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A Method for Simulating Subsurface Injection of Herbicides[1]

J.M. Cupello and A.L. Young[2]

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. ~~Within uncut treatments,~~ Subsurface herbicide application to uncut plants retarded plant growth, but ~~relative to non-herbicide treated controls~~. No differences in ~~plant height~~ growth were observed between plants having cut roots and exposure to herbicide, and plants having only cut roots.

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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herbicides or their manufacturing wastes will be degraded to innocuous products by the combined action of soil microorganisms and soil hydrolysis (15). 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 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 liters 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 active ingredient/ha (kg ai/ha) of a 50:50 mixture of 2,4-D and 2,4,5-T[3]. Plant height at harvest for control plots was visibly different (greater) than plots receiving either undercutting with herbicide or undercutting without herbicide.

The present study was initiated to develop a laboratory method that would 1) simulate field disposal studies of phenoxy herbicides using subsurface injection and ~~to~~ 2) 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 had dimensions of 30.5 cm x 30.5 cm x 17.8 cm (inside diameter), constructed of 0.64 cm plexiglass. A 29.8 cm x 2918 cm x 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 30.5 cm x 30.5 cm sheet of 10 mesh (1680µ aperture width) stainless steel screen. The screen retained the soil inside the stainless steel insert, while permitting passage of the roots into the

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plexiglass container below. Four plexiglass legs, each 1.3 cm x 1.3 cm x 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 systems for cutting, provided that the space (a layer 5.1 cm deep) beneath the stainless steel 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 systems. Chemical treatment and, hence exposure of the cut roots, was accomplished by removing the vermiculite layer and replacing it with treated soil.

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

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

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

The plants ~~growth boxes~~ which were to be cut were allowed to grow for 22 days after the initial planting, at which time the stainless steel inserts were removed, the vermiculite replaced with either treated or untreated soil, the root systems cut, parallel to the b[illegible] of the insert and the stainless steel inserts replaced. All boxes were maintained in the environmental chamber under the conditions previously described. The boxes were periodically removed for watering and plant height measurements. The arrangement of growth boxes inside the chamber was alternated at the time of watering on a random basis in order to minimize any effects due to nonhomogeneous environmental factors within the chamber. ~~On those days on which the plant heights were measured,~~ A minimum of ten plants per box were randomly selected and the heights of the plants, from the soil surface to the tip of the longest leaf, were recorded at approx 1 wk intervals.

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(7)

Chemical formulations and application. Those plexiglass containers that were to receive chemically treated soil at the appropriate point in the experiment were handled in the following manner. At the time of chemical treatment for both cut (day 22) and uncut (day 3) root systems, the 5.1 cm of vermiculite was removed and replaced by 4.1 cm of uncontaminated soil. The remaining 1.0 cm space was filled with soil which had been previously mixed with sufficient herbicide formulation (a 50:50 mixture of the n-butyl esters of 2,4-D and 2,4,5-T) to be equivalent to 2,240 kg ai/ha. To insure a uniform layering of this 1 cm of contaminated soil, a plastic grid containing a matrix of 1 cm x 1 cm square holes was first spaced in the plexiglass container (over the 4.1 cm of untreated soil), the contaminated soil placed on this grid and spread to fill all of the grid squares equally, and the grid carefully removed. This soil was lightly packed, wetted with 500 ml of tap water, and the stainless steel insert re placed ~~back in position.~~

The 1 cm layer of soil ~~was predetermined to~~ weighed 1,500 grams, and to this ~~weight of soil~~ ~~was added~~ 20.1 ml of the herbicide formulation was added. To guarantee quantitative transfer, two 5 ml acetone rinses of the glassware were also added to the treated soils. The acetone was allowed to evaporate ~~[illegible]~~ a few minutes prior to the time the root systems were placed in contact with the chemically treated soils. The control soils received no herbicide, but did receive the 10 ml of acetone.

RESULTS AND DISCUSSION

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

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

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

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

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

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

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

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

It could be argued that the damp and saturated vermiculite data should be fitted to a growth model different from the general form of equation [1]. Even if this were true it would not alter the conclusions; it would only reduce the MSE of the curve fits.

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

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

Chemical treatment study. 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:

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

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

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 versus 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 [3] was used to determine the coefficients (for the uncut treated growth model) Omit Analysis ~~of the coefficients~~ ~~indicated~~ showed that treatment with herbicide significantly reduced the rate of growth ~~relative to uncut, controls~~ of the plants with intact root systems.

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 [4]. The results of these analyses indicated no difference in growth rate between control and treated ~~plots,~~ Plants ~~bot~~[illegible] which had their root systems severed: i.e., cutting the root systems caused the plants to grow as if the herbicide were not present.

<u>Technique evaluation</u>. 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. When root systems are not cut, herbicide application causes a significant decrease in plant growth, but we find no herbicide effect on growth when root systems are cut. The introduction of this one additional experimental variable, root cutting, may completely alter the conclusions drawn from such studies; it is too important a consideration to ignore.

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

During the course of these experiments a number of interesring physiological phenomena were observed. Those 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 mass 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

Omit?

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.

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 attribute 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 ~~tended to~~ minimized the effect of this bimodal distribution of plant heights. (Another way to reduce this artifact would be to confine plant measurements to plants other than those located adjacent to the four stainless steel) walls.

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The present studies were of a 2 x 2 statistical design, and were not replicated over time under exact environmental conditions. However, 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.

ACKNOWLEDGEMENTS

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

Captions for Figures

Figure 1. Plexiglass growth boxes with stainless steel inserts.

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

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

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

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

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Dr. D.E. Davis, Editor
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Capt Alvin L. Young, PhD
Department of Chemistry and Biological Sciences
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Sincerely

ALVIN L. YOUNG, Capt, USAF, PhD
Research Technical Advisor
Dept of Chemistry and Biological Sciences

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

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

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

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

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

RESULTS AND DISCUSSION

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

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

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

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

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

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

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

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

It could be argued that the damp and saturated vermiculite data should be fitted to a growth model different from the general form of equation [1]. Even if this were true it would not alter the conclusions; it would only reduce the MSE of the curve fits.

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

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

Chemical treatment study. 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:

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

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

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 versus 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 [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 [4]. The results of these analyses indicated no difference in growth rate between control and treated plots which had their root systems severed, i.e., cutting the root systems caused the plants to grow as if the herbicide were not present.

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. When root systems are not cut, herbicide application causes a significant decrease in plant growth, but we find no herbicide effect on growth when root systems are cut. The introduction of this one additional experimental variable, root cutting, may completely alter the conclusions drawn from such studies; it is too important a consideration to ignore.

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

During the course of these experiments a number of interesting physiological phenomena were observed. Those 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.

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 attribute 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 x 2 statistical design, and were not replicated over time under exact environmental conditions. However, 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.

ACKNOWLEDGEMENTS

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

Captions for Figures

<u>Figure 1</u>. Plexiglass growth boxes with stainless steel inserts.

<u>Figure 2</u>. Average height of sorghum plants following root exposure to damp or saturated vermiculite, and moist Ulysses silt loam soil. Data points are averages of ten or more plant heights. Curve fits determined by least squares linear regression. The curves are significantly different at the 0.95 confidence level for all treatment comparisons.

<u>Figure 3</u>. The effect of subsurface root cutting on the growth rate of non-herbicide treated sorghum plants. Data points are averages of ten or more plant heights. Curve fits determined by least squares linear regression. The curves are significantly different at the 0.95 confidence level.

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

<u>Figure 5</u>. The effect of subsurface herbicide application on the growth rate of sorghum plants having cut root systems. Data points are averages of ten or more plant heights. Curve fits determined by least squares linear regression. The curves are not significantly different at the 0.95 confidence level.

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Submission of Manuscript

Dr. D.E. Davis, Editor
WEED SCIENCE
Dept of Botany and Microbiology
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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:

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Sincerely

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1 Atch
Manuscript (3)

JAMES M. CUPELLO, Capt, USAF, PhD
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Dept of Chemistry and Biological Sciences

A Method for Simulating Subsurface Disposal of Herbicides[1]

J.M. CUPELLO, A.L. YOUNG AND J.C.H. SMITH[2]

Abstract. Specially designed growth boxes were used to simulate field subsurface injection of phenoxy herbicides. Sorghum [_Sorghum bicolor_ (L.) Moench] 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

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[2] Assoc. Prof. Biol. Sci., Assoc. Prof. Biol. Sci., and Asst. Prof. Math. Sci., respectively, U.S. Air Force Academy, CO 80840.

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[3]. 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

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

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 steel 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 accomplished by removing the vermiculite layer and replacing it with treated soil.

Moisture studies. To determine the sensitivity of this new technique to variations in the amount of water contained in the vermiculite layer, a preliminary experiment was conducted. Two plexiglass containers were filled with moistened vermiculite. The first, referred to as the damp vermiculite container, contained 128% water (w/w). A second container, the saturated vermiculite container, held vermiculite containing 502% water (w/w). As noted, vermiculite will absorb five times its weight in water. A third plexiglass container was filled with 5.1 cm of moist (11% water) Ulysses silt loam soil (pH 7, 1.3% organic matter, and 33, 44, and 23% sand, silt and clay, respectively) as a control, and was used to indicate whether plants grown in vermiculite had different growth rates than those grown in soil only. The stainless steel inserts for the three plexiglass boxes were filled with 10.2 cm of the Ulysses silt loam soil and placed in their respective plexiglass boxes. A cardboard template with the same surface area as the stainless steel inserts was pre-punched with 100 holes in a 10 by 10 square matrix, each hole separated from adjacent holes by a distance of 2.54 cm. The template was placed over the soil in the stainless steel inserts. One hundred seeds of 'Pioneer 846' grain sorghum, selected at random, were placed in the 100 holes and the seeds were pushed into the soil to a depth of 2.54 cm using a wooden dowel rod. The cardboard was then removed and the soil lightly raked and packed. The growth boxes were placed in an environmental chamber for 46 days. The chamber was maintained at a 14-h photoperiod, diurnal temperature of 35±2 C and 15±1 C, and a relative humidity of 60 and 85%, day and night, respectively.

Chemical treatment study. Based on the results of the moisture study, further experiments were designed to study the effects of chemical treatment on cut versus uncut root systems. The experiment was of a 2 by 2 statistical design involving four treatment variables: (a) cut control; (b) uncut control; (c) cut treatment; and (d) uncut treatment. Experimental replication during the course of this study consisted of two replicates of each control growth box and three replicates of each treated growth box; for a total of 10 growth boxes. Additional replication was not possible due to space limitations inside the environmental chamber. All 10 stainless steel inserts were filled with a 10.2-cm layer of the Ulysses silt loam soil. The 10 plexiglass containers were handled somewhat differently depending on whether the root systems were to be cut or uncut. Initially, however, all 10 plexiglass containers were filled with 5.1 cm of damp vermiculite, their stainless steel inserts carefully positioned inside the plexiglass containers and 100 seeds planted in each of the 10 inserts. Those plants whose root systems were to remain uncut were allowed to grow for 3 days, at which time the stainless steel inserts were removed from the growth boxes, the vermiculite layer replaced with moist "treated" or "untreated" soils, and the stainless steel insert replaced. Prior work indicated that by the third day after planting, the roots were just approaching the stainless steel screen.

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

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

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

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

RESULTS AND DISCUSSION

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

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

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

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

gave the smallest MSE, and was selected as the appropriate growth model against which to compare the data resulting from root growth in vermiculite. In the above equation, and those equations which will be introduced later, Y represents the plant height above the soil surface in cm, at various times, X, after planting of the seeds. Various powers of the X term are included in the equation in order to give a reasonable fit. The constant coefficients such as α, β_1, β_2, and β_3 are statistically selected to provide the minimum MSE between the empirical data and the growth model being fitted to the empirical data. Additional statistical testing of Equation No. 1 indicated that no additional terms needed to be included. Utilizing a matrix inversion technique, the coefficients for Equation No. 1 were determined as shown below:

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

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

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

It could be argued that the damp and saturated vermiculite data should be fitted to a growth model different from the general form of Equation No. 1. Even if this were true it would not alter the conclusions; it would only reduce the MSE of the curve fits.

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

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

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:

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

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

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 systems 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 systems. 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. Stn., Garden City, Kansas, for furnishing the seed and soil used in this study.

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Captions for Figures

Figure 1. Plexiglass growth boxes with stainless steel inserts.

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

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.

TCDD ANALYSIS, LIQUID ORANGE SAMPLES

Analysis Performed by ARL/LJ, WPAFB, Ohio

Samples submitted: 1 February 1975

Data Received: 11 March 1975

Sample Source	Sample Number	Date Sampled	TCDD PPM	
*Johnston Island	1	1 Aug 74	< 0.25	(a)
" "	2	"	1.3	(a)
" "	3	"	0.3	(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
DAY	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 (1.5)	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 *

1 vs 3 ns

3 vs 4 NS

2 vs 4 ns

5 v 6 NS

7 vs 8 *

9 vs 10 *

7 vs 9 * ns

5 vs 9 ns

3 vs 9 *

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
Fee Payment: Enclosed $________ Account No.________

For Laboratory Use Only
Fee Payment: ____________________
Date Received: ____________________19____
Date Results Reported: ____________________19____

SAMPLE IDENTIFICATION					SOIL DESCRIPTION			IRRIGATION		PREVIOUS TREATMENTS							TEST DESIRED (✓) Refer to Fee Schedule				
										Last Year					Lime						
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—goid E—excessive	Soil Type i.e. Geary Silt Loam	Type Sprinkler Surface	Leveling None Moderate Severe	Nitrogen (N) lbs./A.	Phosphate (P_2O_5) lbs./A.	Potash (K_2O) lbs./A.	Zinc (Zn) lbs./A.	Manure or Other Nutrients T./A. or lbs./A.	Last Three Years	Rate E.C.C. lbs./A.	General Fertility	Available Nitrogen	Available Zinc	Organic Matter	Lawn or Garden
GCK proposed															19__						
test site															19__						
															19__						
															19__						

MAP OF SAMPLE LOCATION ON FARM

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

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 ± 1°C from approximately 1800 to 0800 hours, and a maximum temperature of 35 ± 2°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 ± 5% relative humidity (RH) (1800 to 0800 hours) and 60 ± 5% RH, respectively.

In addition, fluorescent and incandescent light fixtures inside the chamber were programmed to provide a realistic exposure to sunlight throughout the daytime 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°C ± 1°C/29°C ± 2°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
AGRICULTURAL RESEARCH SERVICE
Southern Weed Science Laboratory
P.O. Box 225
Stoneville, Mississippi 38776

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

C. G. McWhorter
Associate Editor
WEED SCIENCE

cc:
D. E. Davis

Young et al have conducted studies on soil biodegradation and have shown that

POSSIBLE

^TCDD Uptake IN Sorghum bicolor

INTRODUCTION — Disposal
Accidents

LITERATURE REVIEW —
Kansas — Unpublished
Eglin — No uptake in seed
Isensee / Jones

① [illegible]
② Literature

METHODS & MATERIALS

a. Technique in Press

b. Analysis — At Day 64

PARAMETERS
EFFECTS of TCDD on PLANT GROWTH

PLANT HEIGHT
PLANT WEIGHT
FRESH
DRY
ROOT WEIGHT

NUMBER ALIVE / DEAD
PERCENT GERMINATION

BOXES
(1, 2), (3, 4), (9, 10)

1, 3, 5, 7, 9
2, 4, 6, 8, 10
Control

germi[illegible]/no cut ?

PLANT UPTAKE

Cut + Herbicide — SYN.
Cut + No Herbicide

Uncut + Herb. = Uncut + No Herb.

A Method for Simulating Subsurface Disposal of Herbicides[1]

J.M. CUPELLO, A.L. YOUNG and J.C.H. SMITH[2]

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[3]. 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

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

[2] Assoc. Prof. Biol. Sci., Assoc. Prof. Biol. Sci., and Asst. Prof. Math. Sci., respectively, U.S. Air Force Academy, CO 80840.

[3] 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 less 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-

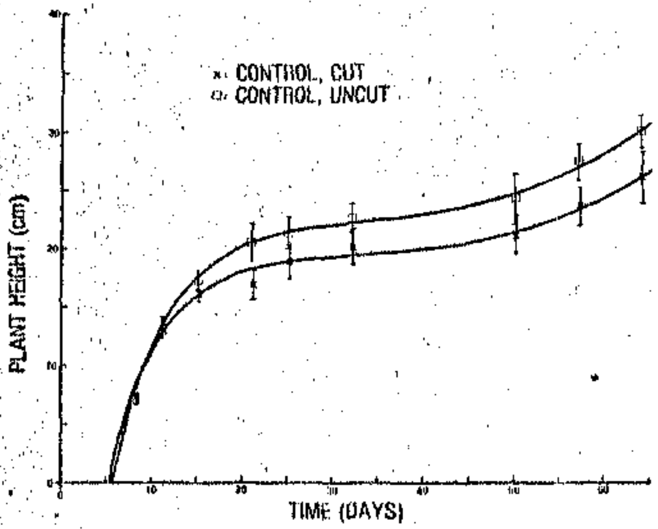

Figure 3. The effect of subsurface root cutting on the growth rate of sorghum. Data points represent the mean of at least 20 plant heights. Curve fits determined by least squares linear regression. Error bars represent the 95% confidence limits of the mean plant height. The curves are significantly different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

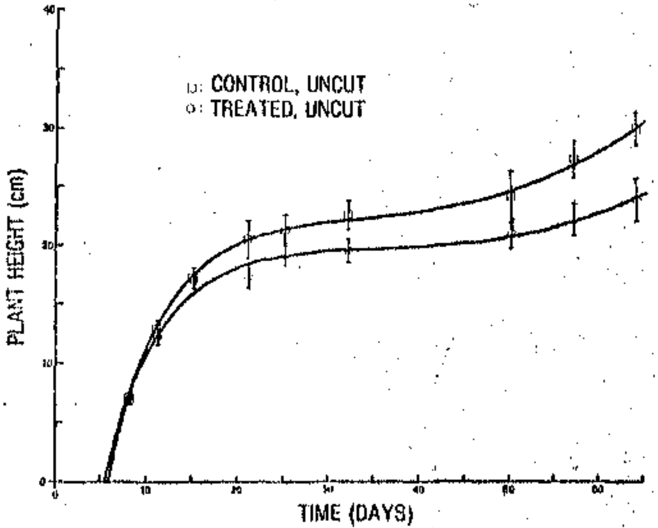

Figure 4. The effect of subsurface herbicide application on the growth rate of sorghum plants having uncut root systems. Control and treated data points represent the mean of at least 20 and 30 plant heights, respectively. Curve fits determined by least squares linear regression. Error bars represent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

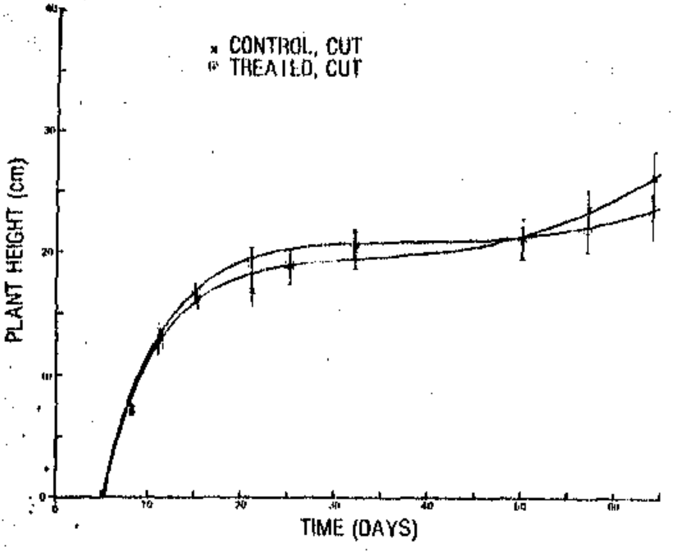

Figure 5. The effect of subsurface herbicide application on the growth rate of sorghum plants having *cut root systems.* Control and treated data points represent the mean of at least 20 and 30 plant heights, respectively. Curve fits determined by least squares linear regression. Error bars represent 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 commerical 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. Stn., Garden City, Kansas, for furnishing the seed and soil used in this study.

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

Voorhees ( ) ~~[illegible]~~ measured root elongation rate through center of soil cores and along plexiglas-soil interfaces. Containers ~~those~~ ~~[illegible]~~ with roots placed at the ~~plastic~~ plexiglas-soil interface were inclined 25° 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[1]

W. B. Voorhees[2]

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 ≤ 2 mm diam. size fraction of a Nutley clay (*Udertic Haploboroll*) from the surface 25 cm. Three sets of five soil cores were prepared by compressing a known weight of slightly moist soil into Plexiglas[3] 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.

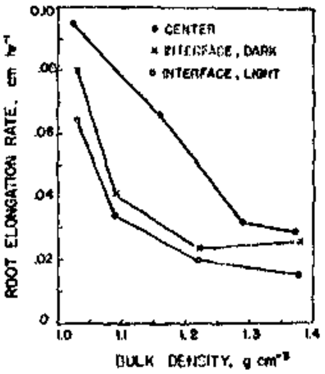

Fig. 1. Root elongation rate through center of soil core and along plexiglas-soil interface.

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 indolacetic 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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[3] 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.