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From 1962 to 1970, massive quantities of herbicides were sprayed on test areas at Eglin AFB Reservation, Florida, to evaluate developmental aerial spray systems. Questions were recently raised about the potential ecological and environmental effects on the test areas. Therefore, a study was conducted to determine if soil that had received massive quantities of herbicide would support seed germination, vegetative growth, and fruit production of seven selected agronomic crops. The study showed that the level of herbicides which remained in the soil was not high enough to prevent germination, plant growth, or fruit production.

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PREFACE

This technical report discusses a portion of the work performed at the Air Force Armament Laboratory, Armament Development and Test Center, under Exploratory Development Project 5066 during the period May 1974 through August 1974.

The sources and manufacturers of materials and equipment used in this study are identified for reference only and do not constitute endorsement of the companies or products by the United States Air Force.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Joe A. Farmer

JOE A. FARMER
Chief, Environics Office

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SECTION I

INTRODUCTION

Test Area (TA) C-52A, Eglin AFB Reservation, Florida, was used to evaluate developmental aerial spray systems from 1962 to 1970. During this period massive quantities of herbicides were sprayed in order to study various spray parameters. This repetitive application of large quantities of herbicides has raised questions about the potential long-term ecological and environmental effects on the test site and surrounding areas of TA C-52A.

Four herbicides accounted for most of the materials used. These are White (10.2 percent triisopropanolamine salt of picloram, 39.6 percent triisopropanamine salt of 2,4-D, and 50.2 percent inert ingredients), Blue (22.6 percent sodium cacodylate, 3.9 percent dimethylarsinic acid, 73.5 percent inert ingredients, and 5.0 percent surfactant), Purple (50 percent n-butyl ester of 2,4-D, 30 percent n-butyl ester of 2,4,5-T and 20 percent isobutyl ester of 2,4,5-T), and Orange (50-50 mixture of 80 percent n-butyl ester of 2,4-D and 2,4,5-T). It was subsequently determined that two of the herbicides, Purple and Orange, contained the impurity called TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) which is highly toxic and a teratogen, causing fetal deformities (Reference 1).

The actual herbicide deposition levels on the test area are not known and can only be estimated using the spray-aircraft flight paths, application rates, and the meteorological conditions under which test spraying was done. Subsequent to the actual testing, some of the herbicide residue has undoubtedly been translocated by wind and water movement.

It was reported in Reference 2 that the test area was ecologically recovering and that the active herbicide ingredients were down to the parts-per-billion range.

The purpose of the effort reported here was to determine if the soil that had received massive quantities of herbicide had recovered sufficiently to support seed germination, vegetative growth, and fruit production of selected agronomic crops.

Reference:

1. Sparschu, G. L., et al: "Teratogenicity of 2,3,7,8-tetrachlorochibenzo-p-dioxin in the Rat." Food Cosmet Toxicol 9 (3) 405 (1971).
2. Young, A. L.: Ecological Studies on a Herbicide Equipment Test Area (TA C-52A), Eglin AFB Reservation, Florida, AFATL-TR-74-12, Air Force Armament Laboratory, January 1974.

SECTION II

MATERIALS AND METHODS

An area on the sourth end of TA C-52A was selected as the site for an experimental vegetative plot (Figures 1 and 2). This area was estimated to have received approximately 2,000 pounds per acre of active herbicide ingredients (mostly 2,4-D and 2,4,5-T) and was devoid of vegetation. The soil of this area was well drained, acid soil of the Lakeland Association. The area surrounding the plot had a sparse population of broomsedge (Andropogon virginicus), switchgrass (Panicum virgatum), wooly panicum (Panicum lanuginosum), and low growing grasses and herbs.

A spot on the northeast section of TA D-51, (3 miles west of TA C-52A) (Figure 1 through 3), where no herbicide had been applied, was selected as a control site. This area was selected because of its similarity to the experimental plot with respect to terrain, soil type, rainfall, and wind conditions.

Since TA C-52A had been used as a bombing and gunnery range prior to its use as a herbicide test area, the experimental plot had to be checked and cleared of hazardous material by Explosive Ordnance Disposal (EOD) personnel. As mentioned above, there was no vegetation on the experimental plot, however the control plot at TA D-51 had to be cleared of a few scattered grasses. A 3 by 7 meter section was fenced with 1/4-inch mesh hardware cloth at each of the locations. A canopy of cheesecloth was placed over each plot to provide partial shade during seed germination and seedling establishment. A sheet of rubberized material was stretched around the windward sections of the fence to protect young plants from wind damage and to reduce moisture stress.

The soil on both plots was prepared by tilling to a depth of approximately 30 centimeters. The experimental plot was prepared on 6 May 1974 and the control plot on 9 May 1974. Soil from each plot was taken to the laboratory where the pH was found to be 4.0 for the experimental plot and 4.6 for the control. It was determined that the soil at both plots was too acidic for the plants to be used in this study and that it should be elevated to a pH more conducive to growth of the selected plants. On 9 May 1974, 5.8 kilograms of lime and 1.4 kilograms of commercial fertilizer (13-13-13) were applied to both plots prior to planting. After 12 days, the pH readings of the soils in the experimental and control plots were 5.6 and 5.8, respect vely. An additional 453 grams of fertilizer were applied to each plot at monthly intervals thereafter.

On 13 May 1974, seven rows 3 meters in length were prepared on each plot. On the same day one row each of Bahia grass (Paspalum notatum variety Pensacola), millet (Panicum miliaceum variety Browntop), radishes (Raphanus satuvus variety Scarlet), and beans (Phaseolus vulgaris variety Tennessee Bunch) was planted. Tomato seeds (Lycopersicon esculentum variety Spring Giants) had been germinated in a greenhouse and allowed to grow for 3 weeks prior to planting. On 14 May 1974, one row of peanuts (Arachis hypogaea variety Starr) and on 15 May 1974, one row of cotton (Gossypium hirsutum variety Dixie King III) were planted.

One hundred and ninety liters of water were applied to each plot twice weekly. This water volume was calculated to be equivalent to 1.91 centimeters of rainfall weekly. Examination of the soil indicated that this amount of water was sufficient to saturate the soil and provide

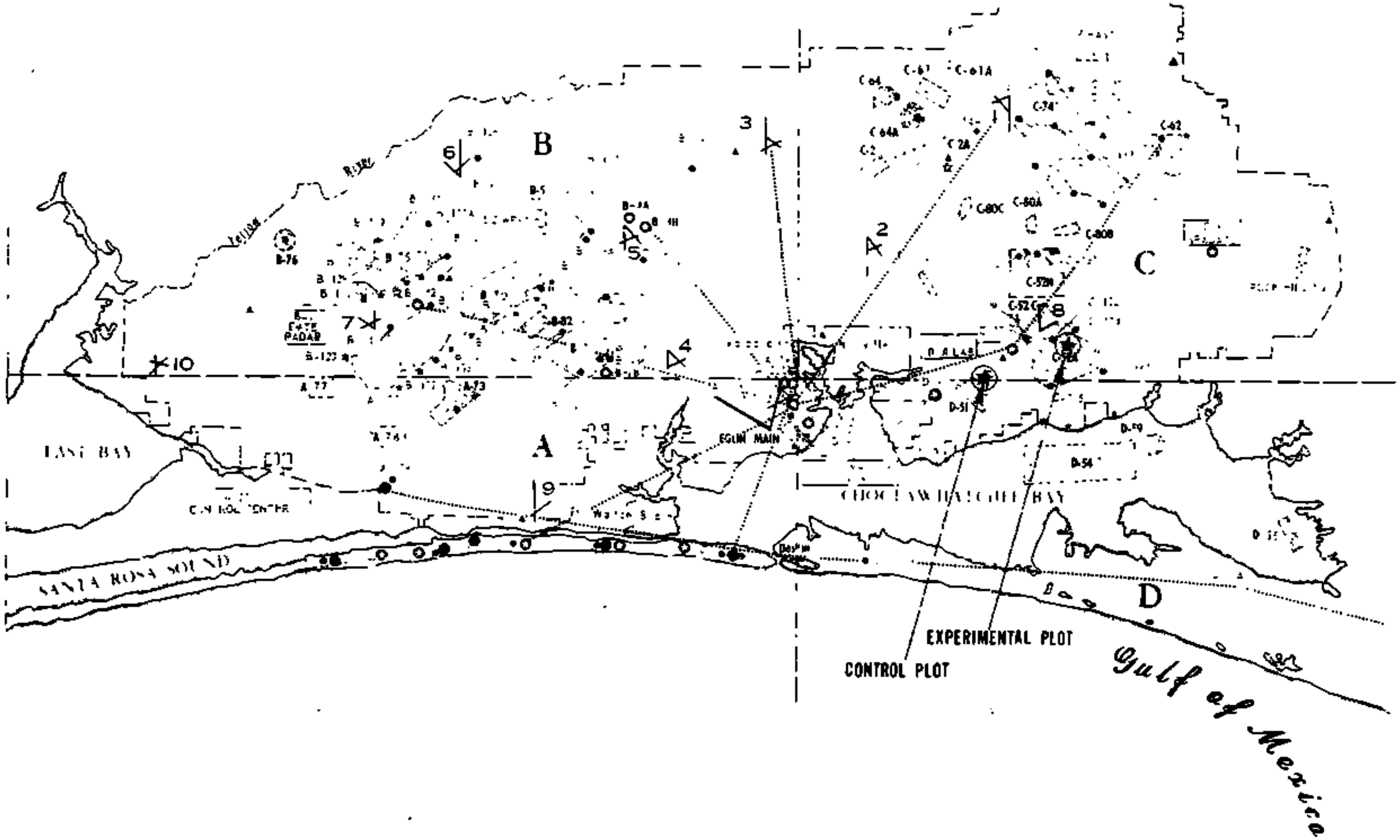

Figure 1. Eglin AFB Reservation Showing Locations of Control and Experimental Plots

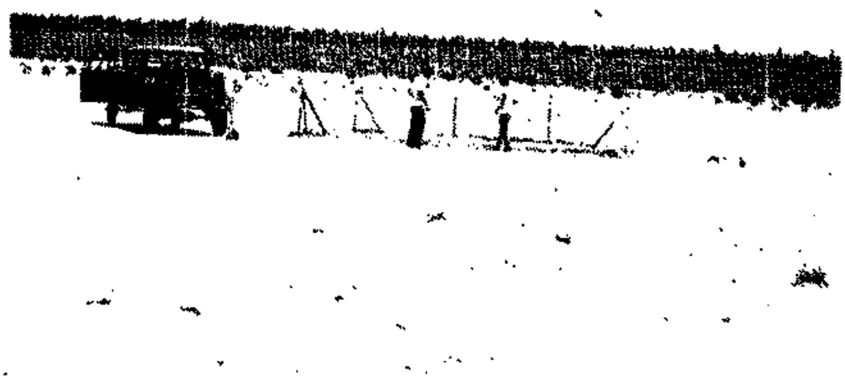

Figure 2. Experimental Plot Located on TA C-52A

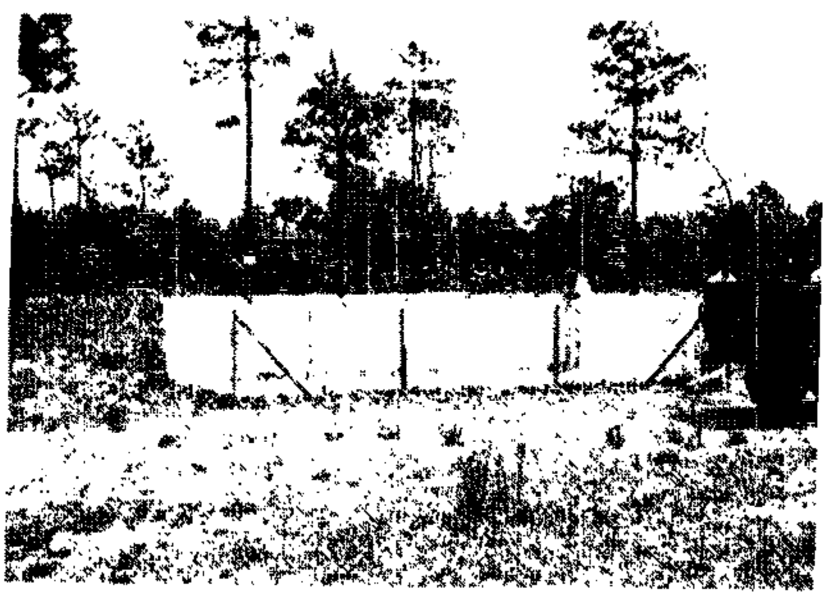

Figure 3. Control Plot Located on TA D-51

enough moisture for the plants during early stages of development. Natural rainfall was supplemented as needed to provide this amount of water as a minimum until the plants reached a point in development where the demand exceeded the amount being applied, and then the amount of water was doubled. Rainfall data recorded during this investigation are shown in Table 1.

TABLE 1. RAINFALL DATA ON CONTROL AND EXPERIMENTAL PLOTS
[Each value is the total amount since the previous date]

DATE	CONTROL (cm)	EXPERIMENTAL (cm)
20 May	1.02	1.27
24 May	1.52	3.81
28 May	0.51	0.25
3 Jun	2.54	1.52
6 Jun	1.02	0.76
17 Jun	0.51	0.00
5 Jul	1.27	2.03
6 Jul	3.81	0.76
7 Jul	1.78	0.51
12 Jul	1.02	0.25
20 Jul	3.30	1.27
23 Jul	3.81	1.27
26 Jul	1.52	4.06
31 Jul	6.35	6.86
5 Aug	5.08	3.30
9 Aug	1.78	0.76
11 Aug	0.00	1.78
16 Aug	1.52	4.06
20 Aug	0.00	1.27

Plant-height measurements were taken 31 May, 7 June, 18 June, 9 July, 19 July, and 13 August. Height was considered to be from ground level to tip of uppermost leaf. Twenty plant heights were measured along rows where more than 20 plants were present; the plants to be measured were selected randomly except that no more than two measurements per foot were taken. If 20 plants or less were present, then all plants were measured except on 3 May when only 10 were measured. Diameter and weight of radish roots were determined on 18 June when they were considered to be mature. The weight of beans produced was determined as they matured and were harvested, and a cumulative total was run for each plot. No production data were available on tomatoes or peanuts because of insect and worm damage on tomatoes and vandalism of peanuts. No data were gathered on Bahia grass seed production because these plants had not matured at the scheduled termination of the study. The data on experimental and control plants were statistically compared using the analysis of variance test at the 95, 99, and 99.5 percent confidence levels.

SECTION III

RESULTS

A reasonable percentage of the seeds of all species planted for this study germinated. Quantitative data were not recorded, but no gross differences were detectable in the germination time or percentages between the control and experimental plots. The germination of cotton seed on both plots was less than that expected, but subsequent tests of seeds from the same lot under the controlled environmental conditioning of a greenhouse resulted in only 80 percent germination. Seedlings of all species appeared healthy (Figures 4 through 15).

Without exception, the plants on the control plot grew larger, produced more and larger fruits, and yielded higher weights than those from the experimental plot (Tables 2 and 3). The differences observed were all statistically significant except those of the bean fruit weight. Growth measurements of several species showed a reversal on the 19 July measurements, but this apparently was due to temporary conditions. The first two measurements taken of millet showed the control to be slightly smaller. This difference was not statistically significant and the initial growth rate differential was reversed during the remainder of the time. Control plants overtook the experimental plants in all growth and reproduction aspects before the test period was ended.

Figure 4. Control Browntop Millet

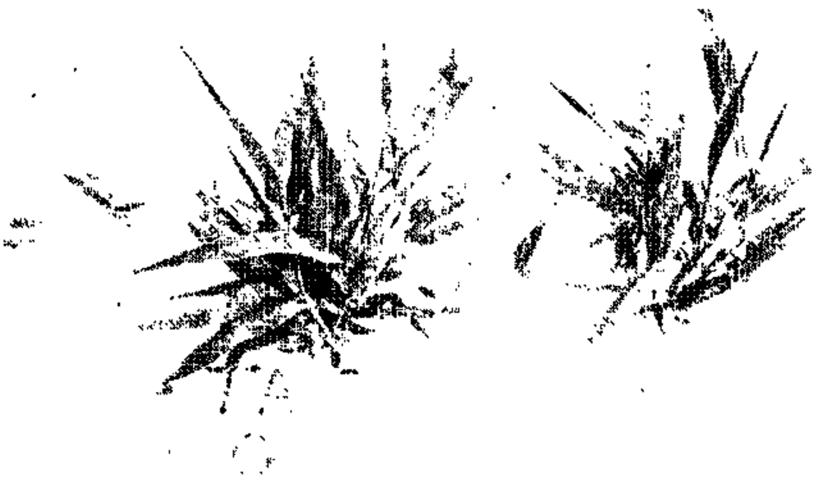

Figure 5. Experimental Browntop Millet

Figure 6. Control Peanuts

Figure 7. Experimental Peanuts

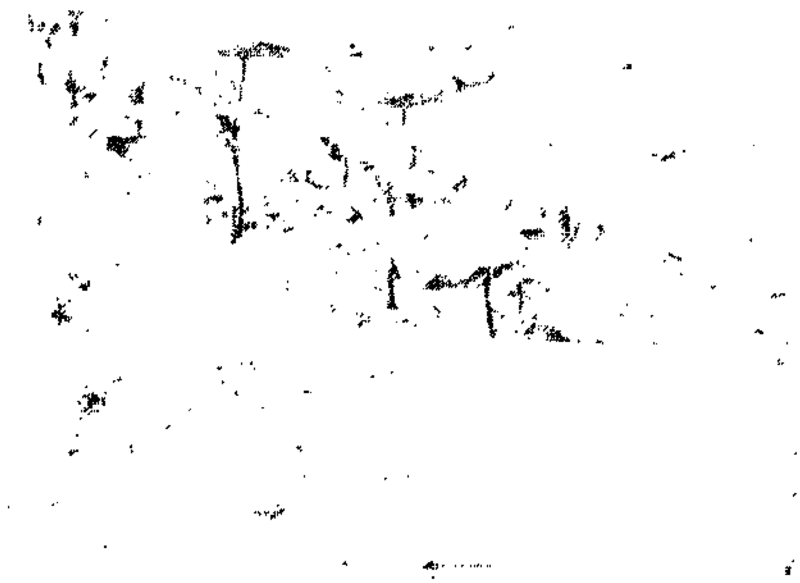

Figure 8. Control Beans

Figure 9. Experimental Beans

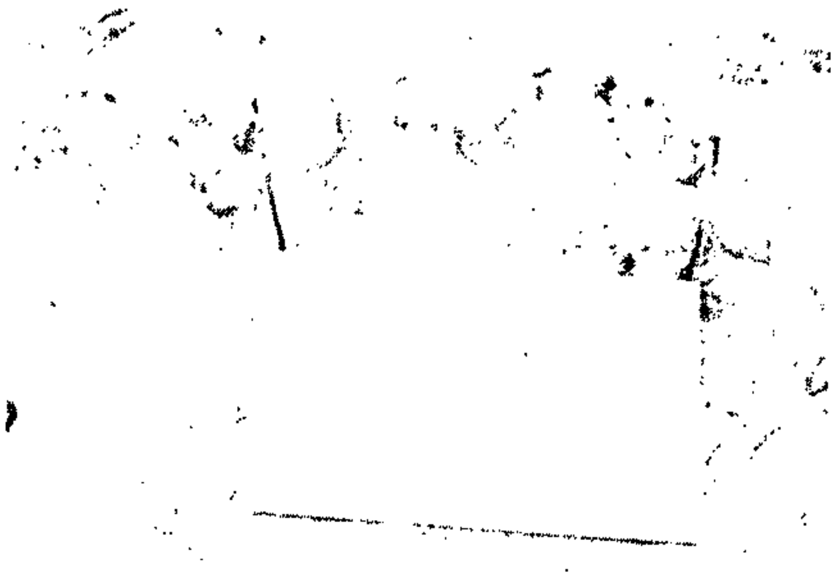

Figure 10. Control Cotton

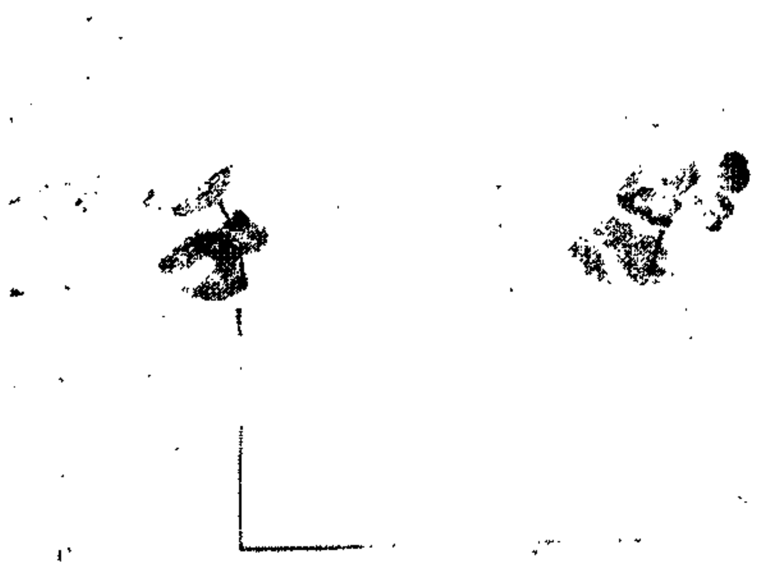

Figure 11. Experimental Cotton

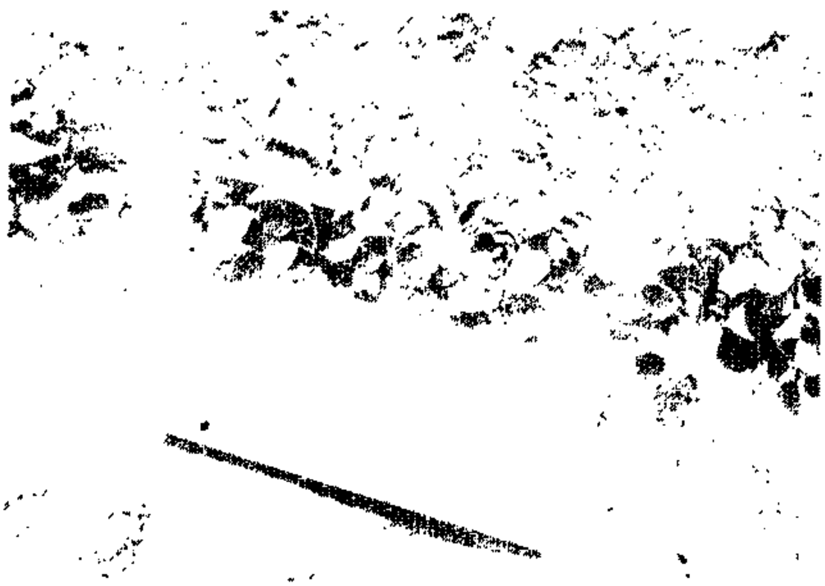

Figure 12. Control Radishes

Figure 13. Experimental Radishes

Figure 14. Control Tomatoes

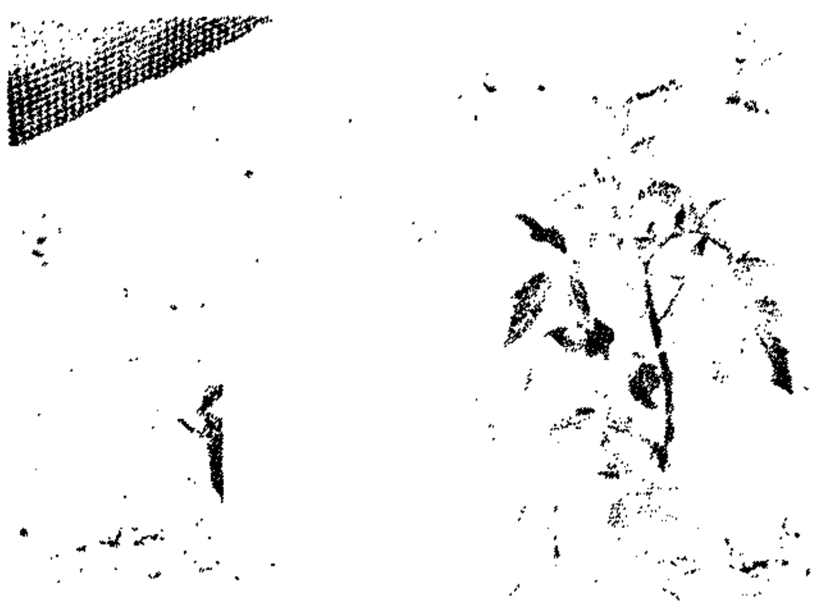

Figure 15. Experimental Tomatoes

TABLE 2. PLANT HEIGHT IN CENTIMETERS

	DATES OF MEASUREMENTS					
	30 May Control vs Experimental	7 June Control vs Experimental	18 June Control vs Experimental	9 July Control vs Experimental	19 July Control vs Experimental	13 July Control vs Experimental
Bahia Grass	4.9 ± 2.4(10) vs ns 4.0 ± 1.2(10)	12.3 ± 2.3(20) vs Δ 7.7 ± 3.9(20)	18.2 ± 3.9(20) vs Δ 13.0 ± 2.2(20)	25.1 ± 3.6(20) vs Δ 19.6 ± 4.5(20)	28.7 ± 4.1(20) vs Δ 23.6 ± 4.5(20)	41.5 ± 4.8(20) vs Δ 28.0 ± 5.9(20)
Millet	9.1 + 5.3(10) vs ns 9.9 ± 3.0(10)	25.7 ± 10.0(20) vs ns 28.2 ± 11.1(18)	44.9 ± 10.6(20) vs ns 39.8 ± 6.4(20)	60.4 ± 9.6(20) vs Δ 50.6 ± 6.8(20)	No new growth	
Cotton	6.7 ± 2.5(11) vs ns 4.5 ± 3.2(7)	11.1 ± 5.4(11) vs ns 6.4 ± 3.4(7)	18.5 ± 9.4(11) vs * 8.0 ± 3.9(7)	41.2 ± 18.1(11) vs Δ 11.9 ± 5.1(5)	40.0 ± 21.2(11) vs † 14.2 ± 5.3(5)	46.9 ± 24.1(11) vs Δ 17.0 ± 5.6(5)
Peanuts	6.6 ± 2.0(10) vs Δ 4.2 ± 1.1(10)	12 0 ± 3.5(20) vs Δ 7.6 ± 1.3(20)	19.7 ± 5.3(20) vs Δ 9.3 ± 1.4(20)	37.2 ± 5.3(20) vs Δ 19.1 ± 2.9(20)	39.8 ± 5.5(20) vs Δ 18.1 ± 3.9(20)	43.5 ± 4.7(15) vs Δ 26.4 ± 4.7(20)
Radishes	6.2 + 3.8(10 vs * 3.0 ± 1.5(10)	13.0 ± 3.6(20) vs Δ 7.4 ± 1.9(20)	32.5 ± 3.7(18) vs Δ 19.0 ± 3.4(20)			
Beans	15.3 ± 3.0(10) vs Δ 9.5 ± 1.6(10)	27.1 ± 3.8(20) vs Δ 16.3 ± 3.0(20)	36.1 ± 5.1(20) vs Δ 19.6 ± 3.1(20)	40.9 ± 5.0(20) vs Δ 24.5 ± 2.3(20)	No new growth	
Tomatoes	20.5 ± 3.9(6) vs ns 20.2 ± 2.6(5)	33.0 ± 7.5(6) vs ns 27.4 ± 3.3(5)	52.1 ± 5.5(6) vs Δ 37.6 ± 1.1(6)	80.4 ± 10.1(6) vs * 64.8 ± 12.7(6)	No new growth	

LEGEND
ns = not statistically significant
* = $P < 0.05$
† = $P < 0.01$
Δ = $P < 0.005$
P is the probability that the statistically significant difference is due to change.
Numbers are represented as mean ± standard deviation
Number in parenthesis is number of plants measured.

TABLE 3. ADDITIONAL MEASUREMENTS OF RADISHES AND BEANS

	Vegetative Structure Weight (g)	Fruit Weight (g)	Root Length (cm)	Root Diameter (cm)
Radishes Control vs Experimental	17.5 ± 5.3(17 vs Δ 7.1 ± 4.1(20)		10.0 ± 2.6(17) vs † 7.9 ± 1.6(20)	20.4 ± 4.9(17) vs Δ 15.1 ± 5.1(20)
Beans Control vs Experimental	30.1 ± 23.9(20) vs * 15.2 ± 6.1(20)	5.4 ± 2.0(20) vs ns 4.8 ± 1.9(20)		
Millet Control vs Experimental		0.19 ± 0.05(5)@ vs 0.18 ± 0.04(5)@		

LEGEND
ns = not statistically significant
* = $P < 0.05$
† = $P < 0.01$
Δ = $P < 0.005$
@ = Each of the five weights contained 100 seeds.
P is the probability that the statistically significant difference is due to change.
Numbers are represented as mean ± standard deviation.
Number in parenthesis is number of plants measured.

SECTION IV

DISCUSSION AND CONCLUSIONS

This experiment was designed to determine if seeds could germinate, develop into mature plants, and produce fruit in a barren area that had received heavy applications of herbicides over a long period of time. It was determined by this work that the level of herbicide remaining in the area is not high enough to prevent seed germination or growth of sensitive agronomic plants. The prime factor limiting the seed germination and growth observed during this experiment was the physical and physiological stresses related to wind and drought on the plants. The experimental test area was much more open than the control area and strong winds and moisture stress were more prevalent. Despite measures taken to alleviate the effects of the wind and artifically supplementing rainfall, the experimental plants frequently were excessively dry, and the protective windscreen was blown down on several occasions. The prevailing winds on the experimental plot caused quick evaporation of moisture and formation of a crust on the upper layer of the soil. Therefore, wind appeared to be the major factor preventing vegetation from returning to the barren area. Seeds falling to the soil from the surrounding plants either do not adhere to the soil sufficiently for germination or the moisture is not sufficient to support germination and growth.

It is not known if the fruit from the crops grown in this experiment could be used for human consumption. There is evidence that TCDD can be taken up and translocated in oats and soybeans (Reference 3). However, no plants or fruit from this experiment were tested for TCDD uptake. Additional experiments are needed to determine if TCDD or other herbicide-related residues were present in these plants.

This experiment determined that a wide variety of agronomic plants can reach maturity and produce fruit in an area that previously has received massive applications of herbicides. It was believed that environmental conditions other than the residual effects of herbicides were responsible for less growth of plants on the experimental plot.

Reference:
3. Isenbee, Alan R. and Gerald E. Jones. "Absorption and Translocation of Root and Foliage Applied 2,4-Dichlorophenol, 2,7-Dichlorodibenzo-p-dioxin, and 2,3,7,8-Tetrachlorodibenzo-p-dioxin." J. Agr. Food Chain. 19 (6) 1210 (1971.

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