GCK proposal</i>																				
<i>test site</i>																				

MAP OF SAMPLE LOCATION ON FARM

		CROP HISTORY				DESIRED RECOMMENDATION			REMARKS
Field Number or Letter	Sample Number or Letter	Last Year		Two Years Ago		Crop to be Fertilized	Yield Goal bu./A. or T./A.	Crop Next Year	Note any special conditions of the soil or crops.
		Crop	Yield bu./A. or T./A.	Crop	Yield bu./A. or T./A.				

DO NOT DETACH

RETURN ALL 4 PARTS. THE ORIGINAL WILL BE RETURNED TO YOU.

RESEARCH SUMMARY  
PLANT UPTAKE STUDY

"Translocation of Subsurface Applied C-14-TCDD By  
Cut and Uncut Roots of Sorghum vulgare L."

Methodology

Five different treatments or combinations of Herbicide Orange and TCDD were applied below the soil surface of specially designed, individual growth boxes containing 100 plants per box. The attached Table indicates the five treatments (the first two being replicates), and the Orange and TCDD concentrations used. This study simulates the field work done by Captain Young, et al in Kansas in 1972, in which 2,000 lbs/ acres of Herbicide Orange were injected subsurface with a Noble blade; the Orange containing 14 ppm TCDD.

The growth boxes were designed to include a 12" x 12" x 7" (I.D.) plexiglass outer container, and a 12" x 12" x 5" (O.D.) stainless steel insert with a stainless steel mesh screen in the bottom.

Each of the ten stainless steel inserts were filled with 4" of Kansas soil, and 100 seeds planted (Sorghum vulgare L., var. Pioneer) at a depth of 1" beneath the soil surface in a 10 x 10 matrix arrangement. Each plexiglass outer container was filled with 2" of Vermiculite to permit access to the root systems as they grew through the soil, crossed the mesh in the stainless steel insert, and grew to a depth in excess of 4".



using a random number table to select the plants to be measured. A preliminary study indicated that measurement of more than ten plants did not significantly decrease the variance in plant height measurements.

The experiment was terminated on day 64 and all plants cut and finely ground in preparation for TCDD extraction and analysis.

Table II indicates the observed average plant heights as a function of time and treatment. Although the statistical analysis of the data is not completed, statistically significant differences have been detected at the 95% confidence level between treatments on all days except day 8. Analysis of the treatment differences on a given day indicate that significant differences between treatments cannot be readily explained as a result of the cut versus uncut treatment, but rather on the Orange and TCDD contamination levels.

A TDY is currently planned (16-20 February) to the Dow Chemical Laboratories, Midland, Michigan, in order to analyze the plant tissue for TCDD content. It is anticipated that a manuscript will be prepared before March, 1976.

Investigators

Captain James Cupello, Ph.D.

Captain Alvin L. Young, Ph.D.

the soil lightly packed.

Environmental Conditions. All four growth boxes were placed inside a Sherer Model CEL 37-14 growth chamber throughout the course of the experiment. The chamber was programmed to provide a minimum temperature of  $15 \pm 1^{\circ}\text{C}$  from approximately 1800 to 0800 hours, and a maximum temperature of  $35 \pm 2^{\circ}\text{C}$  for the remainder of the diurnal cycle. Over these same time periods, the relative humidity inside the chamber as determined by a \_\_\_\_\_ hygrometer was  $85 \pm 5\%$  relative humidity (RH) (1800 to 0800 hours) and  $60 \pm 5\%$  RH, respectively.

In addition, fluorescent and incandescent light fixtures inside the chamber were programmed to provide a realistic exposure to sunlight throughout the day-time hours of the experiment.

On or about day 20 after planting, it was observed that the Sorghum leaves were turning brown and wilting at the tips of the longest shoots; red or reddish-brown spots were becoming evident on the leaf surfaces. Whether this was some type of plant disease and/or a reaction to excessive temperatures or humidities in the chamber was not known. In an attempt to prevent the plants from dying, the environmental conditions in the chamber were altered. The temperature inside the chamber was reduced to provide a minimum/maximum temperature regime of  $15^{\circ}\text{C} \pm 1^{\circ}\text{C}/29^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , while at the same time removing six of the 12, 50 W incandescent bulbs from the chamber. To combat any possible nutritional deficiency that might be occurring, 1000 cc of nutrient solution was added to the soil surface of all ten growth boxes on day 28. By day 31, new growth was observed on those plants previously showing browning and dessication. Steady improvement in the health of the plants continued throughout the study with no recurrence of the aforementioned symptoms.

UNITED STATES DEPARTMENT OF AGRICULTURE  
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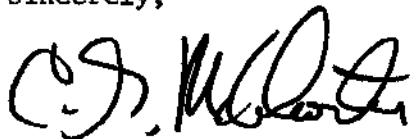
February 3, 1977

Dr. A. L. Young  
Department of Chemistry and Biological Sciences  
U.S. Air Force Academy, Colorado 80840

Dear Dr. Young:

Manuscript 76-130 "A Method for Simulating Surface Injection of Herbicides" was mailed to you on October 24, 1976, with a return due date of January 30, 1977. I hope that it will be possible for you to return this manuscript in revised form within the next few days. If the manuscript cannot be returned in the near future, it will be necessary to resubmit it to the Editor and have the manuscript reviewed again for further consideration. This letter is simply to remind you of this deadline, and to urge you to return the manuscript if at all possible. Please let me know if you do not intend to resubmit.

Sincerely,



C. G. McWhorter  
Associate Editor  
WEED SCIENCE

cc:

D. E. Davis

Young et al have conducted studies on soil  
biodegradation both have shown that.

POSSIBLE

# TCDD UPTAKE IN Sorghum bicolor

INTRODUCTION - Disposal  
Accidents

LITERATURE REVIEW -

KANSAS - Unpublished  
EQUIN - NO UPTAKE IN SEED  
JENSEN / JONES

- ① IMPACT
- ② LITERATURE

## METHODS & MATERIALS

a. TECHNIQUE IN PIPES

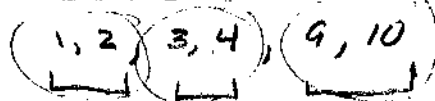
b. ANALYSIS - AT DAY 64

PARAMETERS  
EFFECTS OF  
TCDD ON  
PLANT AND  
GERMINATION

PLANT HEIGHT  
PLANT WEIGHT  
FRESH  
DRY  
ROOT WEIGHT

NUMBER ALIVE / DEAD  
PERCENT GERMINATION

BOXES



1, 3, 5, 7, 9



PLANT  
UPTAKE

germi  
cut

CUT + HPLC/MS --- SYN  
CUT + NO HPLC/MS

UNCONTROLLED = ANALYSIS NO HPLC

## A Method for Simulating Subsurface Disposal of Herbicides<sup>1</sup>

J.M. CUPELLO, A.L. YOUNG and J.C.H. SMITH<sup>2</sup>

**Abstract.** Specially designed growth boxes were used to simulate field subsurface injection of phenoxy herbicides. Sorghum (*Sorghum vulgare* Pers.) seedlings were grown in stainless steel containers (inserts) which were placed in plexiglass boxes containing a soil layer that had received 2,240 kg/ha of a 50:50 mixture of the *n*-butyl esters of 2,4-D [(2,4-dichlorophenoxy)-acetic acid] and 2,4,5-T [(2,4,5-trichlorophenoxy)-acetic acid]. Plant height data were collected periodically for all treatments. Subsurface herbicide application to both intact and cut root systems significantly altered root growth. Plants with treated, intact root systems showed retarded growth which became more pronounced with time. Plants whose root systems were treated, and cut on day 22, showed an initial acceleration of growth; a trend which eventually reversed itself and resulted in control plant height exceeding that of treated plants.

### INTRODUCTION

Interest in disposal methodology for selected herbicides or herbicide manufacturing wastes has prompted field studies where herbicides have been subsurface injected at massive concentration rates (2, 9). The premise for such studies is that high concentrations of herbicides or their manufacturing wastes will be degraded to innocuous products by the combined action of soil microorganisms and soil hydrolysis.

Numerous field methods and incorporation equipment have been described for the subsurface placement of herbicides (4, 5, 14). Laboratory simulation of these field techniques has been confined to "normal" rates for herbicide application and to studies of root versus shoot uptake. A double pot technique first described by Eshel and Prendeville (7) has been most frequently used (3, 12); however, the layering of treated soil in pots or cans has also been popular (8, 10). A few other techniques have also been described. Parker (13), for example, used a double-dish technique using petri dishes to separately expose roots and shoots to sand or soil containing herbicides, while Appleby and Furtick (1) developed a plastic envelope device for allowing separate exposure of seeds, roots, and coleoptiles of emerging grass seedlings to soil-incorporated herbicides. Techniques to observe the growth of roots and the effects of root-active chemicals have been described by Muzik and Whitworth (11) and Duffy (6). The latter study involved chemical treatment of isolated portions of root systems without disturbance or injury to the untreated root mass.

All of the above techniques have been limited to the study of intact (uncut) root or shoot systems. In a field situation where an agricultural subsoiler would be used, many roots and stems would be severed by the shank or blade. Goulding (9) undercut a 4.05 ha plot of sparse to moderately dense greasewood [*Sarcobatus vermiculatus* (Hook.) Torr.] and injected a total of 62,457 L of liquid waste from the manufacture of 2,4-D. Slow recovery of the shrubs was observed, principally between the injection points. In a unique experiment in Southwest Kansas, growing grain sorghum was undercut with Noble blade equipment and simultaneously treated with 2,240 kg/ha of a 50:50 mixture of 2,4-D and 2,4,5-T<sup>3</sup>. The sorghum plants on the treated plots survived to produce grain. Plant height at harvest, however, was visibly less for the treated plots than for control plots receiving neither herbicide nor undercutting. Unfortunately, no other comparisons were made in this study.

The present study was initiated to develop a laboratory method that would (a) simulate field disposal studies of phenoxy herbicides using subsurface injection and (b) quantify the effects of undercutting sorghum with and without the addition of massive quantities of phenoxy herbicides.

### MATERIALS and METHODS

Special growth boxes (Figure 1) were designed to permit simultaneous cutting and exposure of plant root systems to herbicides. The boxes have dimensions of 30.5 cm by 30.5 cm by 17.8 cm (inside diameter), constructed of 0.64 cm plexiglass. A 29.8 cm by 29.8 cm by 12.7 cm (outside diameter) insert constructed of 0.16 cm stainless steel was made to fit loosely inside the plexiglass boxes. The insert had three 0.16 cm diameter stainless steel rods welded across its bottom as support for a 29.8 cm by 29.8 cm sheet of stainless steel screen containing 4 mesh/cm. The screen retained the soil inside the stainless steel insert, while permitting passage of the roots into the plexiglass container below. Four plexiglass legs, each 1.3 cm by 1.3 cm by 5.1 cm were placed in the four corners of the plexiglass box to support the stainless steel insert at a fixed distance from the bottom of the plexiglass box. This design permitted easy access to the root system for cutting, provided that the space (a layer 5.1 cm deep) beneath the stainless screen was loosely packed with vermiculite or a similar growth medium which permitted removal of the insert from the plexiglass box without damaging the root system. Chemical treatment and, hence exposure of the cut roots, was

<sup>1</sup> Received for publication September 21, 1976. Work was supported by Frank J. Seiler Res. Lab. (Air Force Systems Command) and Air Force Logistics Command.

<sup>2</sup> Assoc. Prof. Biol. Sci., Assoc. Prof. Biol. Sci., and Asst. Prof. Math. Sci., respectively, U.S. Air Force Academy, CO 80840.

<sup>3</sup> Condray, J.L. 1972. Annual report of the weeds research project. Garden City Branch Exp. Stn., Kansas State Univ., Garden City, KS 67846.

Figure 3 illustrates the data and best curve fits for the cut and uncut control treatments (no herbicide). Both curve-fits in Figure 3 were independently determined using the procedures described in the moisture studies.

The growth models which provided the best fit to the data for the uncut control and cut control treatments were, respectively:

$$Y = -50.01 - 1.983X + 0.01541X^2 + 34.58 \ln X$$

[Equation No. 3]

$$Y = -37.90 - 1.655X + 0.01310X^2 + 27.96 \ln X$$

[Equation No. 4]

Statistical analysis confirmed that the growth models for cut and uncut controls were significantly different. However, whether this observed difference was due to the physical act of root cutting, growth rate alterations induced by handling the uncut and cut treatments differently (changing vermiculite on day 3 vs day 22, respectively), other experimental variables, or a combination of all of these could not be determined. Therefore, further discussion is limited to comparisons within the categories of cut and uncut treatments.

Figure 4 shows the comparison of uncut control and the uncut treated growth models. The general form of Equation No. 3 was used to determine the coefficients for the growth model. Analysis showed that subsurface herbicide treatment of

intact root systems significantly reduced the rate of plant growth.

Figure 5 illustrates the comparison between cut control and the cut treated growth models. The same procedures were used to make statistical inferences as were used to analyze the data in Figure 4; the only difference being that the general form of the growth model being tested was of the form shown in Equation No. 4. The results of these analyses indicated a lesser growth rate effect between control and treated plots than that shown in Figure 4, but the difference is still statistically significant for those plots which had their root system severed; i.e., cut root systems that are chemically treated with this herbicide grow at a different rate than cut controls. The initial growth rate of cut, treated plants is greater than cut controls, with a reversal in this trend occurring somewhere around day 50 of the experiment. There is no apparent explanation for this growth rate reversal with the data presently available.

*Technique evaluation.* The development of this laboratory method for simulating massive subsurface field disposal of herbicides was prompted by our need for a subsurface application method that mimicked actual field methodology; namely, cutting of root systems during application. The addition of this refinement over existing methods is the principal justification for preferential use of this technique. Frequent employment of subsurface herbicide placement techniques in the field, to enhance chemical persistence and to place the agent in the zone of plant uptake, seems to warrant continued improvement of laboratory simulation methods. Our work has shown that subsurface application of massive amounts of herbicide does affect the growth rate of both cut and uncut root sys-

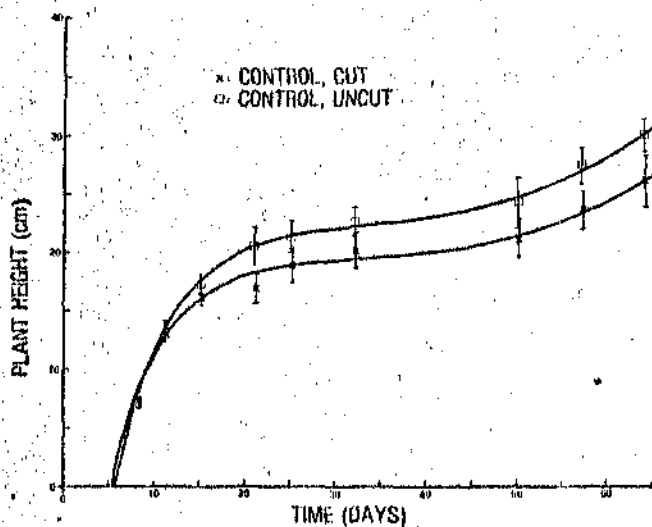


Figure 3. The effect of subsurface root cutting on the growth rate of sorghum. Data points represent the mean of at least 20 plant heights. Curve fits determined by least squares linear regression. Error bars represent the 95% confidence limits of the mean plant height. The curves are significantly different at the 5% level.

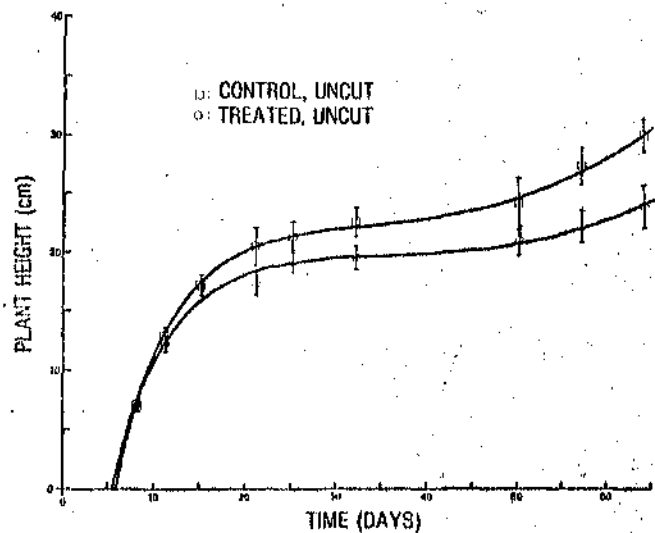


Figure 4. The effect of subsurface herbicide application on the growth rate of sorghum plants having uncut root systems. Control and treated data points represent the mean of at least 20 and 30 plant heights, respectively. Curve fits determined by least squares linear regression. Error bars represent the 95% confidence limits of the mean plant height. The curves are significantly different at the 5% level.

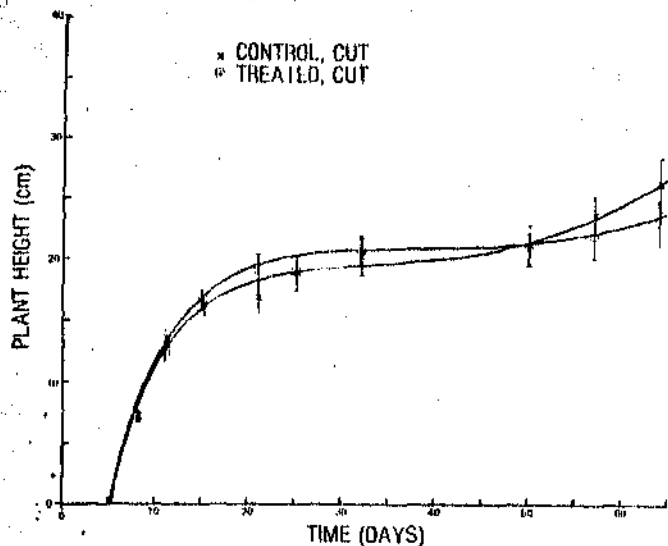


Figure 5. The effect of subsurface herbicide application on the growth rate of sorghum plants having cut root systems. Control and treated data points represent the mean of at least 20 and 30 plant heights, respectively. Curve fits determined by least squares linear regression. Error bars represent the 95% confidence limits of the mean plant height. The curves are significantly different at the 5% level.

tems. The quantitative effect does differ between cut and uncut root systems, however.

Our use of the technique for massive quantities of phenoxy herbicide would not preclude its use at rates commonly found in commercial applications.

During the course of these experiments a number of interesting physiological phenomena were observed. The plexiglass growth boxes treated with subsurface herbicide showed little, if any, root penetration into or beyond the chemically treated soil layer. Boxes with untreated soil showed significant root penetration throughout the soil. If similar results can be verified in field studies, one could argue that the presence of this chemical barrier to root penetration would limit or prevent plant uptake of herbicide. Were this so, massive subsurface placement of herbicides for biodegradation purposes could be accomplished without fear of animal food chain contamination. A reduction in plant yields due to a shallow root system might also be expected if the herbicide injection points were not deep enough.

As anticipated, rates of seed germination were similar for all treatments. Since the seeds were placed beneath the soil surface, and 7.6 cm above the site of herbicide placement, no germination effects were expected.

Those plants directly adjacent to the walls of the stainless steel inserts, and especially those plants located in the corners of the inserts, were visibly taller than those more centrally

located. We attributed this growth advantage to the fact that the outer perimeter of plants had less root competition for nutrients due to the lack of an adjacent row of plants on one of their two sides. The plants located in the corners would have such an advantage from two sides, rather than just one. A random selection of plants for height determinations minimized the effect of this bimodal distribution of plant heights.

The present studies were of a 2 by 2 statistical design. A number of other studies using the identical growth boxes were conducted to study the effects of temperature and relative humidity, alternative methods of cutting the roots, subsurface watering, etc. In all cases, the technique was reliable. Although the mathematical plant growth models will obviously change as the technique is modified (altering soil composition, type of plants, etc.) we are confident that results obtained within a given set of experimental conditions will permit quantitative comparisons between treatments.

ACKNOWLEDGMENTS

The authors are grateful to Mr. Jerry L. Condray, Kansas Agric. Exp. Sta., Garden City, Kansas, for furnishing the seed and soil used in this study.

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CUPELLO ET AL : SUBSURFACE DISPOSAL OF HERBICIDES  
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Voorhees, W.B. 1976. Root elongation along a soil-plastic container interface. *Annals* 68(1): 143.

Root ~~elongation~~ <sup>growth</sup> is often studied under carefully controlled laboratory conditions with seedlings grown in transparent plastic or glass containers in the desired soil environment.

Voorhees ~~measured~~ <sup>( )</sup> measured root elongation rate through center of soil cores and along plexiglas-soil interfaces. ~~These~~ <sup>Containers</sup> ~~with~~ <sup>roots</sup> placed at the ~~plexiglas~~ plexiglas-soil interface were inclined  $25^\circ$  from vertical and subdivided so that part received light and part wrapped with opaque black paper.

As expected, elongation of primary roots along the interface in the presence of light was lower than for roots shielded from the light. Both sets of primary root elongation rates along the interface were statistically lower than through the center. He suggested these differences were probably due either to higher soil strength at the interface or to an attracting electrical charge on the container surface, or both.

## ROOT ELONGATION ALONG A SOIL-PLASTIC CONTAINER INTERFACE<sup>1</sup>

W. B. Voorhees<sup>2</sup>

### ABSTRACT

Root growth is often studied in glass-fronted containers which allow continuous observation of several growth parameters. These observations are generally assumed to extrapolate to root growth under field conditions. However, laboratory studies reported here show that root elongation rates along a soil-container interface in soil cores are significantly lower than those measured within the bulk soil mass. These differences are probably due either to higher soil strength at the interface or to an attracting electrical charge on the container surface, or both.

*Additional index words:* Growth roots, Soil density, *Pisum sativa* L.

ROOT elongation rate is often studied under carefully controlled laboratory conditions with seedlings grown in transparent plastic or glass containers in the desired soil environment. The containers are frequently inclined from the vertical to force roots to grow against the interface to permit continuous observation and measurement. However, these growth characteristics are assumed to be the same as those within the bulk soil mass. Observations reported here show the error of this assumption.

The soil used in these experiments was  $\leq 2$  mm diam. size fraction of a Nutley clay (*Udertic Haploloroll*) from the surface 25 cm. Three sets of five soil cores were prepared by compressing a known weight of slightly moist soil into Plexiglas<sup>3</sup> cylinders (7.6 cm diam. by 7.6 cm long) to obtain the desired bulk density. The cores were saturated with water and equilibrated on ceramic plates to a matrix suction of 1 bar. Germinated pea seedlings (*Pisum sativa* L. 'Alaska') with 1-cm-long primary roots were placed on top of the cores (one/core) in root channels, made by inserting a 1-mm diam. dissecting needle 1-cm deep into the soil core and anchored in place. Cores were then placed in an enclosed chamber within a constant temperature (22 C) room. The atmosphere was kept near saturation by a free water surface at the chamber bottom. Seedlings were centered in one set of cores and allowed to grow vertically through the core center for up to 150 hours. In another core set, the seedlings were placed near the core edge so that roots grew along the soil-container interface. To insure root growth along this interface, the cores were inclined 25° from vertical. These cores were wrapped with opaque black paper which was removed for root elongation measurements. A second set of inclined cores were treated as above but without the wrap and were exposed to light 24 hours a day.

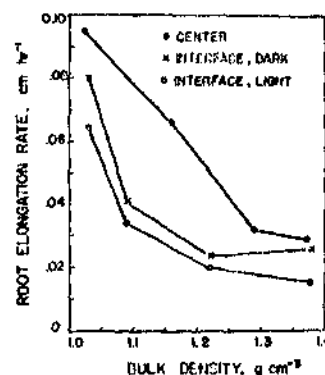


Fig. 1. Root elongation rate through center of soil core and along plexiglas-soil interface.

Figure 1 is a graph of measured primary root elongation rates for all three core sets over a range of bulk densities. Each plotted point has a typical coefficient of variation of about 6%. As expected, elongation of primary roots along the interface in the presence of light was lower than for roots shielded from the light since light inhibits root elongation (Street, 1968). Both sets of primary root elongation rates along the interface were statistically lower than those through the center of the soil core.

There are several possible explanations for this observation. The soil bulk density at the container wall-soil interface is likely higher than for the total soil core since soil doesn't behave exactly like a fluid when initially compressed into the cylinders. Thus, the soil at the interface would have a higher physical resistance to root elongation. The indirect effect of soil physical resistance to root elongation is well documented (Taylor, 1971). Another possible explanation is the presence of an electrical charge on the Plexiglas surface that either repels or attracts root surfaces which also have an electrical charge. Tanada (1972) reported that root tips were electrically attracted to a phosphate-charged glass surface. This attraction depended on concentration of indol-acetic and abscisic acids and the proper combination of red and far-red illumination. It is not known to what extent this attraction would affect root elongation rate.

In the inclined cores, roots growing along the interface tended to grow parallel to the interface, about 1 to 2 mm away from it. Since Plexiglas is nonwetting, the percent of air filled voids at the interface may be quite high, thereby causing roots to elongate a short distance away where water was more abundant and soil physical resistance less.

Thus, based on these data, extrapolations of root elongation rates along a glass or plastic interface to the field should be carefully reevaluated.

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<sup>1</sup>Contribution from the North Central Region, ARS, USDA, Morris, Minn., in cooperation with the Minn. Agric. Exp. Sta., scientific journal paper no. 8966. Received Mar. 6, 1975.

<sup>2</sup>Soil scientist, USDA, Morris, Minn.

<sup>3</sup>Trade names and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the product listed by the USDA.