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ECOLOGICAL STUDIES ON A HERBICIDE-EQUIPMENT TEST AREA (TA C-52A) EGLIN AFB RESERVATION, FLORIDA

JANUARY 1974

FINAL REPORT: January 1967 to November 1973

ENVIRONICS AND FLAME MUNITIONS BRANCH
FLAME, INCENDIARY AND EXPLOSIVES DIVISION
AIR FORCE ARMAMENT LABORATORY
AIR FORCE SYSTEMS COMMAND • UNITED STATES AIR FORCE
EGLIN AIR FORCE BASE, FLORIDA

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This report attempts to answer the major questions concerned with the ecological consequences of applying massive quantities of herbicides (346,117 pounds), via repetitive applications, over a period of eight years, 1962 - 1970, to an area of approximately one square mile. Moreover, the report documents the persistence, degradation, and/or disappearance of the herbicides from the Test Area's soils and drainage waters and their subsequent effects (direct or indirect) upon the vegetative, faunal, and microbial communities. The active ingredients of the four military herbicides (Orange, Purple, White, and Blue) sprayed on Test Area C-52A were 2,4-dichlorophenoxyacetic acid (2,4-D),
(continued)

Item 19. Continued
Ecological Investigations
Fish
Herbicide
Herbicide Equipment Test Grid
Histology
Mammals
Microbial Survey
Military Defoliation Program
Necropsy
Orange
Purple
Reptiles
TCDD
Teratogenic
Test Area C-52A, Eglin AFB Reservation
2,3,7,8-Tetrachlorodibenzo-p-dioxin
2,4,5-Trichlorophenoxyaetic Acid
Vegetative Coverage Survey
White

Item 20. 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 4-amino-3,5,6-trichloropicolinic acid (picloram), and dimethylarsinic acid (cacodylic acid). It is probable that the 2,4,5-T herbicide contained the highly teratogenic (fetus deformina) contaminant 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). Significant herbicide residues were found in 1969. However, analysis of soil cores in 1971 indicated residues of 2,4-D, 2,4,5-T, picloram, and arsenic to be in the parts per billion range; no TCDD was found at this detection limit. Soil samples collected in June and October 1973 showed TCDD (or a TCDD-like chemical) levels ranging from less than 10 parts per trillion (ppt) to 710 ppt. Direct effects of the herbicides on the vegetative community were temporary. With the disappearance of residue, vegetative succession was initiated. By 1973, the majority of the test area could not be distinguished from control sites. Analysis of sample animal populations indicated that no histological or gross abnormalities were present in adults or their progeny.

PREFACE

The Air Force project directly related to the information in this report is Air Force Systems Command Project 5154-02, Ecological Survey of Test Area C-52A, Eglin AFB Reservation, Florida. This report documents five years of ecological investigations performed between 1967 and 1973.

The assistance provided during portions of this report by the Air Force Environmental Health Laboratory, Booz-Allen Applied Research, Dow Chemical U.S.A., United States Air Force Academy, United States Department of Agriculture, University of Alabama, University of Florida, and Vitro Services is gratefully acknowledged.

Information on the test grid monitoring system and types and amounts of defoliants disseminated on Test Area C-52A from July 1962 to April 1969 was obtained from Armament Development and Test Center working papers, "Defoliant History of Test Area C-52A", by Helen Biever. After April 1969, this same information was obtained from Vitro Services, Vitro Corporation of America. Information on soils of Test Area C-52A was obtained from a July 1969 soil survey of Eglin AFB Reservation prepared by the Soil Conservation Service of the United States Department of Agriculture.

This technical report has been reviewed and is approved.

F. Ramon Bonnano

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SUMMARY

In support of programs testing aerial dissemination systems, a one square mile test grid on Test Area (TA) C-52A, Eglin AFB Reservation, Florida, received massive quantities of military herbicides. The purpose of these test programs was to evaluate the capabilities of the equipment systems, not the biological effectiveness of the various herbicides. Hence, it was only after repetitive applications that test personnel began to express concern over the potential ecological and environmental hazards that might be associated with continuance of the test program. This concern led to the establishment of a research program in the fall of 1967 to measure the ecological effects produced by the various herbicides on the plant and animal communities of TA C-52A. This report documents 6 years of research (1967 - 1973) on TA C-52A and the immediately adjacent streams and forested areas.

This report attempts to answer the major questions concerned with the ecological consequences of applying massive quantities of herbicides (346,117 pounds), via repetitive applications, over a period of 8 years (1962 - 1970) to an area of approximately one square mile. Moreover, the report documents the persistence, degradation, and/or disappearance of the herbicides from the soils and drainage waters of TA C-52A, and the subsequent effects (direct or indirect) of the herbicides upon the vegetative, faunal, and microbial communities.

The active ingredients of the four military herbicides (Orange, Purple, White, and Blue) sprayed on TA C-52A were 2,4-dichlorophenoyxacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 4-amino-3,5,6-trichloropicolinic acid (picloram), and dimethylarsinic acid (cacodylic acid). It is probable that the 2,4,5-T herbicide contained the highly teratogenic (fetus deforming) contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). From 1962 to 1964, 92 acres of the test grid received 1,894 pounds 2,4-D, 2,4,5-T per acre while, in 1964 to 1966, another 92 acres received 1,168 pounds per acre. From 1966 to 1970, a third distinct area of over 240 acres received 343 pounds per acre of 2,4-D and 2,4,5-T, and 6 pounds per acre of picloram; and from 1969 to 1970, this same area received 53 pounds per acre of cacodylic acid (28 pounds per acre of arsenic as the organic pentavalent form; calculated on weight of Blue applied per acre).

From the rates of herbicides that were applied during the years of testing spray equipment, it was obvious that TA C-52A offered a unique opportunity to study herbicidal persistence and soil leaching. Yet the problem of how best to assess the level of herbicide residue was a difficult one. The herbicides could be chemically present but because of soil binding might not be biologically active. Thus, both bioassay techniques and analytical analyses were employed. The first major bioassay experiment was conducted in April 1970. By considering the flightpaths, the water sources, and the terracing effects, it was possible to divide the one square mile test grid into 16 vegetation areas. These areas formed the basis for the random selection of 48 soil cores taken from the surface to a depth of 3 feet. Soybean bioassays indicated that 27 of the 48 cores were significantly different from control cores (95% probability level). The results indicated that soil leaching or penetration was much more prevalent along the dissemination flightpaths than in other areas of the test grid. Efforts to quantitate (chemically) the bioassay were confined to only the top 6 inch increment because of within-core variations. By considering that all phytotoxic effects resulted from 2,4-D and 2,4,5-T, the average value for the top 6 inches of the eight cores showing greatest herbicide concentration was 2.82 ppm (parts per million) herbicide. Chemical analyses of soil cores collected from the eight sites showing greatest phytotoxic concentrations were performed in December

1970. Results indicated that the maximum concentration of either 2,4-D or 2,4,5-T was 8.7 ppb (parts per billion). A 1970 analysis of soil cores from areas receiving the greatest quantities of Blue indicated maximum arsenic levels of 4.70, 1.30, and 0.90 ppm, respectively, for the first three 6 inch increments of the soil profile. These same increments were again collected and analyzed in 1973, and the levels of arsenic were 0.85, 0.47, and 0.59 ppm for the three consecutive 6 inch increments. Leaching of the arsenical from the soils may have occurred. In November 1969, picloram analysis of soil cores from areas receiving greatest quantities of White indicated maximum levels of 2.8 ppm picloram present in the 6 to 12 inch increment. An analysis of the same sites in 1971 indicated the picloram had leached further into the soil profile but concentrations were significantly less (ppb). The analyses of soil cores in 1971 showed no residue of TCDD at a minimum detection limit of less than 1 ppb, even in soils previously treated with 947 pounds 2,4,5-T per acre. However, data from soil analysis (via mass spectrometry) of four total samples collected in June and October 1973 indicated TCDD levels of < 10, 11, 30, and 710 parts per trillion (ppt), respectively. These levels were found in the top 6 inches of soil core. The greatest concentration (710 ppt) was found as a sample form the area that received 947 pounds 2,4,5-T in the 1962 - 1964 test period.

A comparison of vegetative coverage and occurrence of plant species on the one square mile grid between June 1971 and June 1973 showed that areas with 0 to 60% vegetative cover in 1971 had a coverage of 15% to 85% in June 1973. Those areas having 0 to 5% coverage in 1971 (areas adjacent to or under flightpaths used during herbicide equipment testing) had 15% to 54% coverage. The rate of coverage seemed to be dependent upon soil type, soil moisture, and wind. There was no evidence to indicate that the existing vegetative coverage was directly related to herbicide residue in the soil: some dicotyledonous or broadleaf plants that are normally susceptible to damage from herbicide residues occurred throughout the entire one square mile grid except in a few irregularly spaced barren areas. The square-foot transect method of determining vegetative cover indicated that the most dominant plants on the test area are the grasses - switchgrass (Panicum virgatum), woolly panicum (Panicum lanuginosum), and the broadleaf plants - rough buttonweed (Diodia teres), poverty weed (Hypericum gentianoides), and common polypremum (Polypremum procumbens). In 1971, 74 dicotyledonous species were collected on the one square mile grid; in 1973, 107 dicotyledonous species were collected. All of the plant species collected were pressed, mounted, and placed in the Eglin AFB Herbarium.

An evaluation of the effects of the spray equipment testing program on faunal communities was conducted from May 1970 to August 1973. The extent of any faunal ecological alteration was measured by assessing data on species variation, distribution patterns, habitat preference (and its relationships to vegetative coverage), and the occurrence and incidence of developmental defects as well as gross and histologic lesions in postmortem pathological examinations.

A total of 73 species of vertebrate animals (mammals, birds, reptiles, and amphibians) were observed on TA C-52A and in the surrounding area. Of these 73 species, 22 were observed only off the grid, 11 were observed only on the grid, and 40 were observed to be common to both areas. During the early studies, no attempts were made to quantitate animal populations in the areas surrounding the grid; however, in 1970 preliminary population studies by trap-retrap methods were performed on beach mouse (Peromyscus polionotus) populations for a 60 day period to confirm the hypothesis that it was the most prevalent species on the grid. The hypothesis was supported by the capture of 36 beach mice from widely distributed areas on the grid, except in areas with less than 5% vegetation. Eight pairs of eastern harvest mice were taken to the laboratory and allowed to breed. Six of the eight pairs had litters totaling 24 mice. These progeny were free from any gross external birth defects. During February - May 1971, population densities of the beach mouse were studied at eight different locations on the grid and in two different areas off the grid which served as controls. Populations were estimated

on the basis of the trap-retrap data. There was no difference in mouse population densities in herbicide treated and untreated control areas affording comparable habitats. All indications were that any population differences in other animal species between the test area and the surrounding area were due to differences caused by the elimination of certain plants, and therefore, certain ecological niches, rather than being due to any direct toxic effect of the herbicides on the animal populations present on TA C-52A.

During the last day of the 1971 study, 9 mice were captured and taken to the laboratory for postmortem pathological examination. There were no instances of cleft palate or other deformities. Histologically, liver, kidney, and gonadal tissues from these animals appeared normal. In the 1973 study, several different species of animals were caught, both on and off the test grid. These included beach mice (Peromyscus polionotus), cotton mice (Peromyscus gossypinus), eastern harvest mice (Reithrodontomys humulis), hispid cotton rats (Signodon hispidus), six-lined racerunners (Cnemidophorus sexlineatus), a toad (Bufo americanus), and a cottonmouth water moccasin (Ankistrodon piscivorus). A total of 89 animals were submitted to the Armed Forces Institute of Pathology, Washington, D. C., for complete pathological examination including gross and microscopic studies. Liver and fat tissue from 70 rodents were forwarded to the Interpretive Analytical Services, Dow Chemical, U.S.A., for TCDD analyses. The sex distribution of the trapped animals was relatively equal. The ages of the animals varied, but adults predominated in the sample. No gross or histological developmental defects were seen in any of the animals. Several of the rats and mice from both groups were pregnant at the time of autopsy. The stage of gestation varied from early pregnancy to near term. The embryos and fetuses were examined grossly and microscopically, and no developmental defects or other lesions were observed. Gross necropsy lesions were relatively infrequent and consisted primarily of lung congestion in those animals that had died from heat exhaustion prior to being brought to the laboratory. The organ weights did not vary significantly between the test and control animals when an animal with lungs and kidneys showing inflammatory pathological lesions was removed from the sample. Histologically, the tissues of 13 of the 26 control animals and 40 of the 63 animals from the test grid were considered normal. Microscopic lesions were noted in some animals from both groups. For the most part, these were minor changes of a type one expects to find in any animal population. One of the most common findings was parasites. A total of 11 controls and nine grid animals were affected with one or more classes of parasites. Parasites may be observed in any wild species, and those in this population were for the most part incidental findings that were apparently not harmful to the animal. There were exceptions however; protozoan organisms had produced focal myositis in one rat and were also responsible for hypertrophy of the bile duct epithelium in a six-lined racerunner.

Moderate to severe pulmonary congestion and edema was seen in several rats and mice. All of these animals were found dead in the traps before reaching the laboratory, and the lung lesions were probably the result of heat exhaustion. The remainder of the lesions in both groups consisted principally of inflammatory cell infiltrates of various organs and tissues. These lesions were usually mild in extent and although the etiology was not readily apparent, the cause was not interpreted as toxic. The analysis of TCDD from the rodents collected in June and October 1973 indicated that TCDD or a chemically similar compound accumulated in the liver and fat of rodents collected from an area receiving massive quantities of 2,4,5-T. However, based on the pathological studies, there was no evidence that the herbicides produced any developmental defects or other specific lesions in the animals sampled or in the progeny of those that were pregnant. The lesions found were interpreted to be of a naturally occurring type and were not considered related to any specific chemical toxicity.

In 1970, beach mice were not found on the more barren sections of the grid (0 to 5% vegetative cover). However, some areas of the grid had a population density that exceeded that of the species most preferred habitat as reported in the literature. In 1973, in an attempt to correlate distribution of the beach mouse with vegetative cover (i.e., habitat preference) a trapping-retrapping program of 8 days duration was conducted. The majority of animals (63) were found in areas with 5% to 60% vegetative cover: Within this range, the greatest number of animals trapped (28) was from an area with 40% to 60% cover. A similar habitat preference has been observed along the beaches of the Gulf Coast. In this study, it appeared that the beach mouse used the seeds of switchgrass (Panicum virgatum) and woolly panicum (Panicum lanuginosum) as a food source.

Trapping data from 1971 and from 1973 were compared to determine whether an increase in the population of beach mice had occurred. The statistical evidence derived from that study showed that the 1.64 beach mice per acre population (based on the Lincoln Index for 1973) was slightly higher than the 0.8 and 1.4 mice per acre reported for a similar habitat. The population of beach mice was also higher in 1973 than in 1971 in the area of the test grid. The apparent increase in beach mouse population on the grid for 1973 over 1971 was probably due to the natural recovery phenomenon of a previously disturbed area (i.e., ecological succession). Some areas of the test grid have currently exceeded the preferred percentage of vegetative coverage of the beach mouse habitat, and other areas were either ideal or fast developing into an ideal habitat. If the test grid remains undisturbed and continues toward the climax species, a reduction in the number of beach mice will probably occur simply due to the decline of preferred habitat.

A 1973 sweep net survey of the Arthropods of TA C-52A resulted in the collection of over 1,700 specimens belonging to 66 insect families and Arachnid orders. These totals represented only one of five paired sweeps taken over a one mile section of the test grid. A similar study performed in 1971 produced 1,803 specimens and 74 families from five paired sweeps of the same area using the same basic sampling techniques. A much greater number of small to minute insects were taken in the 1973 survey. Vegetative coverage of the test area had increased since 1971. The two studies showed similarities in pattern of distribution of arthropods in relation to the vegetation, number of arthropod species, and arthropod diversity. Generally, the 1973 study showed a reduction of the extremes found in these parameters during the 1971 study. This trend was expected to continue as the test area stabilizes and develops further plant cover, thus allowing a succession of insect populations to invade the recovering habitat.

Two classes of aquatic areas are associated with TA C-52A; ponds actually on the one square mile area and streams which drain the area. Most of the ponds are primarily of the wet weather type, drying up once in the last 5 years; however, one of the ponds is spring fed. Three major streams and two minor streams drain the test area. The combined annual flow of the five streams exceeds 24 billion gallons of water. Seventeen species of fishes have been collected from the major streams and three species from the spring fed pond on the grid. Statistical comparisons of 1969 and 1973 data of fish populations in the three major streams confirm a chronologically higher diversity in fish populations. However, the two control streams confirm a similar trend in diversity. Nevertheless, from examining all of the aquatic data, certain observations support the idea that a recovery phenomenon is occurring in the streams draining TA C-52A. These observations are difficult to document

because of insufficient data. For example, in 1969 the southern brook lamprey (Ichthyomyzon gagei) was never collected in one of the streams immediately adjacent to the area of the grid receiving the heaviest applications of herbicides; however, in 1973 this lamprey was taken in relatively large numbers. These observations may or may not reflect a change in habitat due to recovery from herbicide exposure. Residue analyses (1969 to 1971) of 558 water samples, 68 silt samples, and 73 oyster samples from aquatic communities associated with drainage of water from TA C-52A showed negligible arsenic levels. A maximum concentration of 11 ppb picloram was detected in one of the streams in June 1971, but this level had dropped to less than 1 ppb when sampled in December 1971. TCDD analysis of biological organisms from streams draining TA C-52A or in the ponds on the test area were free from contamination at a detection limit of less than 10 parts per trillion.

In analyses performed 3 years after the last application of 2,4-D and 2,4,5-T herbicides, the test grid exhibited population levels of soil microorganisms identical to those in adjacent control areas of similar soil and vegetative characteristics not exposed to herbicides. There were increases in Actinomycete and bacterial populations in some test site areas over levels recorded in 1970. This was possibly due to a general increase in vegetative cover for those sampling sites and for the entire test grid. No significant permanent effects could be attributed to exposure to herbicides.

Data on aquatic alga populations from ponds on the one square mile grid (previously exposed to repetitive applications of herbicides) indicated that the genera present were those expected in warm, acid (pH 5.5), seepage, or standing waters.

SECTION I

INTRODUCTION

The Eglin AFB Reservation has served various military uses, one of them having been the development and testing of aerial spray equipment (e.g., herbicide spray equipment). It was necessary for this equipment to be tested under controlled conditions that were as near to being realistic as possible. For this purpose a testing installation was established in 1962 on the Eglin Reservation with the place of direct aerial application restricted to an area approximately one mile square within Test Area C-52A (TA C-52A) in the southeastern part of the reservation.

In support of programs testing aerial dissemination systems, TA C-52A received massive quantities of military herbicides. The purpose of these test programs was to evaluate the capabilities of the equipment systems, not the biological effectiveness of the various herbicides. After repetitive applications, personnel involved with the test program expressed concern about potential ecological and environmental hazards that might be associated with continuance of these test programs. This concern led to the establishment of an "Environmental Pollution Control and Monitoring System Task Team". One of the purposes of this report is to document the efforts of this task team and other personnel who were assigned to or were associated with the Air Force Armament Laboratory and the Armament Development and Test Center. Their efforts should serve as an indication of the interest and concern on the part of the Air Force for pollution abatement as an integral part of weapon systems development. In view of the controversy associated with the use of herbicides, TA C-52A offers a unique opportunity for evaluating the ecological effects of repetitive applications of herbicides. Data obtained during the past six years of research, plus the current research effort, may be of significance in dictating future programs involving herbicides in military programs, civic action applications, and the public acceptance of herbicides for continued use in weed and brush control programs.

1. DESCRIPTION OF GEOGRAPHICAL AND ENVIRONMENTAL FACTORS

a. General Area

The Eglin AFB Reservation is located in Northwest Florida where it occupies a portion of Santa Rosa Island, Okaloosa Island, the southeastern part of Santa Rosa County, the southern half of Okaloosa County, and the southwestern quarter of Walton County. It covers an area of approximately 750 square miles. To the south the Reservation is adjacent to Choctawhatchee Bay and the Gulf of Mexico, while to the north and east it is bordered roughly by the Yellow River and Alaqua Creek.

The Reservation lies on generally level or gently rolling terrain, all under 300 feet elevation and sloping to sea level on the west and south. It is drained by small tributaries of the Yellow River and Alaqua Creek and by smaller streams that flow directly into Pensacola Bay and Choctawhatchee Bay. The valleys of these streams often are steep sided and terminate abruptly. The soil of most of the Reservation consists of somewhat excessively drained, deep, acid sands of the Lakeland series. In the stream bottoms, and particularly along the Yellow River, the soils are much more heavily organic.

b. Test Area C-52A

Test Area C-52A is located in the southeastern part of the Eglin Reservation. It covers an area of approximately three square miles (Figure I-1) and is a grassy plain surrounded by a forest stand that is dominated by longleaf pine (Pinus palustris), sand pine (Pinus clausa), and

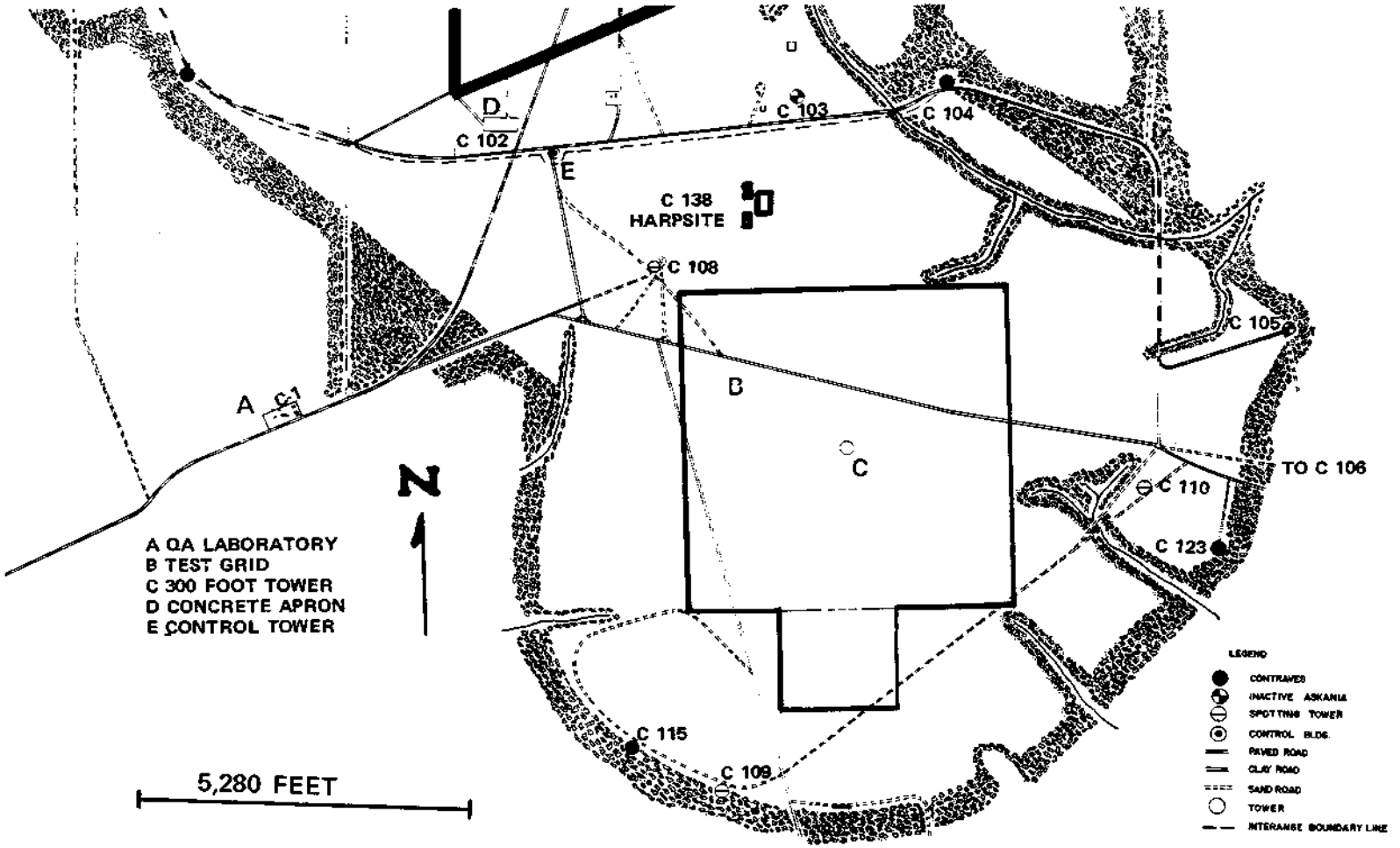


Figure I-1. Map of Test Area C-52A, Eglin AFB Reservation, Florida

turkey oak (Quercus laevis). The actual area for test operations which occupies an area of two square miles, is a cleared area occupied mainly by broomsedge (Andropogon virginicus), switchgrass (Panicum virgatum), and low growing grasses and herbs. Much of the center of the range was established prior to 1960, but the open range as it presently exists was developed in 1961 and 1962.Figures I-2(a)and I-3 are aerial photographs of the one square mile test grid and the immediate adjacent area as it appeared on 16 March 1971 and 14 June 1973, respectively. The test grid is approximately 93 feet above sea level with a water table of six to ten feet. The major portion of this test area is drained by five small creeks whose flow rates are influenced by a 60.4-inch average annual rainfall (Table I-1). The average temperature for the area is 64.9°F (Table I-2). The average maximum and minimum temperatures (°F) by month for the test area are shown in Table I-3. For the most part, the soil of the test grid is a fine white sand on the surface changing to yellow beneath. The profile composition for a typical 3-foot soil core is shown in Table I-4. The soils of the range are predominantly well drained, acid sands of the Lakeland Association with 0 to 5% slope. Figure I-2(b) shows the location of the Lakeland, Chipley, and Rutledge sand series of the Lakeland Association as found on the one square mile grid. The Lakeland sand that covers most of the grid area forms excessively drained thick deposits that extend to a depth of about seven feet. This sand is characteristically very dry, even with 60 inches of annual rainfall. The Chipley sand is moderately well drained, and the water table in this soil may rise to within 20 to 40 inches of the surface for three months during the year. The Rutledge sand is poorly drained, strongly acid (pH 4.5 to 5.0) soil. The water table in this sand is within ten inches of the surface for several months during the year.

2. DESCRIPTION OF THE TEST FACILITY

a. Description

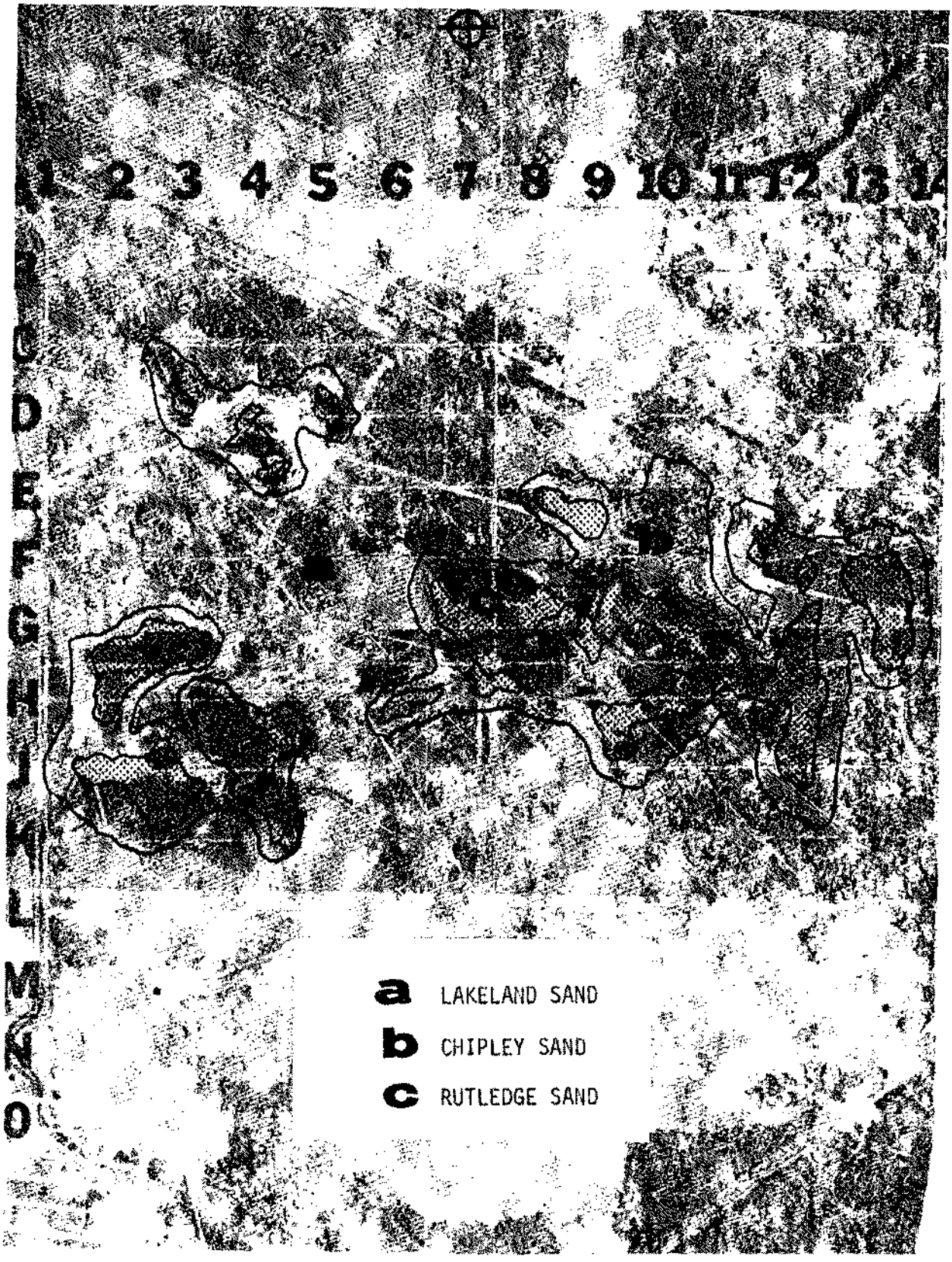
Test Area C-52A, the southern most portion of the TA C-52 range complex, is a 3 square mile cleared area on which a one square mile micrometerological and aerosol/particulate sampling grid was located (Figure I-1). Test Site C-1, on the western edge of TA C-52A, was the control center for operation of the sampling instrumentation, grid support and test data assessment. Test Site C-102 at TA C-52 Central, provided cinetheodolite time-space-position support and fixed and mobile communications for TA C-52A mission aircraft control. This test area was closed January 1971.

b. Capabilities and Uses

Test Area C-52A was used for assessing the dissemination and deposition characteristics of aerially delivered liquid and particulate materials from spray tanks and other systems of a similar nature. Micrometeorological conditions existing below 300 feet over the test area were continuously described by the Automatic Meteorological Data Acquisition and Processing System (AMDAPS) which included wind, temperature, and dew point sensors on a 300-foot tower at grid center and wind sensors on 12-foot masts located at each of the four corners of the one square mile grid. A complex of defoliant grids, intersecting near the central AMDAPS tower and oriented to eight major compass headings, provided 16 discrete sampling grids which could be selected for the most advantageous wind conditions prior to and during mission time. These grids employed glass plates and Kromekote cards for physical collection of test materials in droplet form. Each of the 250 permanent sampling stations of the TA C-52A basic grid array employed a wide variety of sampling devices including the above but were also equipped with individual commercial power and sequencing control lines for remote operation of automatic vacuum type samples which collected small particle and aerosol test materials. These sampling stations were arranged on 400-foot centers to form the one square mile grid (see sampling station array in Figure I-4). Remotely controlled, battery operated, portable samplers were also available to gather data in special purpose grid configurations anywhere in a 10 square mile area.

(a) Photograph of the One Square Mile Grid Taken at 5,000 Feet Above Ground Level on 16 March 1971

Figure I-2. Test Area C-52A



(b) Soil Types of the One Square Mile Grid on Test Area C-52A

Figure I-2. Concluded

Figure I-3. Photograph of the One Square Mile Grid Taken at 4,300 Feet Above Ground Level on 14 June 1973

TABLE I-1. ANNUAL TOTAL PRECIPITATION FOR EGLIN AFB AND NICEVILLE, FLORIDA, FROM 1964 TO 1969

YEAR	PRECIPITATION, Inches	
	EGLIN AFB	NICEVILLE
1964	68.10	72.68
1965	61.85	65.29
1966	51.10	66.95
1967	62.76	73.05
1968	31.68	42.33
1969	60.01	68.96
Average[a]	55.92	64.88

[a]Average of the two locations is 60.40 inches

TABLE I-2. AVERAGE MONTHLY TEMPERATURES (^{o}F) FOR FOUR YEARS AT NICEVILLE, FLORIDA

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1966	45.4	50.1	56.1	65.3	73.8	76.1	81.7	79.3	77.1	67.7	56.6	49.6
1967	49.4	48.9	60.8	70.6	72.2	79.2	78.7	72.4	63.5	55.2	56.8	65.4
1968	48.7	45.9	54.5	67.9	72.3	79.9	79.2	81.5	75.5	66.6	55.0	49.3
1969	49.8	52.0	52.1	66.9	73.2	80.5	81.2	78.1	75.2	69.6	54.5	49.2
Average[a]	48.3	49.2	55.9	67.7	72.9	78.9	80.2	78.3	72.8	64.8	55.7	53.4

[a]Average temperature for 4 years is 64.9^{o}F

TABLE I-3. AVERAGE MAXIMUM AND MINIMUM TEMPERATURES (°F) BY MONTH FOR FOUR YEARS (1966 - 1969) TAKEN FROM THE SIX FOOT LEVEL AT THE CENTER TOWER OF TEST AREA C-52A, EGLIN AFB, FLORIDA

MONTH[a]	TEMPERATURE, °F	
	MINIMUM	MAXIMUM
January	40.05	58.86
February	40.55	60.69
March	48.15	68.32
April	59.96	81.52
May	62.67	82.15
June	68.38	86.04
July	68.82	87.48
August	71.04	87.71
September	66.90	84.06
October	54.24	77.41
November	46.09	68.03
December	42.08	61.67

[a]Average temperature for 4 years is 65.54°F

TABLE I-4. SOIL PROFILE (6-INCH INCREMENTS) FOR TEST AREA C-52A, EGLIN AFB RESERVATION, FLORIDA[a]

DEPTH, Inches	SAND, %	SILT, %	CLAY, %	O.M., %[b]	C.E.C.[c]
1 - 6	91.6	4.0	4.4	0.46	1.19
6 - 12	90.1	4.3	5.6	0.20	0.81
12 - 18	92.1	4.3	3.6	0.20	0.73
18 - 24	92.9	3.5	3.6	0.00	0.69
24 - 30	93.1	2.8	4.1	0.07	0.69
30 - 36	92.8	3.6	3.6	0.07	0.69

[a]As determined by the Soils Department, University of Florida, Gainesville, Florida. Soil sample taken within 50 feet of K-9 permanent sampling station.

[b]Percent organic matter.

[c]C.E.C. (cation exchange capacity) is the ability of a cation to be displaced or exchanged from the soil by another cation. The cation exchange capacity of a typical greenhouse potting soil is 11.43. A soil with a cation exchange capacity of 1 can "bind" or "fix" 10 ppm of a given cation(s).

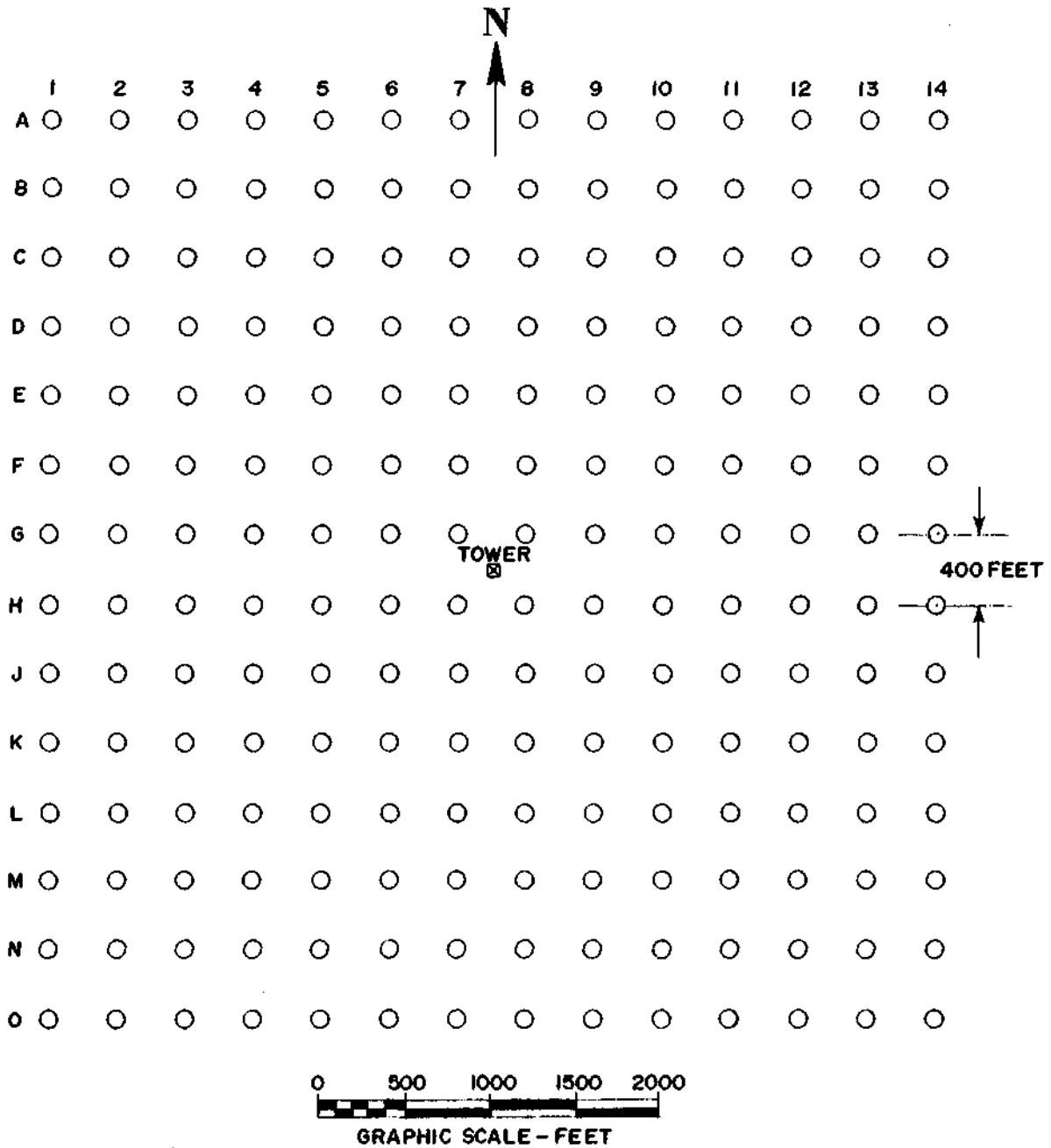


Figure I-4. Location of the Permanent Sampling Stations on the One Square Mile Grid

Fixed and portable illuminated flight line markers were available for missions during hours of darkness. A Quantitative Assessment Laboratory facility, collocated with the Test Site C-1 control center, received the collected samples and performed appropriate chemical analysis or biological assays providing data for assessment of test item performance. Micrometeorological data from TA C-52A was both recorded at the AMDAPS master station located at Test Site C-1 and transmitted to the main base Staff Meteorologist Control Center, Eglin AFB, via teletype on a 24-hour basis.

3. DESCRIPTIONS OF SAMPLING GRIDS AND HERBICIDE DEPOSITION

Descriptions of the sampling grids located on TA C-52A and individual mission data including herbicide and total gallons sprayed have been compiled by Biever[1].

a. Grid Descriptions

Figure I-5 shows the location of the various herbicide grids that were located on TA C-52A. The original sampling grid (Grid 1) for spray equipment testing became operational in June 1962. It consisted of four intersecting straight lines in a circular pattern, each line being at a 45^{o} angle from those adjacent to it. This grid was discontinued after two years. It was located immediately south of the one square mile grid.

The second sampling grid (Grid 2) consisted of three parallel lines intersected at right angles by another set of three parallel lines. These lines were 800 feet apart, thus forming four equal quadrants. The southwest corner of this grid corresponded to the southwest corner of the one square mile grid. The parallel line grid was operational during the period May 1964 to November 1965.

The third sampling grid (Grid 3) consisted of three concentric circles, with respective diameters of 1200 , 1600, and 2000 feet. This grid was located in the northeast quadrant of the one square mile grid. The concentric circles grid was operational between October 1967 and April 1968; however, difficulty in interpreting data from this sampling array caused use of this grid to be discontinued.

The fourth sampling grid (Grid 4) is a one square mile grid, the center of which was marked by a 300-foot tower. This was the last testing grid used on TA C-52A and its inwind and crosswind sampling arrays extended into Grid 2 and Grid 3. Figures I-6, I-7, I-8, and I-9 show various views of Grid 4 at the time the grid was under construction.

The two inwind and four crosswind sampling arrays of Grid 4 became operational in May 1968. Each inwind array consisted of three parallel rows spaced 400 feet apart, with 297 sampling stations per row. The aircraft flight path crossed the midpoints of the sampling lines. The crosswind sampling arrays consisted of three parallel rows 400 feet apart, with 253 sampling stations per row.

b. Deposition Rate

The total amounts of chemicals (including herbicides, insecticides, oils, and simulants) applied to TA C-52A are shown in Table I-5. All of these materials were disseminated during the period from June 1962 to December 1970 (Figure I-10). The total pounds of actual herbicides

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[1]Defoliant History of Test Area C-52A, Working Papers, Vitro Corporation of America and Armament Development and Test Center, Eglin AFB, Florida, December 1969.

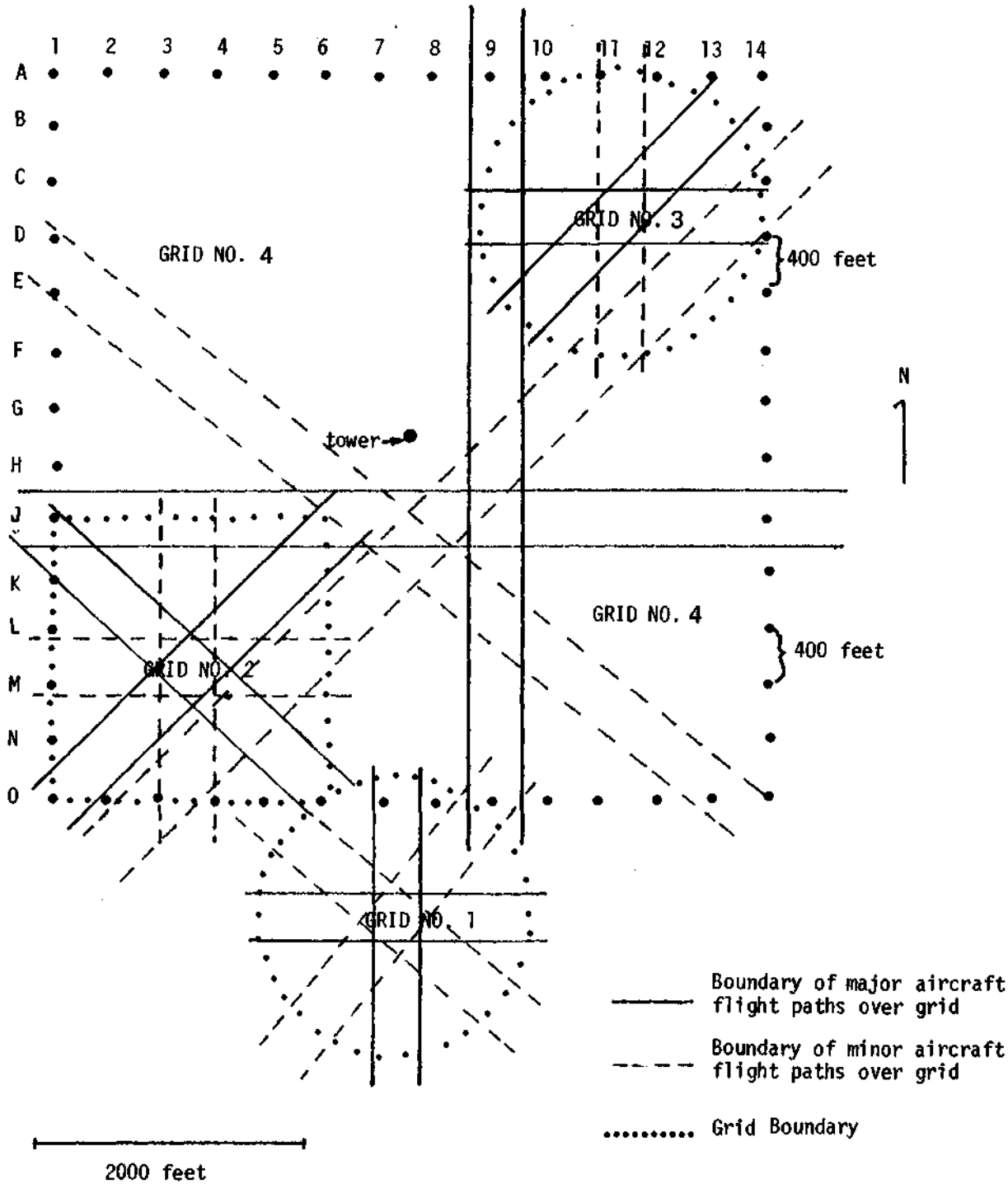

Figure I-5. Location of Test Grids and Major Flight Paths Used during Dissemination of Herbicides Over Test Area C-52A

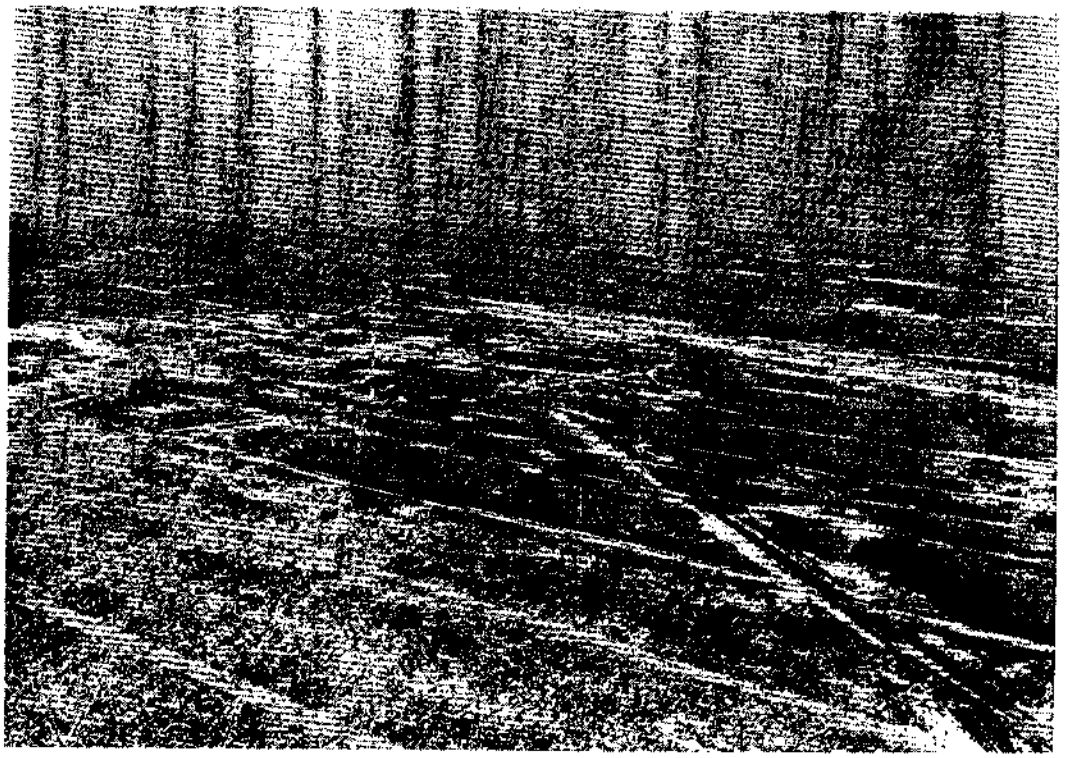

Figure I-6. A View of Grid Number 4 Looking from the Southeast to the Northwest, 1964

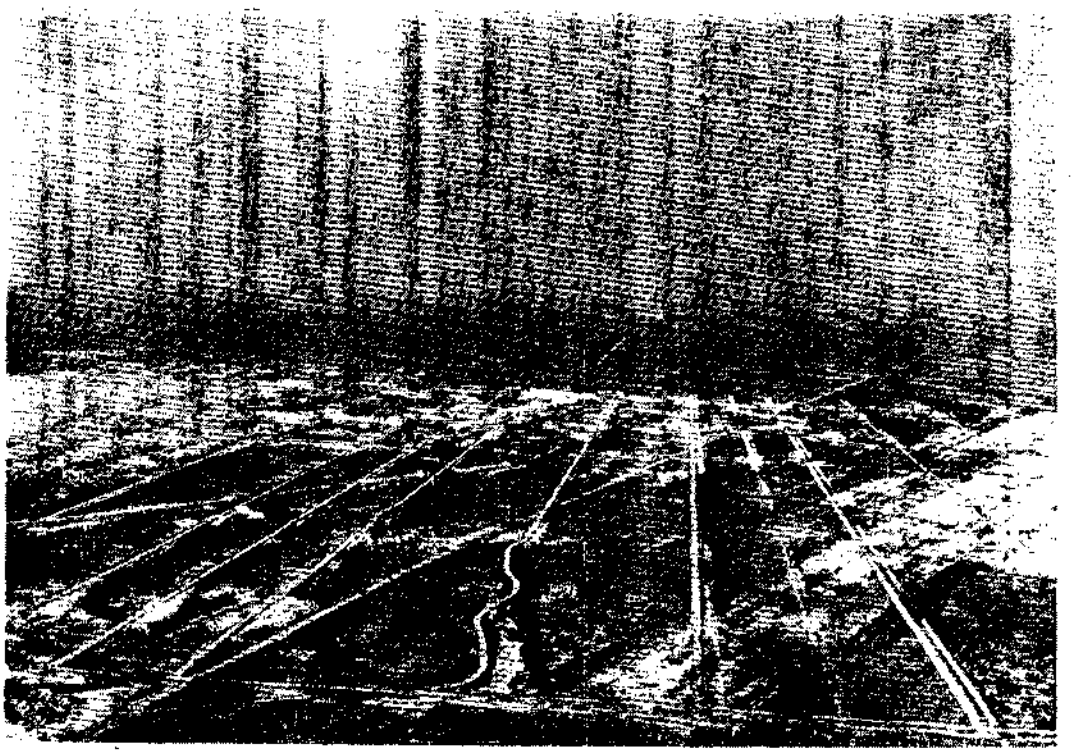

Figure I-7. A View of Grid Number 4 Looking from East to West, 1964

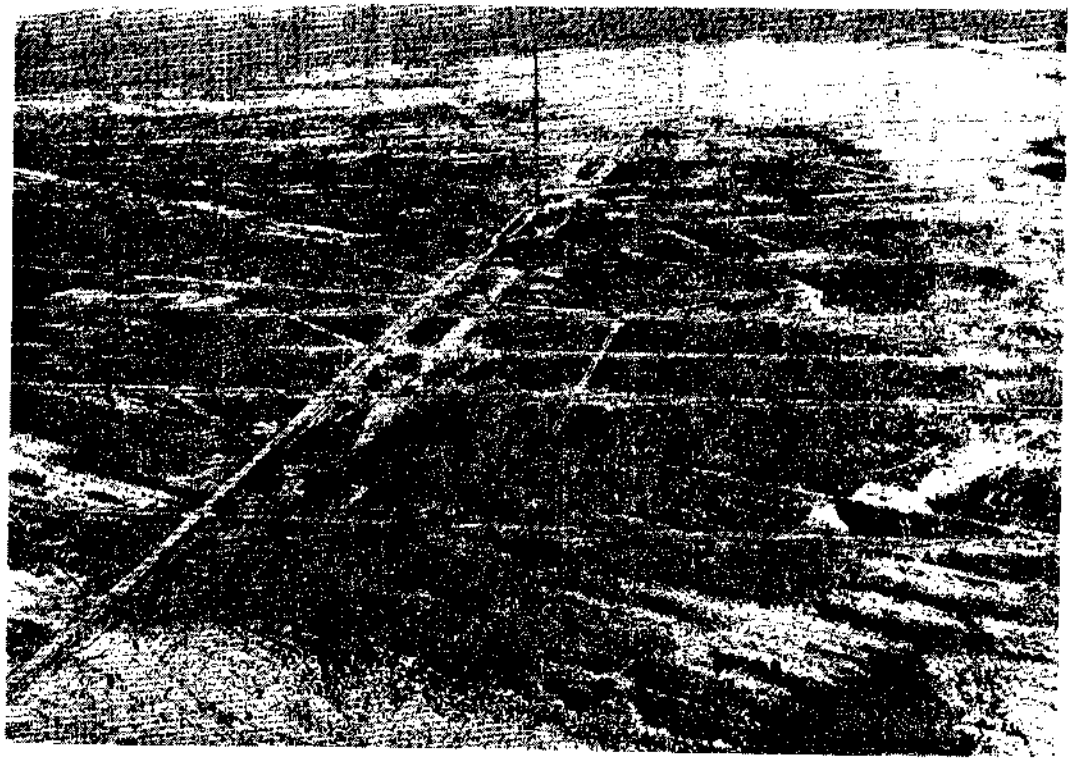

Figure I-8. A View of Grid Number 4 Looking from North to South, 1964

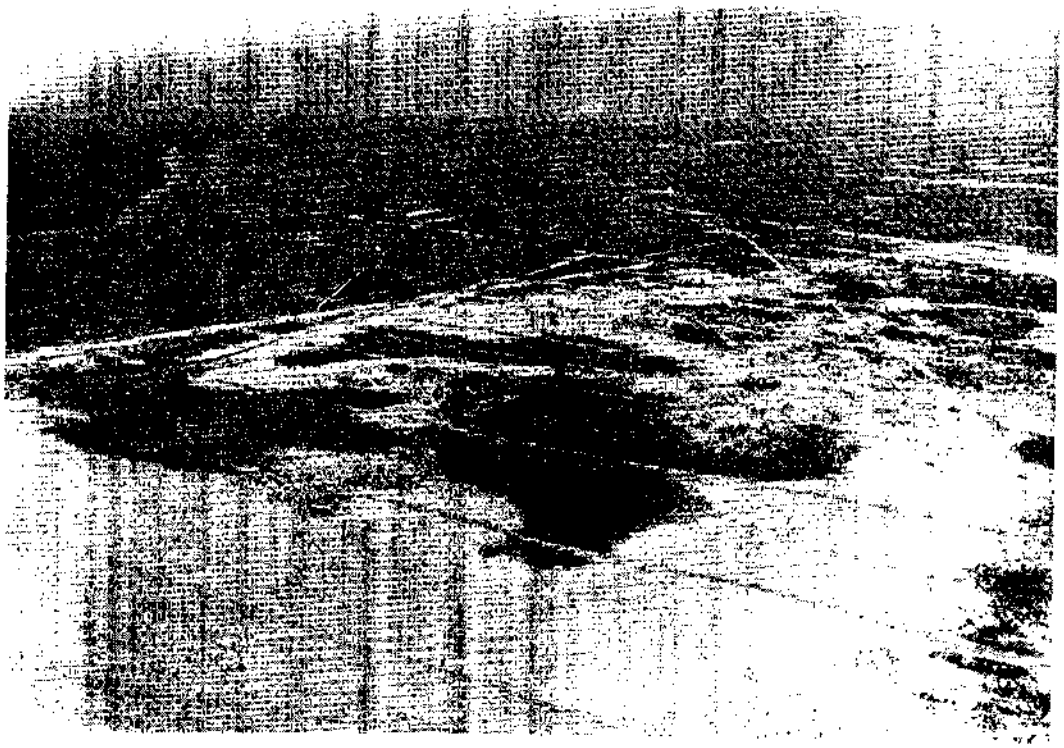

Figure I-9. A View of the Western Portion of Grid Number 4 Looking from the Southeast to the Northwest, 1964

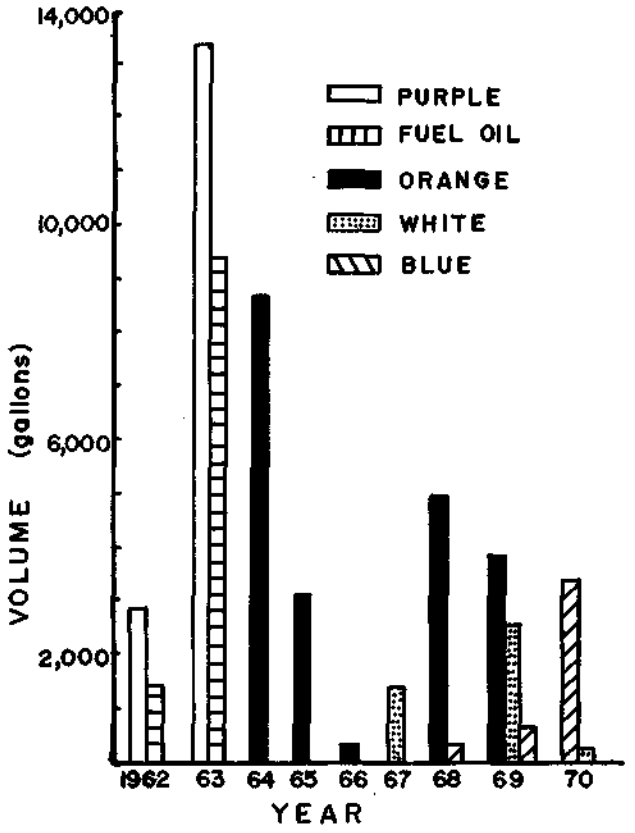

Figure I-10. Annual Dissemination of Herbicides on Eglin AFB Test Area C-52A

deposited on the test area (total of all grids) is shown in Table I-6. The approximate deposition rate of herbicides, pounds active ingredient per acre, for each grid is shown in Table I-7.

4. DESCRIPTION OF PESTICIDES

As closely as possible the equipment utilized on TA C-52A was tested under realistic yet controlled conditions. Most testing programs involving military herbicides and insecticides actually included the pesticides themselves rather than simulants. The low toxicity associated with these pesticides was the salient justification for such action.

a. Orange

Orange was a reddish-brown to tan colored liquid soluble in diesel fuel and organic solvents, but insoluble in water. One gallon of Orange contained 4.21 pounds of the active ingredient of 2,4-dichlorophenoxyacetic acid (2,4-D) and 4.41 pounds of the active ingredient of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Orange was formulated to contain a 50:50 mixture of the n-butyl esters of 2,4-D and 2,4,5-T. The percentages of the formulation were:

n-butyl ester of 2,4-D	49.49%
free acid of 2,4-D	0.13%
n-butyl ester of 2,4,5-T	48.75%
free acid of 2,4,5-T	1.00%
inert ingredients (e.g., butyl alcohol and ester moieties)	0.62%

Some of the physical, chemical, and toxicological properties of Orange are listed in Table I-8.

TABLE I-5. APPROXIMATE TOTAL VOLUME OF HERBICIDES, INSECTICIDES, AND/OR SIMULANTS APPLIED TO TEST AREA C-52A, EGLIN AFB RESERVATION, FLORIDA, 1962 - 1970

CHEMICAL	GALLONS DISSEMINATED
Orange	19,807
Purple[a]	16,164
White	4,172
Blue	4,395
Stull Bifluid[b]	1,716
Fuel Oil	10,863
Orange Simulant[c]	1,460
Malathion Insecticide	215
Total	58,792

[a]Purple was a mixture of n-butyl 2,4-D (50%), n-butyl 2,4,5-T (30%), and isobutyl 2,4,5-T (20%). The isobutyl portion was included as a measure to depress the freezing point of 2,4,5-T. This mixture was eventually replaced by Orange.

[b]Stull Bifluid consisted of Orange (85%) plus a chemical additive, which when mixed in the spray system pump during agent dissemination produced a gel defoliant.

[c]Orange simulant consisted of glycerine (68%), sodium thiosulfate (16.8%), and water (15.2%).

TABLE I-6. TOTAL POUNDS OF ACTIVE INGREDIENTS OF HERBICIDES DISSEMINATED ON TEST AREA C-52A, EGLIN AFB RESERVATION, FLORIDA, JUNE 1962 - DECEMBER 1970

HERBICIDE	POUNDS ACTIVE INGREDIENT
2,4-D	169,292
2,4,5-T	160,948
Picloram	2,253
Cacodylic Acid and Sodium Cacodylate	13,624

TABLE I-7. APPROXIMATE DEPOSITION RATE OF HERBICIDES APPLIED TO TEST AREA C-52A, EGLIN AFB RESERVATION, FLORIDA

TEST GRID[a]	GRID AREA[b], Acres	HERBICIDE (POUNDS ACTIVE INGREDIENT/ACRE)				
		2,4-D	2,4,5-T	PICLORAM	CACODYLIC ACID	ARSENIC[c]
1	92	947 (1962-1964)[d]	947 (1962-1964)	--	--	--
2	92	584 (1964-1966)	584 (1964-1966)	--	--	--
3	92	30 (1967)	--	8 (1967)	11 (1968)	6
4	240	183 (1968-1969)	160 (1968-1969)	6 (1969-1970)	53 (1969-1970)	28

[a]The test grids are described in text

[b]In actuality, grids 2 and 3 fall within the confines of the 640 acre grid 4. However, the positioning of the test arrays on grid 4 has resulted in most of the herbicide being disseminated within a 240 acre area, with only slight infringement on the original sites of grids 2 and 3.

[c]Pounds per acre of arsenic as the organic pentavalent form; calculated on weight of Blue applied per acre.

[d]Years when the majority of the herbicide was applied.

TABLE I-8. PHYSICAL, CHEMICAL, AND TOXICOLOGICAL PROPERTIES OF THE THREE MAJOR HERBICIDES AND ONE MAJOR INSECTICIDE

CHEMICAL	SPECIFIC DENSITY (25C)[a]	VISCOSITY CENTIPOISE (23C)	MOLECULAR MASS	WEIGHT OF FORMULATION (lbs/gal)	WEIGHT ACTIVE INGREDIENT (lbs/gal)	SOLUBLE IN WATER	RELATIVE TOXICITY	SPECIFIC TOXICITY FOR WHITE RATS (mg/kg)
Orange	1.282	43	618	10.7	8.62	No	Low	566
White	1.120	125	1,173	9.4	2.54	Yes	Very Low	3,080
Blue	1.324	14	296	10.9	3.10	Yes	Very Low	2,600
Malathion	1.232	36	328	10.3	9.74	No	Low	1,375

[a]As determined by the Air Force Armament Laboratory

b. White

White was a dark brown, viscous liquid that was soluble in water but insoluble in organic solvents and diesel fuel. One gallon of White contained 0.54 pound of the active ingredient of 4-amino-3,5,6-trichloropicolinic acid (picloram) and 2.00 pounds of the active ingredient of 2,4-D. White was formulated to contain a 1:4 mixture of the triisopropanolamine salts of picloram and 2,4-D. The percentages of the formulation were:

triisopropanolamine salt of picloram	10.2%
triisopropanolamine salt of 2,4-D	39.6%
inert ingredient (primarily the solvent triisopropanolamine)	50.2%

Some of the physical, chemical, and toxicological properties of White are listed in Table I-8.

c. Blue

Blue was a clear yellowish-tan liquid that was soluble in water, but insoluble in organic solvents and diesel fuel. One gallon of Blue contained 3.10 pounds of the active ingredient dimethylarsinic acid (cacodylic acid). Blue was formulated to contain both cacodylic acid (as the free acid) and the sodium salt of cacodylic acid (sodium cacodylate). The percentages of the formulation were:

cacodylic acid	4.7%
sodium cacodylate	26.4%
surfactant	3.4%
sodium chloride	5.5%
water	59.5%
antifoam agent	0.5%

Some of the physical, chemical, and toxicological properties of Blue are listed in Table I-8. It should be noted that cacodylic acid and sodium cacodylate contain arsenic in the form of the pentavalent, organic arsenical. This form of arsenic is essentially nontoxic to animals as can be noted by the LD_{50} value for white rats. Of the total formulation, 15.4% is arsenic in the organic form, only trace quantities are present in the inorganic (toxic) form.

d. Malathion

Malathion insecticide (0,0-dimethyphosphorodithioate) was a clear brown to colorless liquid with a slight characteristic odor. The ultra-low-volume (ULV) formulation was very slightly soluble in water (145 ppmw). Malathion ULV had a minimum purity of 95%. One gallon of ULV malathion contained 9.74 pounds active ingredient and 0.51 pound inert ingredients. Some of the physical, chemical, and toxicological properties of malathion are listed in Table I-8.

SECTION II

BIOASSAY AND CHEMICAL RESIDUE STUDIES OF THE SOILS OF TEST AREA C-52A

From the rates that were applied during the years of testing spray equipment, it was obvious that Test Area C-52A at Eglin AFB Reservation offered a unique opportunity to study herbicidal persistence and soil leaching. Yet, the problem of how best to assess the level of residue was a difficult one. The herbicides could be chemically present but because of soil binding might not be biologically active. Moreover, as noted in Section I, many chemicals were applied to the test area, and a biological assessment might be the result of two or more chemicals interacting. Thus, both bioassay techniques and analytical analysis were employed. The results of the bioassay studies by A. L. Young and J. H. Hunter have not been published; however, the methodology developed and the results obtained have played a major role in understanding the ecological succession of the plant and animal communities on the test area. Thus, a detailed synopsis of their work is included in this report.

1. SYNOPSIS OF BIOASSAY RESEARCH, 1969 - 1970

In the late summer of 1969, six 5-foot cores were randomly collected from an area known to intersect spray flight paths used for missions involving the herbicide designated as Orange. The samples were taken to the laboratory and subsampled for bioassay and analytical results. The bioassay technique employed the use of soybean (variety Clarke 63) for detecting phenoxy-herbicide residue. The experiments were conducted under greenhouse conditions. No standard herbicide concentrations were included; instead, cores from treated areas were compared to control cores and the relative differences noted. Comparisons for each depth were made against control plants for the particular depth.

These initial bioassay studies indicated two things. First, significant concentrations of herbicides (or phytotoxic materials) were present on the test grid; and second, these herbicides were definitely leaching or penetrating into the soil (at least to a depth of 3 feet). Moreover, the bioassay analysis indicated different relative concentrations of herbicides both between cores and within a given core.

As noted earlier, the area of interest was an area greater than one square mile. Obviously, this area was too large to completely bioassay or to subject to chemical analyses. Therefore, it was decided to find the areas of greatest herbicide concentration and follow up with detailed bioassay and chemical analyses. To find these specific areas, it was necessary to design an experiment that would allow inferences about herbicidal persistence for the entire test area. Consulting statisticians assisted in designing the experiment and in analyzing the results.

In order to properly evaluate herbicidal persistence and soil leaching, a vegetation chart of the test grid was prepared on 26 March 1970. The greatest amounts of vegetation were found near the water sources of the grid. There were two areas that supported very dense vegetation. A terracing effect of diminishing amounts of vegetation away from these two areas was apparent. The effects of repeated spray could be seen along the flightpaths most frequently used in test programs. In these strips, vegetation occurred only near the water sources and even there it was scant. By considering the flightpaths, the water sources, and the terracing effects, it was possible to divide the test grid into 16 vegetation areas. These areas formed the base for the random selection of soil samples. The statistical null hypotheses that were to be investigated included the following:

1. There were no herbicide concentration differences among the soils of the various vegetative areas.

2. There were no differences in herbicide content among soil depths down to 3 feet.

3. There were no interactions between the vegetative areas and the soil depths.

In order to conduct an experiment that would provide reliable evidence with respect to these hypotheses, three random 3 foot soil cores were taken from each of the vegetative units and three from a control area, an area 0.2 mile northwest of the square mile grid. Figure II-1 shows the sites for the random sampling of these soil cores. These cores provided the replication for the experiment. Because of the time involved in taking the soil cores and the possible effects of the soil drying out if left unplanted for several days, it was necessary to apply the technique of blocking over the days of soil core removal and planting. The experiment was initiated 1 April 1970. Again the bioassay organism was soybean (five seeds per cup and one cup per 6 inch increment of soil core), and the experiment was conducted in a greenhouse.

A series of standards for herbicide Orange was included in this experiment (range of standards was 0.25 to 4.00 ppm Orange). All standards were prepared in soil taken from the top 6 inch increment of control soil. The results indicated that there were herbicidal persistence and leaching; of the 48 treatment cores collected and bioassayed, 27 cores were significantly different from control cores (95% probability level). The results indicated that soil leaching or penetration was much more prevalent along the dissemination flightpaths than in other areas of the test grid. Moreover, there were differences among the soils of the various vegetative areas within a given flightpath. Likewise, differences were found in herbicide content among the increments of many of the soil cores. This was probably due to both the elapsed time since herbicide application and to such factors as rainfall frequency and organic matter content of the soil. It is interesting to note that there were no statistical evidences of differences between wet and dry soils that received approximately the same amounts of herbicide. Efforts to quantitate the bioassay were confined to only the top 6 inch increment because of within-core variations. By considering that all phytotoxic effects were from Orange, the approximate concentration was 2.82 ppm herbicide. This was an average value for the top 6 inches of soil core for the eight cores showing greatest herbicide concentration.

In reference to the statistical null hypothesis, all three were rejected: (1) there were differences in herbicide concentration between cores; (2) there were differences in herbicide content within cores; and, (3) there were interactions between the sampling areas and the soil depths (this indicated non-uniformity in soil strata).

Sixteen of the soil cores (one from each vegetation type) were subsampled for arsenic concentration (hence, a measure of Blue). The arsenic was extracted by a cold-acid extraction technique and analyzed by atomic absorption spectrophotometry. Four of the 16 locations contained arsenic levels above 1.0 ppm in the top 6 inches of soil. A further analysis for arsenic in the soil profile indicated that arsenic readily (and almost uniformly) leached throughout the soil profile. In areas receiving repetitive applications of Blue, the top 6 inches of soil contained arsenic levels of 1.4 ppm, and the additional 6 inch increments down to 5 feet contained from 0.70 to 1.2 ppm arsenic.

From the bioassay study, it was evident that some areas of the test grid contained high levels of phytohormonal herbicide residue (i.e., residue showing plant responses similar to those caused by 2,4-D; 2,4,5-T; and picloram). Thus, 5 foot cores were collected from two areas (dry soils) exhibiting highest herbicide residue. Each core was divided into 6 inch increments, placed in amber bottles, and immediately shipped to the United States Department of Agriculture, Pesticide Degradation Laboratory, Beltsville, Maryland, for analysis of 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD). No TCDD was found in either soil core (the Pesticide Degradation Laboratory reported a detection limit capability of 0.0005 ppm TCDD).

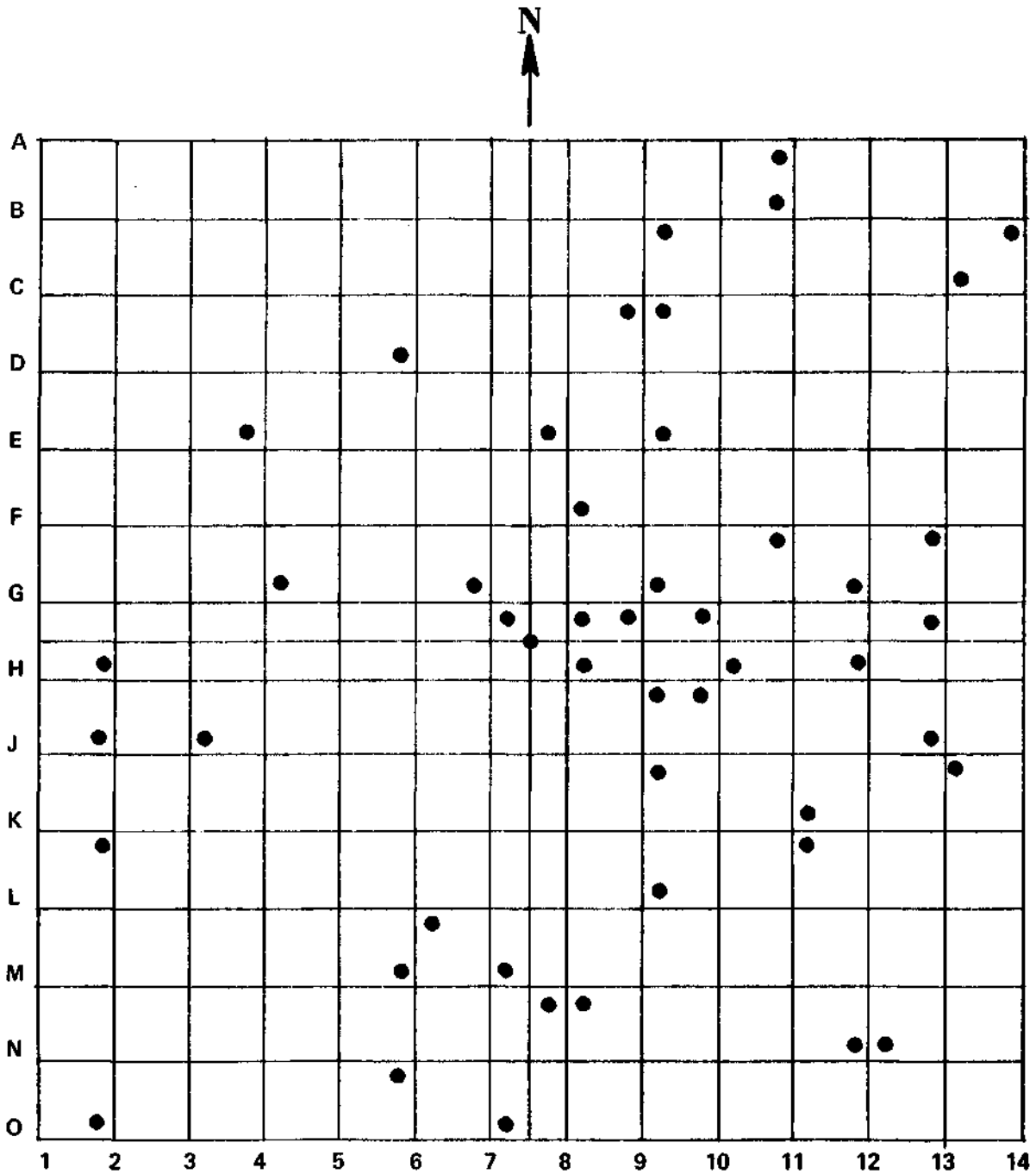

Figure II-1. Collection Sites for the Random Sampling of 3 Foot Soil Cores For Residue Analyses

2. SYNOPSIS OF BIOASSAY RESEARCH, 1970 - 1971

a. Introduction

A follow-up bioassay experiment of six of the field locations studied in the previous bioassay experiments was initiated in December 1970. Three of the samples were selected because they showed leaching to a depth of 36 inches, two because of leaching to 30 inches, and the remaining sample because of leaching to 18 inches. In addition, two samples were obtained from grid 1 (the 1962 - 1964 grid). These samples had not been previously bioassayed, but because of prior history, it was expected that data on persistence could be obtained from both a bioassay and a chemical analysis.

Since the preliminary work indicated that although equal amounts of Orange were introduced into soil cores, the varying organic composition of the soil at different depths influenced the amount of Orange available to the plants. This investigation attempted to determine if differences in amounts of herbicides and in soil composition do affect plant growth and, if so, the magnitude of this effect. This information was then used to estimate the concentration of Orange in the selected sites.

b. Method and Materials

In order to calculate the effects of herbicide concentration upon plant growth and to study the effects of soil depth on herbicide activity, a soil core was taken from a control site 0.2 mile northwest of the one square mile test area. This core was collected in 6 inch depth segments down to 3 feet. After being dried, sieved, and weighed, each depth-segment unit was divided into seven parts and treated so that soil samples from each unit contained the following concentrations of Orange: 0.028 part per million (ppm), 0.057 ppm, 0.113 ppm, 0.226 ppm, 0.454 ppm, and 0.908 ppm; one soil unit was not treated and so had 0.0 ppm concentration of Orange. These soil samples were then used as standards.

Each of these samples was thoroughly mixed and distributed among six cups. Five soybean seeds were planted in each of three of the cups and five cucumber seeds were planted in each of the remaining three cups for each concentration. All bioassays were conducted in an ISCO E-3 environmental chamber maintained at a diurnal temperature regime of 90^{o} to 70^{o}F, a diurnal humidity regime of 65% to 85%, and a 14-hour daylength. The length of the cucumber plants from the root tip to the end of the epicotyl was measured after 6 days. The soybeans were harvested 10 days after planting, and the root length of each plant was recorded.

Samples of soil were also collected from six selected sites on the test grid. These locations were coded using the coordinate of the grid marker and the direction with respect to that marker in which the cores were taken. These six locations were B-14 SW, C-9 SW, J-3 NE, M-8 SW, N-12 NW, and 0-7 NE. A composite of three soil cores, 2-1/2 feet apart, was collected from each site at a distance 50 feet from the designated grid marker and on an imaginary line perpendicular to the specified direction for sampling. These cores were collected in 6-inch segments down to 3 feet, and the soil from the corresponding depth segments for each of the three cores collected from a specified site were throughly mixed. A control soil sample was collected in a similar manner from the same location that the soil for the standards was taken. The soil from each site/depth segment unit was distributed among six cups and a bioassay was conducted in the same manner as that described above for the Orange concentration standards. Again both cucumber and soybeans were used as the test organisms.

This same procedure was used to collect and bioassay soil samples from sites 50 yards south of grid markers O-5 and O-8 which are on the southern border of the present test area (and in the old grid 1 area). Control soil was collected for this bioassay also from the same site as the other controls and the standards, and soybeans and cucumbers were used as the test organisms.

In addition, soil samples from many locations on the test grid were collected and analyzed for organic matter content. Samples were analyzed by the use of a muffle furnace (total combustion of organic matter).

c. Statistical Methods

General. Except as noted, statistical analyses were conducted to test for significant differences at the 0.95 probability level. If a significant difference was indicated at this level, further testing was conducted at the 0.99 probability level.

Wide variations occurred among the measurements of the plants within the individual cups. The seeds actually belonged to two populations: (1) those seeds that would germinate under proper conditions and (2) those seeds that would not germinate. It was impossible to determine which population an individual seed belong to in all cases, and the extreme values (zero length when a seed did not germinate) would have biased the results had the arithmetic mean been used as a representative cup value. Therefore, the median measurement of the plants within a cup was used as the cup value, and the cup became the experimental unit.

Calibration of Standards. An analysis of variance (ANOVA) technique was employed to study the effects upon the plants grown in the soils treated with the standard concentrations of the defoliant Orange. This was done to determine those concentrations which affected plant growth in a significantly different manner. Also, it was hypothesized that because of soil composition variations with depth, a different percentage of the applied defoliant would be bound to the soil or in other ways unavailable to the plants. If this were the case, different standard curves would have to be developed for each depth group.

A test for homoscedasticity indicated unacceptable differences in variances. The transformation:

$$x = \log_{10} (x' + 1)$$

Where :

x = the transformed data

x' = the original cup value

provided homogeneity of variance.

Both the concentration levels and the depth increments were arbitrarily selected and thus are parametric factors. Since each corresponding site/depth-segment was treated alike, the experiment has a cross-classified design, and the statistical model can be expressed:

$$y_{ijk} = a + c_1 + d_j + (cd)_{ij} + e_{ijk}$$

Where:

a = the overall effect

c_i = the effect of the i^{th} concentration (i = 1,2,...,m)

d_j = the effect of the j^{th} depth-segment (j = 1,2,...,n)

e_{ijk} = the error factor for the k^{th} replicate of the i^{th} concentration and the j^{th} depth-segment

y_{ijk} = the cup value for the k^{th} replication of the i^{th} concentration and the j^{th} depth-segment

The ANOVA technique employed was based on this model. In each instance that a significant difference was indicated between the levels of a factor or when a significant interaction was indicated, Duncan's new multiple range test was used to separate the levels into homogenous groups.

A mathematical expression of the form

$$y = a + bx$$

which approximated the relationship between the defoliant concentration and the cup value was determined for each homogenous group by the method of least squares. To find such a relationship a correlation study was undertaken which compared the standard data and square root and logarithm transformations of both the concentration and cup values. Once the proper form of the data was selected, regression analysis was used to study the mathematical expressions. This included tests of the following hypotheses:

(1) The data does not fit the curve (lack of fit test).

(2) One regression line can be used for all the depth groups.

(3) The regression coefficients for the different depth groups are equal.

(4) If all the regression coefficients are equal, the elevations (Y-intercepts) are equal.

Analysis of the Grid Samples. The concentration of Orange present in the soil from each site/depth-segment taken from the testing area was estimated by using the calibration curves developed for the standard concentrations. The bioassay of the standard Orange concentrations and the bioassay of the grid soil samples were done at different times; therefore, an adjustment of the data was necessary to eliminate the bias and confounding introduced by this procedure. Since control plants were grown with each group, the grid soil bioassay data was weighted according to the ratio of the average of the controls for the test area data to the average of the zero standard concentration data. (A paired-observations t-test of these groups indicated they were significantly different at the 0.95 probability level.)

3. RESULTS AND DISCUSSION

a. Calibration of Standard Curves

The results of the ANOVA was the same for both soybeans and cucumbers. The interaction effect between concentration and depth-segments was non-significant; therefore, this effect was pooled with the residual term. The effects of both concentration and depth were highly significant (at a probability level greater than 0.99). These ANOVA tables are presented in Table II-1 along with the means and the results of Duncan's new multiple range tests.

The multiple range tests indicated that for both soybeans and cucumbers each concentration level was significantly different from every other concentration level, and the range of the standard curve could include all the concentration levels tested, i.e., from 0.0 ppm to 0.908 ppm. The

TABLE II-1. ANALYSIS OF VARIANCE - ORANGE STANDARDS							
Concentration	6	5.3013	0.8836	152.34[a]	3.0419	0.5070	144.86[a]
Depth	5	0.6875	0.1375	23.71[a]	0.7185	0.1437	41.06[a]
Conc. x Depth	30	0.1195	0.0040	- - -	0.1620	0.0054	1.93
Residual	84	0.5389	0.0064		0.2314	0.0028	
Adj Error	114	0.6584	0.0058		0.3934	0.0035	

[a]Indicates significance at the 0.99 probability level.

DUNCAN'S NEW MULTIPLE RANGE TEST

Soybean Data Results -							
Conc. Mean Values:	1.06	0.97	0.92	0.80	0.73	0.59	0.43
Depth Mean Values:	0.91	0.85	0.78	0.74	0.71	0.70	
Cucumber Data Results -							
Conc. Mean Values:	1.17	1.04	0.99	0.93	0.83	0.75	0.69
Depth Mean Values:	1.07	0.93	0.91	0.87	0.85	0.85	

Note: Common underscoring indicates homogenous groups at 0.95 probability level.

depth-segment groupings for soybeans were somewhat different that those for cucumbers. At the 0.95 probability level, three distinct depth groups were indicated from the cucumber data set. The groupings indicated by the soybean data set were not as clearcut, and the 18 to 24 inch depth-segment could be placed into two different groups. Since the cucumber data set indicated that this depth-segment should be placed in the last (deepest) depth group, a similar decision was made in the case of the soybean data. These depth groupings are as follows:

	Depth, inches	
Group	Cucumber	Soybean
I	0 to 6	0 to 6
II	6 to 18	6 to 12
III	18 to 36	12 to 18
IV	- - -	18 to 36

In the correlation analysis, the necessity for transformation of both the Orange concentration values and cup values were studied. In addition to the untransformed data, square root and logarithmic transformations were included. The correlation matrices for both the soybean and the cucumber data sets are presented in Table II-2. In both cases, the best correlation (soybeans: -0.8896; cucumbers: -0.8335) was

$$\log_{10}(\text{cup value} + 1) \times \sqrt[2]{\text{concentration (ppm)}}$$

TABLE II-2. CORRELATION MATRICES			
CUP VALUES	CONCENTRATION (ppm)		
	NO TRANS	$LOG_{10}(x+y)$	SQUARE ROOT
SOYBEAN DATA			
No Trans:	-0.7423	-0.7761	-0.9353
$Log_{10}(x+y)$	-0.8405	-0.8637	-0.8896
Square Root	-0.8085	-0.8363	-0.8758
CUCUMBER DATA			
No Trans:	-0.6547	-0.6908	-0.7703
$Log_{10}(x+y)$	-0.7399	-0.7728	-0.8335
Square Root	-0.7049	-0.7385	-0.8094

The method of least squares was used to determine the best fitting linear expression of the relationship between these two transformations for each of the four depth groups identified for the soybean standards and the three depth groups identified for the cucumber standards. These preliminary mathematical equations along with the square root of the percent of deviations from the mean explained by the regression equation (correlation coefficient) are presented in Table II-3.

TABLE II-3. PRELIMINARY MATHEMATICAL RELATIONSHIPS FOR STANDARD CONCENTRATIONS			
GROUP	DEPTH, in.	REGRESSION EQUATION	CORR. COEFF.
SOYBEAN DATA			
I	0 to 6	Y = 1.15 - 0.59X	0.86
II	6 to 12	Y = 1.11 - 0.64X	0.86
III	12 to 18	Y = 1.06 - 0.69X	0.98
IV	18 to 36	Y = 1.01 - 0.72X	0.98
CUCUMBER DATA			
I	0 to 6	Y = 1.27 - 0.49X	0.86
II	6 to 18	Y = 1.13 - 0.52X	0.87
III	18 to 36	Y = 1.06 - 0.50X	0.92

Where:

$Y = \log_{10}(\text{cup value} + 1)$

$X = \sqrt[2]{\text{concentration}}$

F-tests were employed to further analyze these results as follows:

Hypothesis Tested	F-Value	
	Soy Bean Data	Cucumber Data
(1) The data does not fit the curve:		
Depth Group I	[a]55.49	[a]58.14
Depth Group II	[a]53.92	[a]271.84
Depth Group III	[a]420.24	[a]320.00
Depth Group IV	[a]1,184.46	- -
(2) One regression equation can represent all depth groups	[a]11.26	[a]14.00
(3) All the regression coefficients are equal	0.98	0.04
(4) The elevations are equal	[a]28.39	[a]36.82

[a]Indicates significant at the 0.99 probability level; hypothesis rejected.

Except for the hypothesis that all the regression coefficients are equal (which was not rejected), these hypotheses were rejected at the 0.99 probability level for both the soybean and cucumber data sets. Thus within each data set, the slope of the regression equations for the different depth groups is equal. However, rejection of the last hypothesis indicates that the intercept for each depth group is different. A common regression coefficient was computed for each data set, and the final equations are as follows: (These equations are plotted in Figures II-2 and II-3).

Depth Group	Regression Equation for Root Lengths	
	Soybeans	Cucumbers
I	$Y = 1.18467 - 0.68076 \cdot X$	$Y = 1.27936 - 0.50374 \cdot X$
II	$Y = 1.12705 - 0.68076 \cdot X$	$Y = 1.12775 - 0.50374 \cdot X$
III	$Y = 1.05610 - 0.68076 \cdot X$	$Y = 1.06402 - 0.50374 \cdot X$
IV	$Y = 0.99721 - 0.68076 \cdot X$	- -

Where:

$$Y = \log_{10}(\text{cup value} + 1)$$

$$X = \sqrt[2]{\text{concentration (ppm)}}$$

and $0.0 \leq X \leq \sqrt[2]{0.908}$

b. Analysis of Grid Samples

Using the relationships expressed in the previous paragraph, estimates were made of the concentration of herbicide Orange in the soil samples taken from the defoliant testing area. These estimates along with the limits of the 95% confidence intervals are presented in Table II-4. Analysis for organic matter content are presented in Table II-5. A comparison of organic matter content within selected cases is presented in Table II-6.

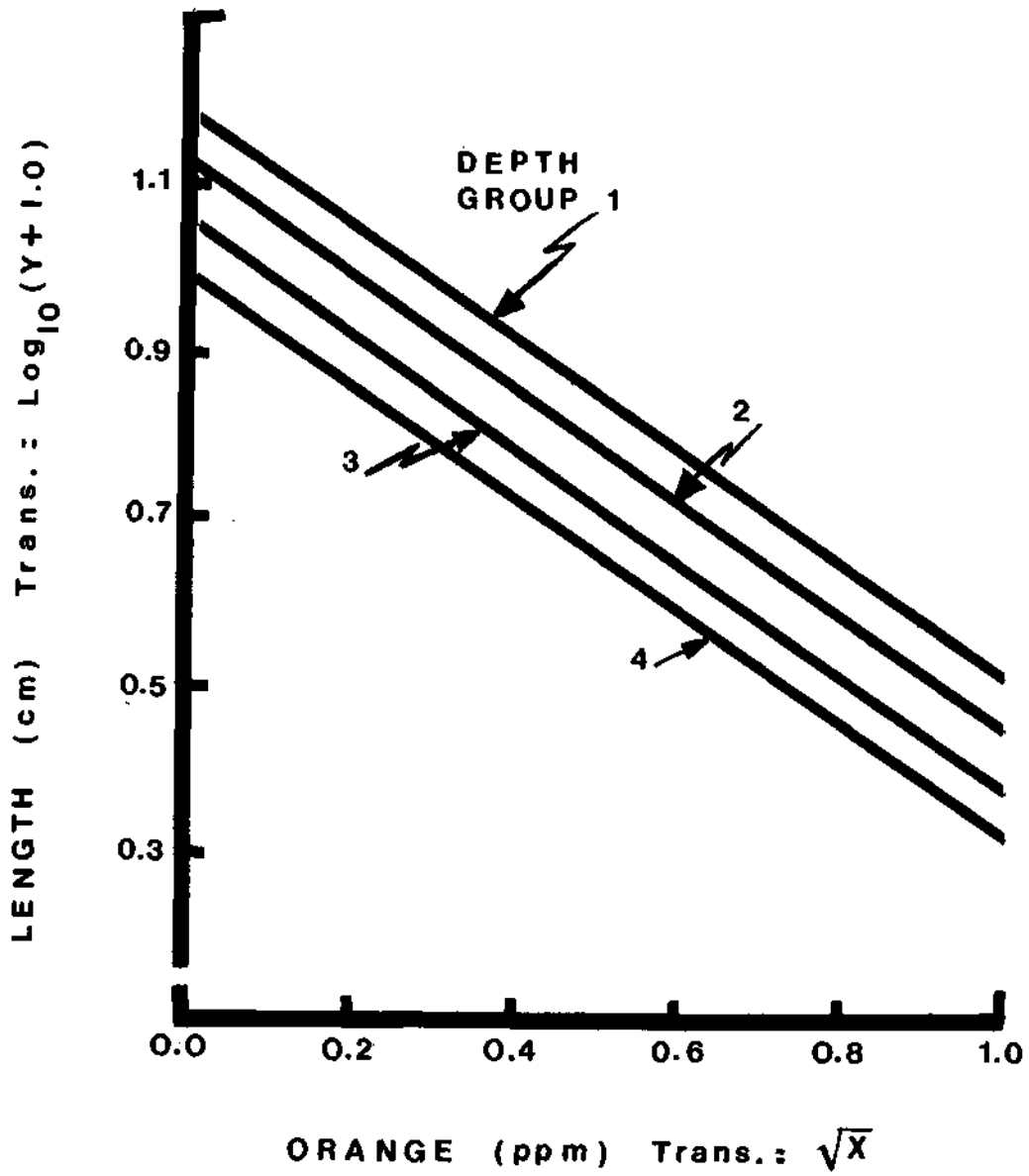

Figure II-2. Concentration Calibration Curves for Soybean Bioassay [see text for description of depth groups]

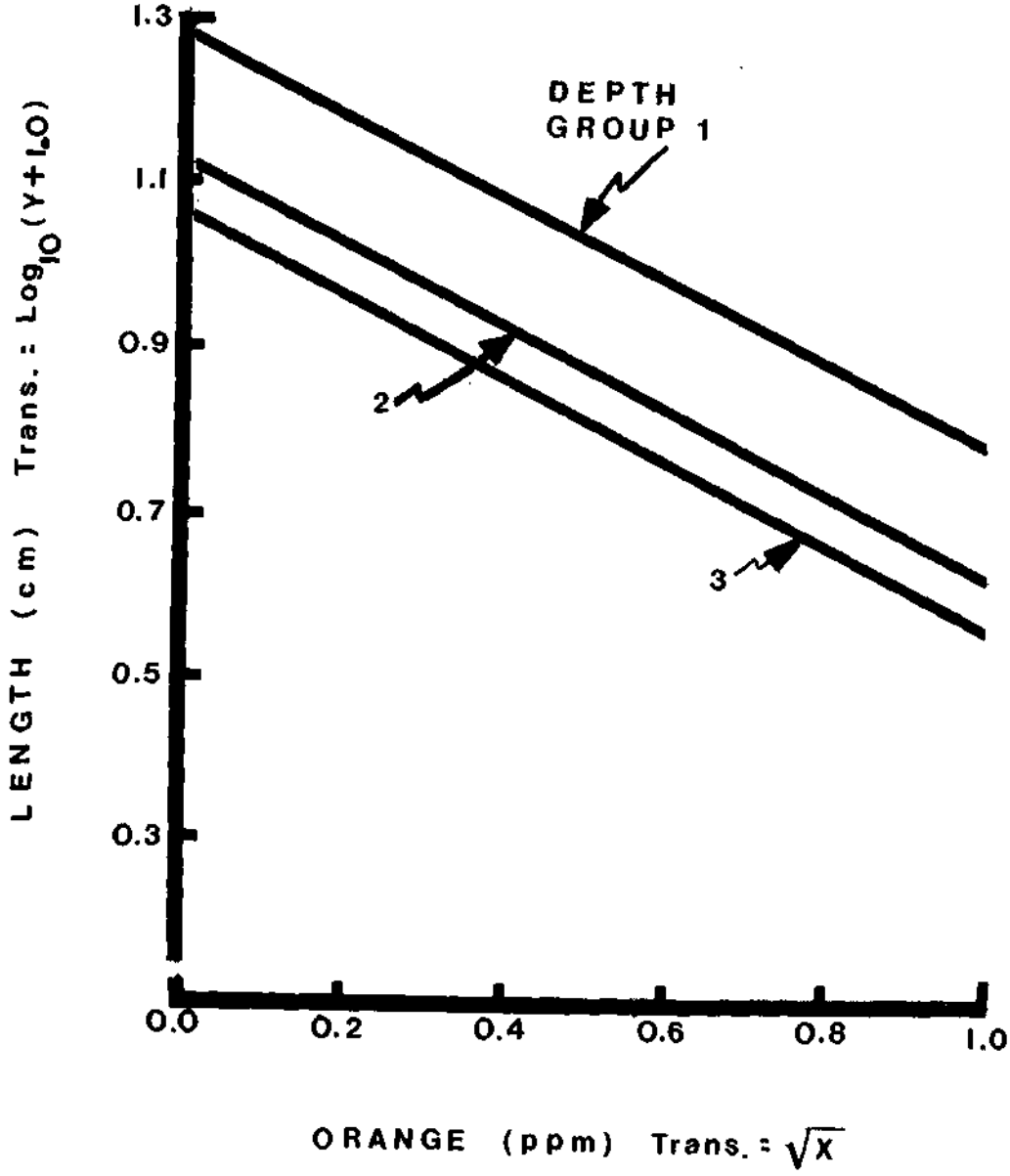

Figure II-3. Concentration Calibration Curves for Cucumber Bioassay [see text for description of depth groups]

TABLE II-4. CONCENTRATION ESTIMATES OF ORANGE AND 95% CONFIDENCE INTERVAL LIMITS VIA BIOASSAY ANALYSES

Site	Depth Segment	Soybean Data			Cucumber Data		
		Lower Limit	Concentration Estimate (ppm)	Upper Limit	Lower Limit	Concentration Estimate (ppm)	Upper Limit
B-14	1	0.0000	.0113	.1638	0.0000	.0032	.1526
B-14	2	0.0000	.0730	.3413	0.0000	0.0000	.0358
B-14	3	0.0000	.0138	.0593	0.0000	0.0000	.0363
B-14	4	.0003	.0130	.0450	0.0000	0.0000	.0344
B-14	5	.0012	.0175	.0528	0.0000	0.0000	.0341
B-14	6	.0148	.0475	.0987	0.0000	.0000	.0341
C-9	1	.0693	.3034	.7030	.0087	.1652	.5183
C-9	2	.0535	.2975	.7389	0.0000	.0161	.0932
C-9	3	.0765	.1579	.2684	0.0000	.0079	.0723
C-9	4	.0344	.0791	.1420	0.0000	0.0000	.0333
C-9	5	.0344	.0791	.1420	0.0000	0.0000	.0326
C-9	6	.0293	.0712	.1315	0.0000	.0003	.0389
J-3	1	.0814	.3297	.7447	.0136	.1851	.5532
J-3	2	.0214	.2094	.5912	0.0000	.0050	.0632
J-3	3	.0263	.0805	.1645	0.0000	.0025	.0540
J-3	4	.0057	.0297	.0725	0.0000	0.0000	.0391
J-3	5	0.0000	.0017	.0196	0.0000	0.0000	.0387
J-3	6	0.0000	0.0000	.0105	0.0000	0.0000	.0393
M-8	1	.0814	.3297	.7447	.0017	.1260	.4473
M-8	2	.0222	.2120	.5957	0.0000	.0032	.0567
M-8	3	.0700	.1485	.2562	0.0000	..0098	.0778
M-8	4	.0367	.0824	.1464	0.0000	0.0000	.0331

TABLE II-4. CONCLUDED

Site	Depth Segment	Soybean Data			Cucumber Data		
		Lower Limit	Concentration Estimate (ppm)	Upper Limit	Lower Limit	Concentration Estimate (ppm)	Upper Limit
M- 8	5	.0477	.0985	.1675	0.0000	0.0000	.0343
M- 8	6	.0426	.0911	.1578	0.0000	0.0000	.0330
N-12	1	.0814	.3297	.7447	.0005	.1136	.4243
N-12	2	.0183	.1993	.5738	0.0000	.0044	.0613
N-12	3	.0567	.1289	.2304	0.0000	.0039	.0594
N-12	4	.0691	.1282	.2054	0.0000	.0005	.0406
N-12	5	.0985	.1672	.2540	0.0000	0.0000	.0339
N-12	6	.0593	.1147	.1883	0.0000	0.0000	.0326
0- 7	1	.0169	.1718	.4885	0033	.1377	.4689
0- 7	2	.0183	.1993	.5738	0.0000	.0111	.0812
0- 7	3	.0441	.1097	.2046	0.0000	.0262	.1151
0- 7	4	.0344	.0791	.1420	0.0000	.0022	.0507
0- 7	5	.0247	.0640	.1217	0.0000	.0007	.0423
0- 7	6	.0293	.0712	.1315	0.0000	0.0000	.0326
0- 5	1	0.0000	.0321	.2225	.0184	.2020	.5826
0- 5	2	0.0000	.0196	.2142	0.0000	.0209	.1040
0- 5	3	0.0000	.0010	.0259	.0025	.0508	.1605
0- 5	4	0.0000	.0018	.0202	0.0000	.0026	.0526
0- 5	5	0.0000	0.0000	.0102	0.0000	.0116	.0806
0- 5	6	0.0000	.0012	.0180	0.0000	.0252	.1110
0- 8	1	0.0000	.0249	.2040	0.0000	.0047	.1611
0- 8	2	0.0000	.0029	.1489	0.0000	0.0000	.0385
0- 8	3	0.0000	0.0000	.0178	0.0000	0.0000	.0347
0- 8	4	0.0000	0.0000	.0103	0.0000	0.0000	.0369
0- 8	5	0.0000	0.0000	.0103	0.0000	0.0000	.0370
0- 8	6	0.0000	.0007	.0160	0.0000	0.0000	.9354

TABLE II-5. PERCENT ORGANIC MATTER OF SOIL FROM TEST AREA C-52A
[Samples Collected between December 1970 and June 1971]

SAMPLE	DEPTH, Inches	ORGANIC MATTER' %	
A-11	0-6	0.68[b]	
B-8	0-6	1.05[a]	
B-14	0-6	1.13[a]	
C-11	0-6	0.55[a]	
D-7	0-6	0.87[a]	
E-3	0-6	0.73[b]	
E-6	0-6	0.37[b]	
F-12	0-6	1.31[a]	
J-1	0-6	0.85[a]	
L-10	0-6	0.68[b]	
N-5	0-6	0.76[a]	
N-8	0-6	0.41[b]	
N-12	0-6	0.72[a]	
O-2	0-6	0.45[b]	
CONTROL (0.2 mile NW of grid)	0-6	1.25[b]	1.99
C-9	0-6	0.96	
C-9	6-12	0.71	
C-9	12-18	0.72	
C-9	18-24	0.67	
C-9	24-30	0.87	
C-9	30-36	0.41	
G-11	0-6	1.65[b]	1.99
G-11	6-12	1.04	
G-11	12-18	1.61	
G-11	18-24	1.49	
G-11	24-30	1.06	
O-7	0-6	0.57	
O-7	6-12	0.52	
O-7	12-18	0.56	
O-7	18-24	0.62	
O-7	24-30	0.54	
O-7	30-36	0.59	
CONTROL (0.2 mile NW of grid)	0-6	2.03[a]	
	6-12	1.35[a]	
	12-18	0.86[a]	
	18-24	0.73[a]	
	24-30	0.83[a]	
	30-36	0.71[a]	

[a]Average of three replicates.

[b]Determined by the Wakley-Black wet digestion method; all others analyzed by weight loss after 30 minute heating in tared crucible.

[c]Samples designated by the closest permanent sampler station. Samples taken 50 feet from sampler.

TABLE II-6. PERCENT ORGANIC MATTER WITHIN TREATMENT AND CONTROL CORES TAKEN FROM TEST AREA C-52A				
DEPTH, inches	CONTROL	ORGANIC MATTER, %		
		C-9	G-11	0-7
0 to 6	2.03	0.96	1.99	0.57
6 to 12	1.35	0.71	1.04	0.52
12 to 18	0.86	0.72	1.61	0.56
18 to 24	0.73	0.67	1.49	0.62
24 to 30	0.83	0.87	1.06	0.54
30 to 36	0.71	0.41	0.96	0.59

4. CONCLUSIONS

The varying effects of different concentrations of herbicide Orange and the changes in soil composition associated with different depths are clearly indicated by the ANOVA and multiple range tests on the bioassay of the standard concentrations. Thus, it should be expected that the same concentration of Orange in different types of soils will be reflected by different plant growth patterns.

An obvious disparity exists between the soybean data and cucumber data estimates of Orange concentration at various test area sites. Confounding occurred because parts of this experiment were conducted at different times without statistical balancing. The attempt to adjust for those differences by weighting the data to reflect the differences in the control plants was not successful. Evidently the differences in the environments could not be explained by such a simple adjustment.

5. CHEMICAL ANALYSES OF SOIL CORES

As a result of the bioassay analyses previously discussed, those soil samples collected in November 1969 and in April and December 1970, which caused the greatest growth inhibition, were analyzed chemically for 2,4-D; 2,4,5-T; picloram; arsenic; and the contaminant TCDD.

a. Methods and Materials

A 25 to 50 gram soil sample was weighed, acidified, and extracted with 1:1 hexane: acetone (see Figure II-4). The hexane:acetone was made basic and the aqueous phase saved for extraction by ether after acidification, butylation via boron trichloride, and the subsequent determination of 2,4-D and 2,4,5-T. The hexane phase was gently shaken repeatedly with sulfuric acid until the sulfuric acid was clear. The hexane phase was then condensed and the extract representing 50 - 100 mg of soil was injected on a 5% OV-225 gas chromatographic (GC) column. The GC was equipped with a Ni^{63} electron capture detector. If a peak was found within $\pm 10\%$ of the retention time of TCDD, the sample was irradiated with ultraviolet light for 16 hours. Column chromatography was also employed.

A gas chromatograph trace of the TCDD samples is shown in Figure II-5. This figure shows an unaltered and a spiked soil sample before and after ultraviolet light treatment for three different soil samples. In no case was any TCDD detected. Notice also that the added TCDD was completely destroyed by UV irradiation.

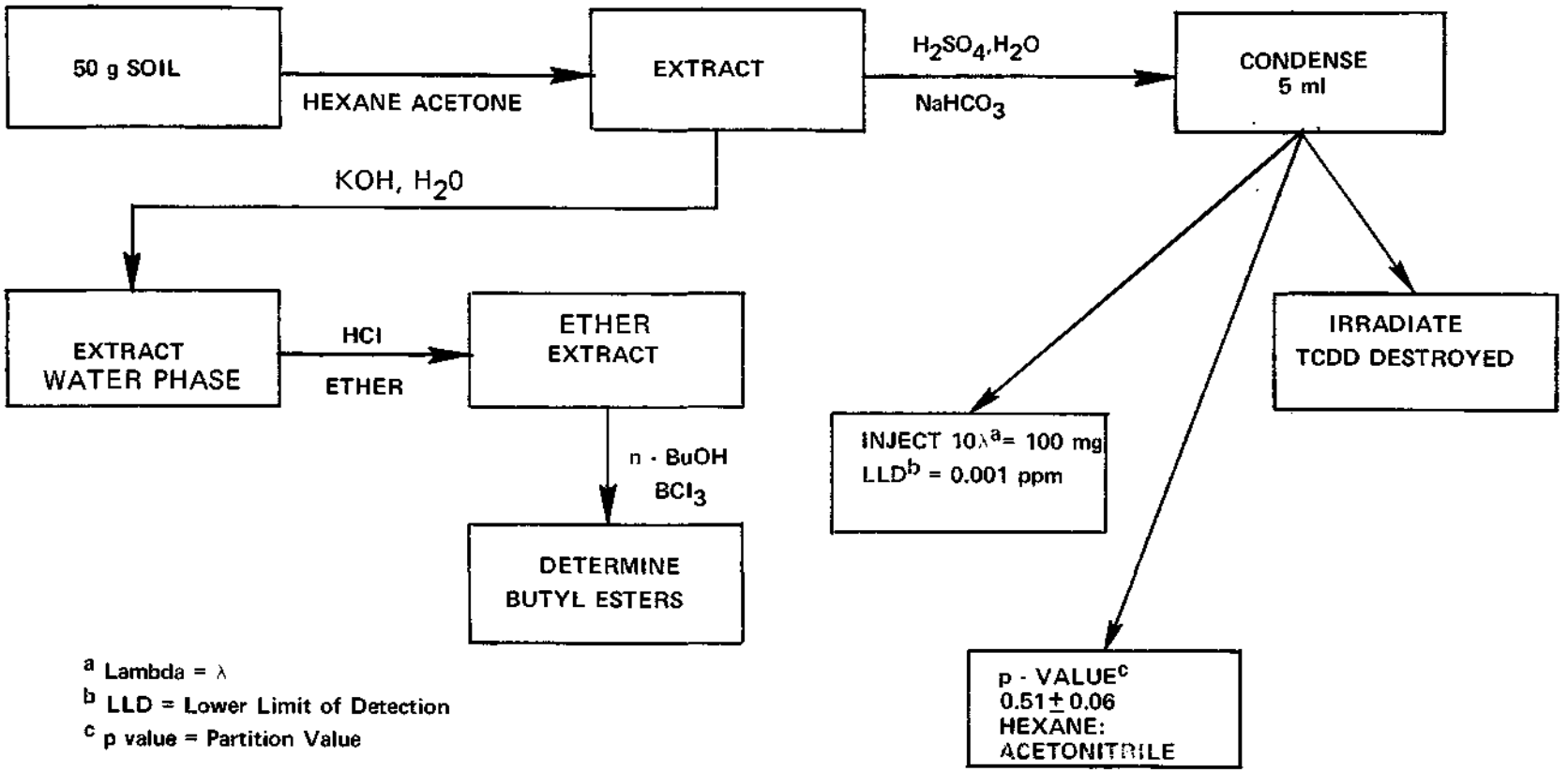

Figure II-4. Analysis Flow Chart for the Extraction of 2,4-D; 2,4,5-T; and TCDD from Lakeland Sand

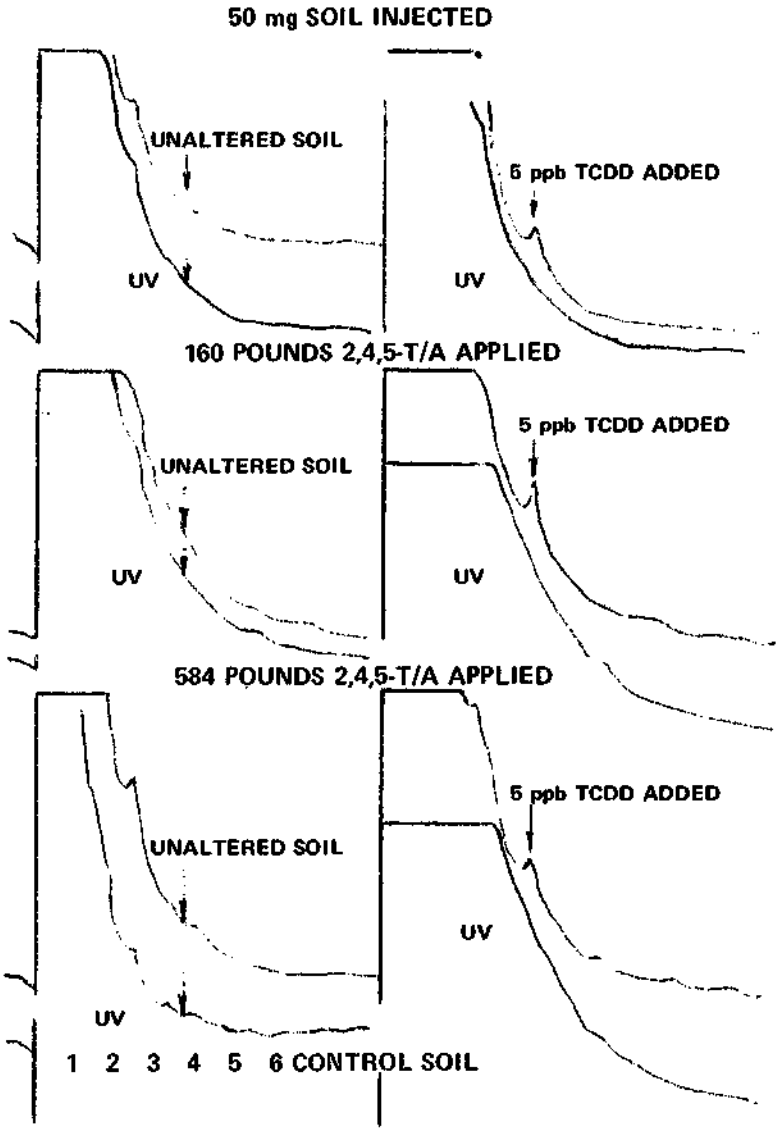

Figure II-5. Gas Chromatograph Traces of TCDD Samples. (The Traces Represent 50 mg of Extracted Soils from Grids 2 and 4. See Table I-7)

The soils were analyzed for arsenic using 6 mls of 1:1 $H_2SO_4/HC10_4$ per 10 g, followed by reductive distillation and development of a molybdenum blue color. Nine soil cores (to the 36-inch depth) were analyzed for picloram by Dow Chemical U.S.A. The minimum detection limit for picloram was 5 ppb.

Additional soil samples were collected in June and October 1973 from near sampler sites C-9, F-6, and 0-7 and from the center of Grid 1 located approximately 1000 feet south of sampler station 0-7 (see Figure I-5). These soil samples were analyzed for TCDD by the Interpretive Analytical Services, Dow Chemical, U.S.A., Midland, Michigan. The method of analysis reported by the Interpretive Analytical Services is as follows:

> 10 gms of soil were extracted with 1:1 acetone-hexane, and the hexane recovered from the extract by the addition of water. (J. Assoc. Offic. Anal. Chemists 56, 728 (1973). (Initially, the soil extracts were subjected to separation procedures involving only silica gel column chromatography, but too many interferences were found for best sensitivity. Hence, all samples were treated by a modification of the techniques developed by Baughman and Meselson, "An Analytical Method for Detecting Dioxin", National Institute of Environmental Health Sciences Conference on Dibenzo-dioxins and Dibenzo furans, Research Triangle Park, NC, April 1973, published in Evnironmental Health Perspectives, No. 5, August 1973). The modified procedure is as follows:
>
> Hexane extracts from above washed with successive 10 ml portions of concentrated H_2SO_4 until H_2SO_4 is colorless (4 - 5 washings).
>
> Hexane evaporated to 0.5 ml, and passed through a silica gel column. Dioxins eluted from the column and 1:5 benzene-hexane. Eluate evaporated to a small volume, and taken up in 0.5 ml hexane. (This step is in addition to the Baughman-Meselson procedure, and was found necessary to achieve best sensitivity.)
>
> Hexane solution from above placed on an Al_2O_3 column. PCB's eluted with 1:4 $CC1_4$-hexane. Dioxin eluted with 1:4 CH_2C1_2-hexane. Eluate evaporated to small volume and injected directly into an LKB-9000 gas chromatography-mass spectrometer combination.
>
> Column Conditions: 6 ft x 1/8 inch stainless steel packed with 3% OV-3 on 80/100 mesh Gaschrom. Z, isothermal at 230°C. Retention time ~ 6.5 min.
>
> Detector: mass spectrometer set to monitor m/e = 320 and 322 simultaneously.

b. Results and Discussions

Table II-7 shows the results of all analyses for soils collected during the period 1969 through 1971. Notice the persistence of 2,4-D and 2,4,5-T in soil core J-3NE from April to December 1970. However, it should be emphasized that if no herbicide degradation had occurred, a concentration of approximately 80 ppm 2,4,5-T might be expected within the top 6 inches (see Table II-7,

assuming the weight of a one acre 6-inch increment weighs 2 million pounds). Notice that cores M-8SW, N-12SW, and 0-5S show leaching of 2,4-D, and 2,4,5-T. This particular region of the grid received 1,168 pounds per acre of Orange from 1964 to 1966. Moreover, O-5S may have received heavy concentrations of herbicides when Grid 1 was in use (1962 to 1964). Table II-8 compares chemical and bioassay data for the same core (M-8S), collected in December 1970 and subsampled for analyses. These data suggest that chemical analysis for 2,4-D and 2,4,5-T alone may not account for all the biologically active phytotoxic components (e.g., degradation products and/or fuel oil residue). Note that the trend is reasonably similar between methods of analysis.

No TCDD residues were found by the Pesticide Degradation Laboratory in any of the soil samples at a minimum detection limit of less than 1 ppb. Recent analyses of drums of Orange in storage suggest that the average concentration of TCDD in Orange may be 2 ppm[2]. If it is assumed that all the 2,4,5-T sprayed on TA C-52A was contaminated with 2 ppm TCDD, then approximately 0.5 pound of TCDD was disseminated in this test area. However, the analyses of TCDD in the part per trillion (ppt) range would be required for detection of potential residue. The results of soil samples collected in June and October 1973 and analyzed by Interpretive Analytical Services, in the parts per trillion range, did in fact indicate the presence of TCDD or a TCDD-like chemical compound. These data are shown in Table II-10. The greatest concentration of TCDD was found in the soil core from the center of Grid 1. This grid received on the average 947 pounds 2,4,5-T per acre in the period 1962-1964. The levels detected in the 6 to 12 and 12 to 18 inch depths were probably due to contamination at the time of sample collection. These data suggest that TCDD or a TCDD-like compound may persist for an extended period of time. Moreover, it would appear that the Orange (Purple) disseminated on this test grid was significantly contaminated with this compound.

Significant levels of picloram were found in November 1969 near Sampler K-9. Notice that at this date the residue was confined to the top 12 inches. However, by May 1970 picloram may have moved to the lower increments within the soil profile.

Table II-7 also shows the levels of arsenic found in selected soil cores. From the data in this table, it is evident that there is no appreciable build-up of arsenic in the soil. Perhaps this is due to leaching or possibly to the reduction and volatilization of dimethylarsine from the cacodylic acid. These observations are further verified by data on arsenic levels from sites J-3NE, C-9SW, K-9N, and 0-8S collected and analyzed in June 1973. Table II-9 compares the arsenic levels from these sites collected in 1970 and again in 1973.

c. Conclusions

Small amounts (in parts per billion) of 2,4-D, 2,4,5-T, and picloram were found persisting on the test area in June 1971. The last application of Orange was December 1969, while the last application of White was May 1970. The last application of Blue was in September 1970; nevertheless, no significant build-up of arsenic has been noted. However, leaching of the arsenical from the soils may have occurred. Significant TCDD residue (or a TCDD-like compound) has been detected in the parts per trillion range from Grid 1.

---

[2]Personal communication with Dr. Walter Melvin, February 1973, Air Force Environmental Health Laboratory, Kelly AFB, Texas.

TABLE II-7. RESULTS OF CHEMICAL ANALYSES FOR PICLORAM; 2,4-D; 2,4,5-T; 2,3,7,8-TCDD; AND ARSENIC IN SOIL SAMPLES FROM EGLIN AFB RESERVATION TEST AREA C-52A

SOIL SAMPLE[a]	DEPTH[b]	PICLORAM[c], ppb	2,4-D, ppb	2,4,5-T, ppb	TCDD[d], ppb	ARSENIC[d], ppm
NOVEMBER 1969						
K-9N	1[e]	34, 21	1.2	2.8	<1.0	2.24
	2	21, 11	7.0	2.0	<1.0	0.86
	3	<10, 6	0.1	0.8	<1.0	0.90
	4	<10, 5	0.1	0.6	<1.0	0.52
	5	<10, <5	0.1	0.9	<1.0	0.62
	6	<10, < 5	0.1	0.3	<1.0	0.54
6 APRIL 1970						
J-3NE	1	ND	1.7	1.2	<1.0	0.55
	2	ND	1.7	1.0	<1.0	0.34
	3	ND	0.1	1.0	<1.0	0.41
	4	ND	0.1	1.0	<1.0	0.41
	5	ND	ND	ND	< 1.0	ND
	6	ND	0.1	0.7	<1.0	0.52
10 DECEMBER 1970						
J3NE	1	<10	<0.1	2.4	< 0.1	4.70
	2	<10	<0.1	1.8	< 0.1	1.30
	3	<10	<0.1	1.1	< 0.2	0.90
	4	<10	<0.1	0.7	< 0.2	0.55
	5	<10	<0.1	1.0	< 0.2	1.13
	6	<10	<0.1	0.3	< 0.2	0.90
6 APRIL 1970						
J-9SE	1	ND	1.6	5.9	<1.0	3.21
	2	ND	1.1	0.1	<1.0	0.48
	3	ND	0.1	0.7	<1.0	0.20
	4	ND	0.1	0.4	<1.0	0.27
	5	ND	0.1	0.3	<1.0	0.27
	6	ND	0.1	0.4	<1.0	0.20

TABLE II-7. CONTINUED

SOIL SAMPLE[a]	DEPTH[b]	PICLORAM[c], ppb	2,4-D, ppb	2,4,5-T, ppb	TCDD[d], ppb	ARSENIC[d], ppm
10 DECEMBER 1970						
B-14SW	1	<10	< 0.1	0.6	< 0.1	0.55
	2	<10	2.4	0.4	< 0.1	0.55
	3	<10	0.6	0.4	< 0.1	0.55
	4	<10	< 0.1	0.2	< 0.2	0.58
	5	<10	< 0.1	< 0.1	< 0.3	0.41
	6	<10	< 0.1	< 0.1	< 0.1	0.48
10 DECEMBER 1970						
C-9SW	1	<10	< 0.1	1.8	< 0.4	1.64
	2	<10	< 0.1	1.2	< 0.3	0.88
	3	<10	< 0.1	0.3	< 0.2	0.48
	4	<10	< 0.1	0.3	< 0.1	0.48
	5	<10	< 0.1	<0.1	< 0.2	0.55
	6	<10	< 0.1	0.4	< 0.4	0.52
10 DECEMBER 1970						
M-8SW	1	<10	5.6	7.0	< 0.2	0.90
	2	<10	5.8	1.4	< 0.2	0.48
	3	<10	7.6	2.8	< 0.2	0.34
	4	<10	15.0	5.6	< 0.1	0.41
	5	<10	5.0	2.8	< 0.5	0.34
	6	<10	13.2	6.8	< 0.2	0.55
10 DECEMBER 1970						
N-12SW	1	<10	7.6	2.2	< 0.2	1.86
	2	<10	10.0	1.0	< 0.2	1.05
	3	<10	11.8	1.2	< 0.2	0.76
	4	<10	4.8	1.4	< 0.3	0.69
	5	<10	6.0	2.6	< 0.2	0.69
	6	<10	7.0	3.0	< 0.2	0.76
10 DECEMBER 1970						
O-5S	1	ND	0.8	1.2	< 0.1	0.90
	2	ND	0.6	0.6	< 0.1	0.80
	3	ND	0.6	1.2	< 0.2	0.76
	4	ND	0.6	0.6	< 0.2	0.41
	5	ND	< 0.1	8.4	< 0.7	0.69
	6	ND	0.6	1.4	< 0.1	0.55

TABLE II-7. CONTINUED

SOIL SAMPLE[a]	DEPTH[b]	PICLORAM[c], ppb	2,4-D, ppb	2,4,5-T, ppb	TCDD[d], ppb	ARSENIC[d], ppm
10 DECEMBER 1970						
0-7NE	1	<10	ND	6.6	<0.2	1.52
	2	<10	2.8	0.2	<0.3	0.76
	3	<10	3.6	0.4	<0.2	0.76
	4	<10	0.8	2.6	<0.2	0.62
	5	<10	5.6	2.6	<0.2	0.62
	6	<10	1.2	0.2	<0.6	0.62
10 DECEMBER 1970						
0-8S	1	ND	<0.1	1.2	<0.1	2.70
	2	ND	<0.1	2.6	<0.1	0.58
	3	ND	<0.1	0.8	<0.1	0.62
	4	ND	<0.1	0.6	<0.1	0.20
	5	ND	<0.1	1.2	<0.1	0.41
	6	ND	<0.1	0.6	<0.2	0.07
13 MAY 1970						
K-9N	1[e]	ND	0.1	8.7	<1.0	3.94
	1	ND	0.1	3.2	<1.0	4.25
	2	17, 7	ND	ND	ND	ND
	3	<10, 5	ND	ND	ND	ND
	4	11, <5	0.1	0.1	<1.0	0.41
	4	ND	0.1	ND	<1.0	0.41
	5	<10,<5	ND	ND	ND	ND
	6	10, 5	0.1	0.9	1.0	0.41
	6	ND	0.1	1.0	1.0	0.48
13 MAY 1971						
G-9N	2	92, 160	ND	ND	ND	ND
10 DECEMBER 1970						
CONTROL	1	<10	<0.1	<0.1	<0.2	0.55
or						
8 APRIL 1971[f]						
	2	<10	<0.1	<0.1	<0.3	0.41
	3	<10	<0.1	<0.1	<0.2	0.55
	4	<10	<0.1	<0.1	<0.4	0.24
	5	<10	<0.1	<0.1	<0.4	0.41
	6	<10	<0.1	<0.1	<0.5	0.48

TABLE II-7. CONCLUDED

[a]Samples designated by the nearest permanent air sampler station on the one square mile test grid. All samples were taken 50 feet from a certain air sampler, except control site was 0.4 mile from the one square mile grid.

[b]Samples taken with a core borer in 6 inch increments. Depth 1 = 0 to 6 inches; 2 = 6 to 12 inches; 3 = 12 to 18 inches; 4 = 18 to 24 inches; 5 = 24 to 30 inches and 6 = 30 to 36 inches. Each increment was uniformily mixed prior to sampling for chemical analysis.

[c]Picloram analysis was performed by International Research and Development Corporation and/or The Dow Chemical Company; Dow Chemical Method ACR 69.10, modified.

[d]Analysis performed by E. A. Woolson, Pesticide Degradation Laboratory, United States Department of Agriculture, Beltsville, Maryland.

[e]Two samples from same depth were taken 10 feet apart.

[f]Control soil for picloram analysis taken 8 April 1971, and other analyses performed after 10 December 1970 sampling.

TABLE II-8. A COMPARISON OF CHEMICAL AND BIOASSAY DATA FOR SOIL CORE M-8[a] COLLECTED DECEMBER 1970

DEPTH, Inches	CHEMICAL ANALYSIS, ppb[b]	BIOASSAY ANALYSIS[c]	
		LOWER LIMIT, ppb	UPPER LIMIT, ppb
0-6	12.6	80	745
6-12	7.2	22	596
12-18	10.4	70	256
18-24	20.6	37	146
24-30	7.8	48	168
30-36	20.0	43	158

[a]Sample collected 50 feet southwest of air sampler station M-8.

[b]Total concentration of 2,4-D and 2,4,5-T.

[c]Data from soybean bioassay (see Table II-4)

TABLE II-9. A COMPARISON OF ARSENIC LEVELS IN SOIL CORES COLLECTED IN 1970 AND 1973 FROM TA C-52A[a]

DEPTH, Inches	LOCATION [b]							
	J-3NE		C-9SW		K-9N		O-8S	
	1970	1973	1970	1973	1970	1973	1970	1973
0-6	4.70	0.85	1.64	1.68	3.94	0.62	2.70	1.46
6-12	1.30	0.47	0.88	0.56	4.25	0.54	0.58	0.42
12-18	0.90	0.59	0.48	0.60	[c]ND	0.41	0.62	0.45

[a]Analysis performed by E. A. Woolson, Pesticide Degradation Laboratory, United States Department of Agriculture, Beltsville, Maryland.

[b]Samples collected 50 feet in the designated direction (e.g., NE) from the permanent air sampler station.

[c]ND = not determined.

TABLE II-10. LEVELS (PARTS PER TRILLION) OF 2,3,7,8 TETRACHLORODIBENZO-p-DIOXIN (TCDD) IN SOIL FROM TA C-52A COLLECTED IN JUNE OR OCTOBER 1973[a]

DEPTH, Inches	LOCATION				
	C-9	F-7	O-7	GRID 1	CONTROL
0-6	<10[b]	11	30	710	<20[c]
6-12	ND	ND	<10	140	<10
12-18	ND	ND	<10	72	<10
18-24	ND	ND	<10	<10	<10
24-30	ND	ND	<10	<10	<10
30-36	ND	ND	<10	<10	<10

[a]Method described in text.
[b]Lower limit of detection in parts per trillion TCDD.
[c]Probable interference from excessive organic matter.

SECTION III

STUDIES OF THE VEGETATION OF TEST AREA C-52A

The first studies of Test Area C-52A were those concerned with vegetation. Testing of aerial spray equipment began in June 1962, and following heavy applications of materials in 1963 and 1964, vegetation surrounding the test site showed changes suggestive of herbicidal damage. In the fall of 1966, concern about the extent of this damage led to the establishment of a contract with the University of Florida, Gainesville, Florida. The purpose of the contract was to conduct a taxonomic study that would quantitatively measure changes in density of vascular plants in the area adjacent to the test grid (References III-1, III-2, and III-3).

Observations of tree growth rings in those reports prompted studies (Reference III-4) concerned with assessment of spray drift upon the forest trees adjacent to the test area. A third study (Reference III-5) was concerned with the histological examination of a plant species growing in the flight lines on the test grid. Synopses of Reference III-1 to III-5 are included in this section. The last report, and the one most concerned with the current research effort (Reference III-6), will be summarized and referred to in this report within the section on current studies.

References:

III-1. Ward, D. B.: Ecological Records on Eglin AFB Reservation - - the First Year. AFATL-TR-67-157, Air Force Armament Laboratory, Eglin Air Force Base, Florida, 1967. Unclassified.

III-2. Ward, D. B.: Ecological Records on Eglin AFB Reservation - - the Second Year. AFATL-TR-68-147, Air Force Armament Laboratory, Eglin Air Force Base, Florida, 1968. Unclassified.

III-3. Ward, D. B.: Ecological Records on Eglin AFB Reservation - - Conclusions. AFATL-TR-70-55, Air Force Armament Laboratory, Eglin Air Force Base, Florida, 1970. Unclassified.

III-4. Hunter, H. H. and B. M. Agerton: Annual Diameter Growth of Conifers Adjacent to Eglin Reservation Test Area C-52A as Related to the Testing of Defoliant Spray Equipment. AFATL-TR-71-52, Air Force Armament Laboratory, Eglin Air Force Base, Florida, 1971. Unclassified.

III-5. Sturrock, T. T. and A. L. Young: A Histological Study of Yucca filamentosa L. from Test Area C-52A, Eglin Reservation, Florida. AFATL-TR-70-125, Air Force Armament Laboratory, Eglin Air Force Base, Florida. 1970. Unclassified.

III-6. Hunter, J. H. and A. L. Young: Vegetative Succession Studies on a Defoliant-Equipment Test Area, Eglin AFB Reservation, Florida. AFATL-TR-72-31, Air Force Armament Laboratory, Eglin Air Force Base, Florida. 1970. Unclassified.

1. SYNOPSIS OF TAXONOMIC STUDIES, 1966 - 1969

a. General Observations

In the fall of 1966, turkey oak, Quercus laevis, adjacent to the test clearing was severely affected, apparently by herbicide driftage; and the large proportion of the trees of this species gave the entire forest an abnormal aspect. Upper branches of all trees had apparently been completely defoliated, and many or even most of the twigs were killed. On the lower trunks a proliferation of small branchlets had appeared; in all cases, these branchlets began growing in the spring of 1965, as determined by the number of bud scale scars present.

Blue-jack oak, Quercus incana, and sand live oak, Quercus germinata, were also heavily damaged near the test clearing but appeared undamaged as little as 1.1 miles from the clearing in an area where turkey oak still exhibited signs of damage.

Longleaf pine, Pinus palustris, and sand pine, Pinus clausa, adjacent to the test clearing appeared unaffected. Needle length and internode length were normal, as compared with similar plants away from the test area.

A sampling was made of the growth rates of four tree species as indicated by width of annual growth rings. Blue-jack oak, turkey oak, and long-leaf pine produced growth rings that appeared to vary independently of spray applications to the adjacent TA C-52A. Sand pine seemingly showed a positive correlation with a marked increase in growth in the years immediately after the 1963 - 1964 period of heavy spray application.

Extensive observation of the vegetation in all directions from TA C-52A indicated a rapid disappearance of damage attributable to spray driftage. The maximum distance that damage was definitely detected was 5 miles, seen on trees at the upper end of Range 52, north of the test area.

Observations made later in this project failed to disclose damage at any distance from the test area that was not attributable to the 1963 - 1964 period of activity. Following a testing period in 1969, particular attention was given to the oaks, pines, and herbaceous plants in various directions from TA C-52A. A few sand pines along the south edge of the test area had visible abnormalities in their early spring growth, but this had largely disappeared by early August. Other vegetation appeared normal, suggesting that herbicide driftage, if any, from the 1969 series of tests was significantly less than in earlier years.

b. Quantitative Sampling

In the fall of 1966, a program was devised to permit the quantitative measurement of changes in density of stand of vascular plants in the area adjacent to TA C-52A. Since changes in the herbaceous plants might be more subtle and more difficult to detect than those in the larger woody plants, an experimental design was developed that would disclose differences of slight magnitude but of statistical significance.

Five stations were selected in apparently homogeneous woodland east of the test area, and at each station four parallel transects of 50 meters were laid out at right angles to an imaginary

radius from the center of TA C-52A. The first of these sets of transects was immediately adjacent to the test clearing, and the fifth was at a distance of 2.0 miles. All plants intercepted by the transects were counted and listed. The resulting data were interpreted by a conventional analysis of variance and F-test.

The 2157 plants of 54 species intercepted by the 1000 meters of transect were found (with a few exceptions) not to differ significantly in their frequency with distance from the test area. This was taken to mean that the supposedly higher spray driftage near the test area had not had a significant effect on the viability of individuals of most species.

A few species were found to differ significantly with distance from the test area. Eleven species fluctuated significantly in number of individuals at varying distances from the test area, but upon examination, these differences were found to result from natural heterogeneities in the supposedly homogeneous woodland. They could not be attributed to spray effects.

Three species were found to be absent near the test area and to increase significantly away from this area. These species were the small, upright legume, Tephrosia mohrii, the persimmon, Diospyros virginiana, and the weeping haw, Crataegus lacrimata. Again, since the legume is a perennial with a deeply buried rootstock and the other two are trees which would have left evidence in the form of dead trunks, it is probable that these species were not exterminated by spray driftage near the test area, but rather reflect an original and natural heterogeneity in their distribution.

Only in the case of four species was there clear evidence of influence by proximity to the test area. The number of individuals of four herbs - Hypericum gentianoides, Solidago odora, Warea sessilifolia, and Rhynchosia cytisoides - decreased significantly away from TA C-52A. More meaningfully, the number of individuals of these species increased significantly adjacent to the test site. These four species are noted for their ability to rapidly colonize cleared land. The observed variation in number of individuals is best explained by these species utilizing the opening in the canopy resulting from the spray-induced loss of foliage by the oaks.

By early August 1969 the oaks in the area of the five transect stations again had normal foliage. At that date, it was not possible to find individuals of the Hypericum, the Warea, or the Rhynchosia, and the Solidago was very reduced in number. The presumption is that the spray-induced damage of 1963 - 1964 to the forest surrounding the test site had largely disappeared and that by 1969 the vegetation of the area was again essentially normal.

2. SYNOPSIS OF GROWTH OF SAND PINE, 1969 - 1970

a. General Comments

One of the observations noted in Reference III-1 was that the growth of sand pine, Pinus clausa, was seemingly related (positive correlation) to the periods of heaviest testing of spray equipment. This observation prompted an investigation, conducted between March 1969 and June 1970, into the growth of tree rings.

The species of trees selected for sampling were sand pine, longleaf pine, P. palustris, and turkey oak, Quercus laevis. A total of 18 sand pines were cut along with two longleaf pines and two turkey oaks. Sand pines were cut at ground level, and all others were cut 2 to 3 feet above ground level. A cross section was cut from the end of each trunk and was sanded so that the annual growth rings could be measured - to the nearest 0.1 mm.

An examination of the relative amount of annual herbicide delivery on TA C-52A revealed that the greatest amount of defoliant drift probably occurred in the following increasing order of years: 1967, 1966, 1962, 1965, 1969, 1968, 1964, and 1963. This order is based primarily on the amount, type, and formulation of materials sprayed per year. The amount of drift and defoliant damage that occurred would also have depended upon climatic and flight conditions. For example, probably very little drift damage occurred in 1967 because relatively little testing was done and all testing occurred during winter months (the time of a reduced level of plant growth). Likewise, damage would have been expected in 1964, not only because of the many Orange missions, but also because all testing occurred from May through July and some of the missions were flown at altitudes greater than the usual 150 to 200 feet.

b. Results

An initial examination of the annual diameter growth data indicated that possibly the growth of some trees was directly or indirectly stimulated by spray drift from defoliant testing. However, an analysis of the data did not substantiate this hypothesis. Growth obtained by trees during individual years of testing was often no greater than growth obtained in some previous year before defoliant spraying started. When the total annual diameter growth for individual pines during years of defoliant testing was compared with the same number of years before testing, no significant differences were apparent. Five of nine treatment trees (those close to TA C-52A) and three of six control trees made less total growth during the years of testing. For longleaf pine, one sample showed a decrease and the other an increase between 1962 and 1968.

Several of the pines located close to TA C-52A made less annual growth in 1963 - 1964 (period of expected greatest damage) when compared to the annual diameter growth of other years of defoliant testing. However, the reduced amount of growth did not seem to be related to defoliant spraying because three control trees also grew less in 1963 than any other year between 1962 and 1969. In addition, the amount of diameter growth in 1963 for trees close to TA C-52A was often no less than the diameter growth for some year previous to defoliant testing.

Difficulties were encountered in measuring annual diameter growth in turkey oak. The older increments could be easily differentiated, but the last three to five increments were not discernable. Gross observations of the diameter growth of the turkey oak sampled close to TA C-52A indicated the tree had grown less during the years of defoliant testing. As discussed in Reference III-3, the annual diameter growth of two turkey oaks that had grown within a few hundred yards of TA C-52A was measured, but without a clear indication that annual diameter growth was inhibited by defoliant testing. The greatest amount of tree defoliation around TA C-52A had been noticed on turkey oak, and a reduction in annual diameter growth was expected to be most evident in individuals of this species. However, the small amount of sampling showed no indication of a general reduction of annual diameter growth during the years of testing.

An attempt was made to relate annual diameter growth to rainfall and temperature. An analysis of average, maximum, and minimum monthly temperatures recorded since 1949 at Niceville, Florida, revealed a high degree of uniformity for the same months from year to year. Rainfall for each month, however, varied greatly over the years; and many attempts

were made to discover what combinations of monthly rainfall data correlated with annual growth. While the combination of April and May rainfalls yielded the highest correlation of any two other months, only one sample showed a significant probability (95 percent confidence level) of this rainfall being related to annual diameter growth. Therefore, the rainfall data available could not be used to explain the annual growth patterns of the trees observed.

3. SYNOPSIS OF HISTOLOGICAL STUDY OF YUCCA, 1970

a. General Comments

The military herbicides Orange (2,4-D and 2,4,5-T) and White (2,4-D and picloram) function as growth regulators in their herbicidal behavior. A study was undertaken to determine whether structural (histological) changes were evident in a plant species (Yucca filamentosa) found in a high herbicide residue area of TA C-52A.

Observations have confirmed that the largest bulk of the various chemicals used in the testing of aerial defoliation spray equipment was released and fell within the instrumented test area (TA C-52A). As a result of these repetitive applications, many plant species (i.e., the dicotyledonous plants) were selectively eliminated. The vast majority of the remaining plant life was monocotyledonous with the only distinct plant association being broomsedge (Andropogon virginicus), switchgrass (Panicum virgatum), and yucca (Yucca filamentosa). Field observations indicated that yucca was the most persistent species occuring in the flight-line areas of the test grid. Observations on gross morphology indicated no differences between plants occurring on and off the test grid; however, crownal areas (the plant part at the soil surface) appeared to be different.

Specimens of yucca were selected from areas of high herbicide residue and from an area sufficiently distant from the treated area to preclude contact with the herbicides and/or herbicide residues. Tissue samples obtained from the crown area of these plants were prepared for microscopic observation.

b. Results

Both cross and radial sections of treated and control plants were examined. The control plants were used to establish the normal development of this species. Yucca is different from most monocotyledonous plants in that it has some secondary growth and develops a periderm-like structure. In addition, repeated divisions of parenchyma cells and the suberization of their products produces tangential bands of cork progressing inward and including some of the fibrovascular bundles which have an uncharacteristic forked habit of growth. All of these structures were quite evident in both the control and the treated samples.

Based on these studies, there is no apparent difference in the formation of structures in Yucca filamentosa specimens obtained from an area subjected to repetitive applications of military herbicides and specimens from an area not receiving the herbicides. Both samples followed the normal structure for this species, as described in the scientific literature.

While these observations were conducted on a very small sample of the plants which did persist in the heavily treated area, no evidence of malformations was found. A larger sample and many more observations of different tissues of the plants might detect abnormalities in this

species under these conditions, and it is also possible that a larger sample of untreated plants would similarly produce individuals with malformations - - such anomalous structures are often found in many species of plants grown under different environmental conditions.

4. CURRENT VEGETATIVE SUCCESSION STUDIES

a. Introduction

The first complete survey of the vegetation existing on the herbicide test grids was initiated in 1971 (Reference III-6). The 1971 survey established a base line from which future observations or surveys could proceed to determine the rate and type (plant species involved) of plant succession on the test grids.

The June 1973 studies of vegetation are a continuation of the 1971 survey and provide precise data and photographs to illustrate changes that have occurred during a 2-year period.

b. Materials and Methods

In May 1971, the one square mile grid (Figure I-4) was divided into 169 sections (each 400 by 400 feet). The percentage plant cover in each 160,000 ft^2 section was visually estimated, and a vegetative coverage map resulted. In June 1973, the same technique was used to construct another vegetative coverage map. Coverage was ranged into five classes as follows: Class O = 0 to 5% cover, Class I = 5 to 20% cover, Class II = 20 to 40% cover, Class III = 40 to 60% cover, Class IV = 60 to 80% cover, and Class V = 80 to 100% cover.

In June 1971, three 400 by 400 foot sections from each coverage class were randomly selected for a detailed collection of dicotyledonous (broadleaf) plant species. A diagonal transect starting 20 feet within the northwest corner of each section was walked to the southeastern boundary. Plants were collected along the transect, and the results were tabulated for the number of dicotyledonous plants occurring in each section. A control area 0.2 mile northwest of the one square mile grid and an area in the center of the plot formerly occupied by Grid 1 were also surveyed. In June 1973, each of these areas was again surveyed by the same method. A square-foot analysis was performed on (1) 15 additional 400 by 400 feet sections, (2) the control area used in 1971, (3) a new control area west of the one square mile grid, and (4) a 160,000 ft^2 section in the center of area occupied by Grid 1. The additional 15 sections were randomly selected, and within each section, nine areas, each measuring one square foot, were analyzed (Figure III-1). The square foot sampling sites were selected by dividing the 400 by 400 foot sections into three strips, each 133 feet wide. A line was drawn in center of each strip and three one-square-foot areas were selected by generating d_1, d_2, and d_3 as distances to be walked. The distances were generated by a random number generator which included constraints that assured one sampling area from each one-third of the transverse with the sampling area being random within the one-third area. After each distance was walked-off, the metal square-foot measuring device was placed, and the percent coverage for each plant species was visually estimated.

d. Results and Discussion

The 1971 and 1973 vegetative coverage maps are shown in Figures III-2 and III-3, respectively. Table III-1 shows the percent coverage that each vegetative class occupied in June 1971 and in June 1973.

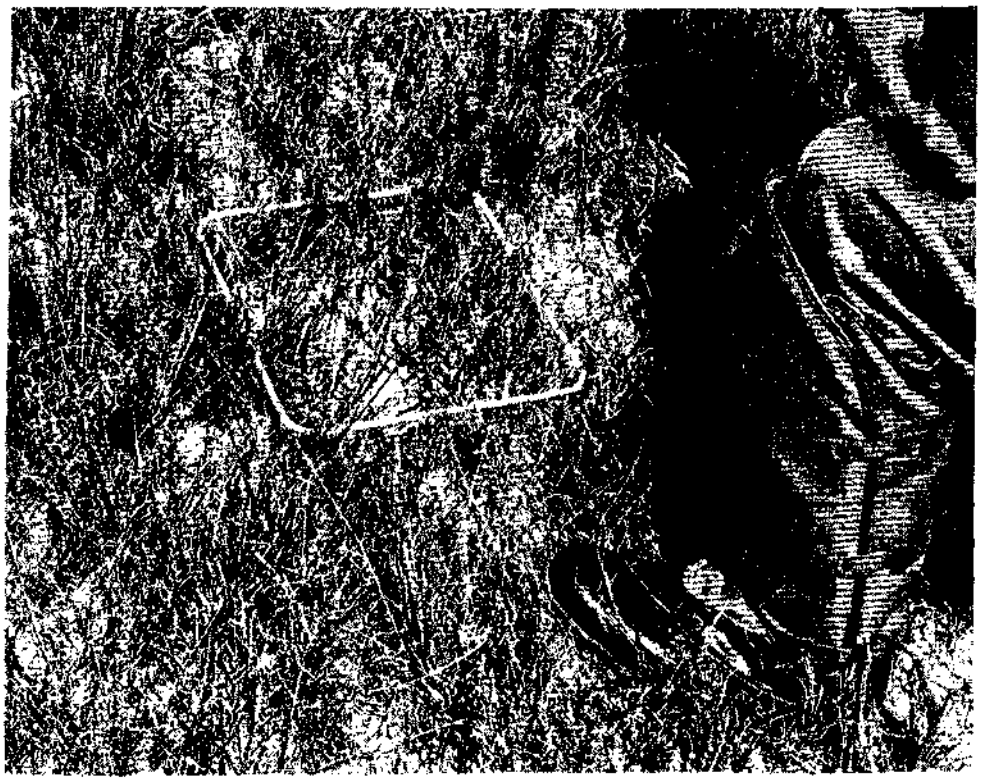

Figure III-1. Square-Foot Measuring Device in Use During Vegetation Survey - June 1973

TABLE III-1. PERCENT OF VEGETATIVE COVER OCCUPIED BY VEGETATIVE CLASS FOR THE 1 SQUARE MILE GRID

VEGETATIVE CLASS	JUNE 1971	JUNE 1973
0 (0 to 5%)	4%	0%
I (5 to 20%)	14%	4%
II (20 to 40%)	29%	12%
III (40 to 60%)	25%	18%
IV (60 to 80%)	21%	42%
V (80 to 100%)	4%	23%

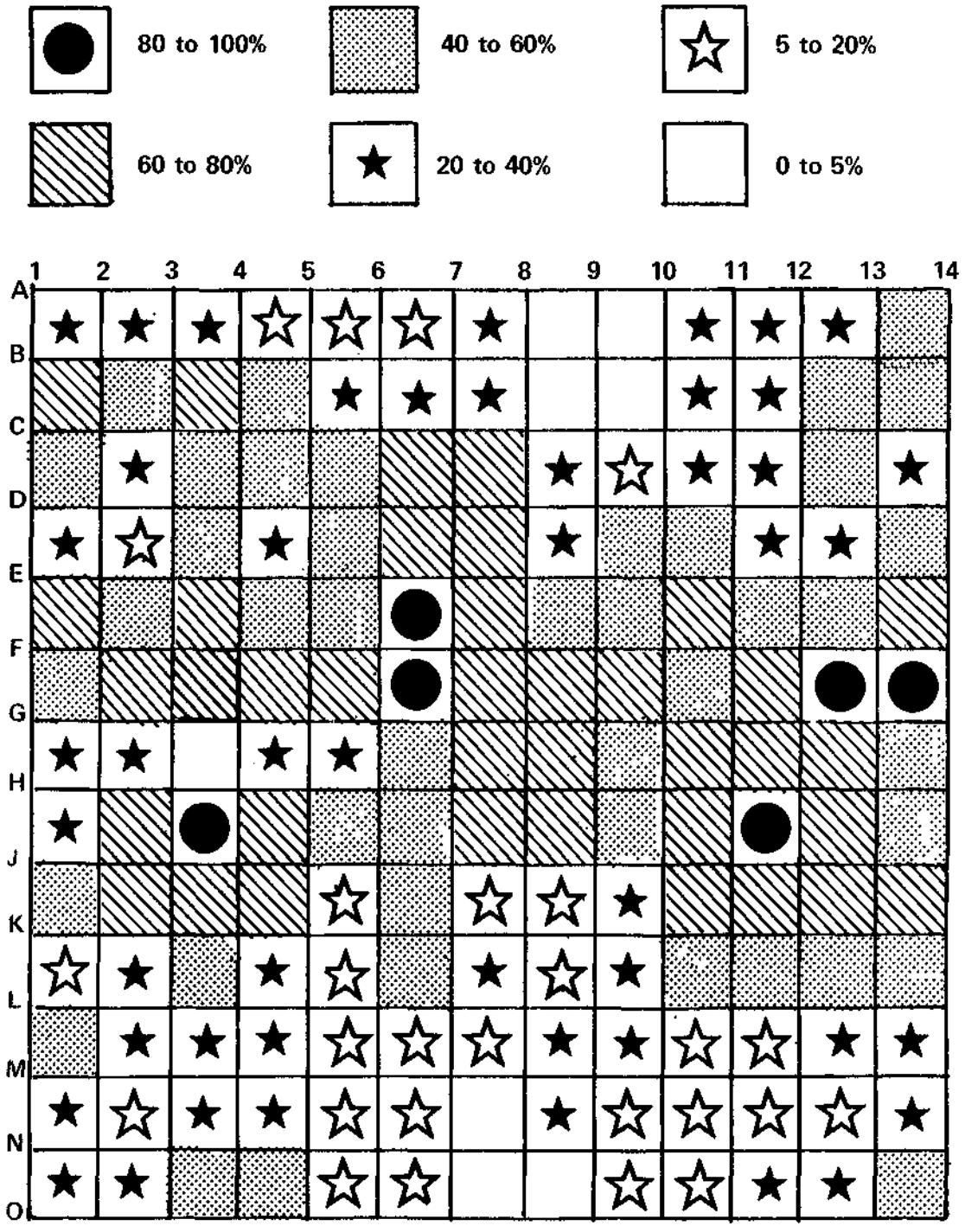

Figure III-2. Vegetative Coverage of the One Square Mile Grid on Test Area C-52A, May 1971

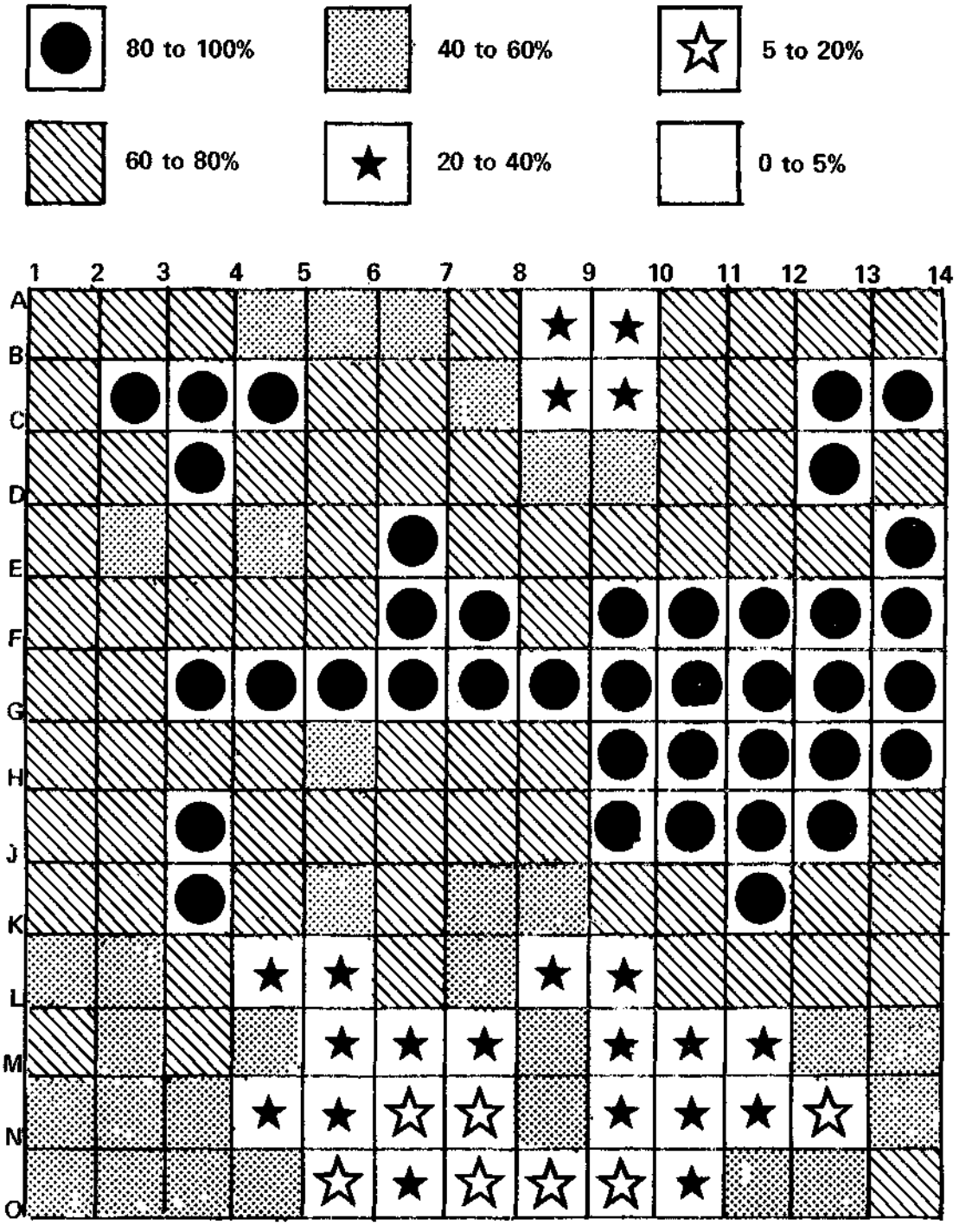

Figure III-3. Vegetative Coverage of the One Square Mile Grid on Test Area C-52A, June 1973

After a 2 year period, no vegetative class O areas were judged to remain on the one square mile grid. All class O areas had developed into class I or II areas. The greatest change in number of individual areas occurred from class II to IV (14% of total) and class III to IV (17% of total). For those areas with less cover than class V, 19 class IV areas, two class III areas, four class II areas and four class I areas did not change in percent cover during the 2 year interval.

Examples of vegetative cover changes are illustrated in Figures III-4 to III-13 by comparing the 1971 and 1973 photographs of identical areas. All of these photographs represent changes from one coverage class to another, except for area N-5 (Figures III-6 and III-7). Although an increase in vegetative coverage can be seen for N-5, this increase was not enough to place the area in Class II. Figure III-14 and Figure III-15 illustrate an increase in number of shrubs for a Class IV area.

In 1971, data on number of dicotyledonous or broadleaf species were assembled, and the same 400 by 400 feet areas were again surveyed in 1973. Table III-2 shows the increase in numbers of broadleaf plants after 2 years. The numbers of broadleaf plants are still significantly increasing in all areas, including Control Area 1. As is expected, the greatest percent change occurs in those areas with the smallest amount of vegetation because these areas have open sections where seeds can germinate free of competition from other plant species. However, these areas are relatively dry, windblown sites, and vegetative succession will continue to be relatively slow as compared to areas on the grid that have more poorly drained soils, and therefore, more available soil moisture. An indication of relative rate of succession for different areas of the one square mile grid is illustrated by observing the speed with which class O, I, II, and III areas change to other classes. For example, all 1971 class O areas on the northern section of the grid are now in class II, but 1971 class O areas in the southern portion of the grid only changed to class I in the 2 year interval.

From June to September 1971, 74 dicotyledonous species were collected on the one square mile grid, and 33 additional species were found during the June 1973 survey. Table III-3 contains a complete list of all species that have been found on the grid. The relative frequency of occurrance of all new species collected in 1973 was rare or infrequent except for Euphorbia maculata and Polygonella gracillis. Plants that were found in Control Area 2 but not found on the grid were rosinweed (Silphium ovatifolium), hairy bedstraw (Galium pilosum), sun flower (Helianthus sp.), and flax (Linum floridanum).

Because the square-foot analysis technique is a more accurate method of determining vegetative cover, the results of the two methods are compared in Table III-4. This shows that visual estimations of 400 by 400 foot areas were 8% to 30% higher than the more accurate square-foot technique. However, class rankings for the two methods are the same except for areas found to have 40% to 60% vegetative cover by the square-foot analysis. The most significant comparison of the two methods is that which shows Control Site 2 and Grid 1 area to be in class III instead of class IV.

As a result of the square-foot analysis, the most important plants on the grid in terms of coverage are the grasses, switchgrass (Panicum virgatum)(Figure III-16), and, woolly panicum (P. lanuginosum) (Figure III-17). These two grasses were found to comprise from 44% to 64% of the existing coverage for all vegetative classes. The most important dicotyledonous plants are rough buttonweed (Diodia teres) (Figure III-18), poverty weed (Hypericum gentianoides) (Figure III-19), and common polypremum (Polypremum procumbens). These three dicots occupy from 3% to 17% of the existing cover in all classes on the grid.

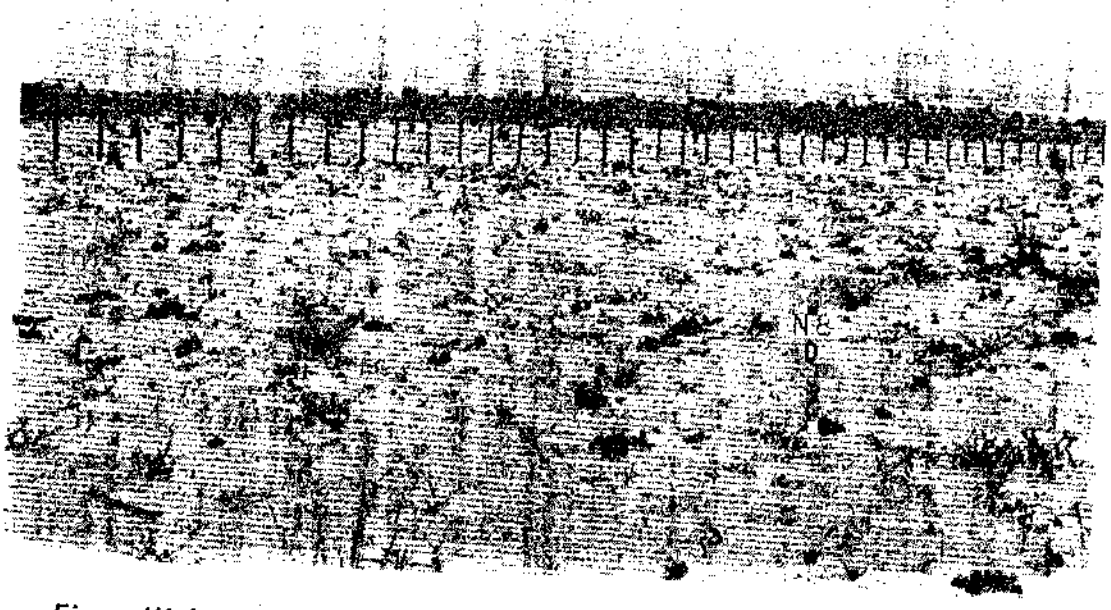

Figure III-4. Area N-8, June 1971, Looking SE from Sampler N-8

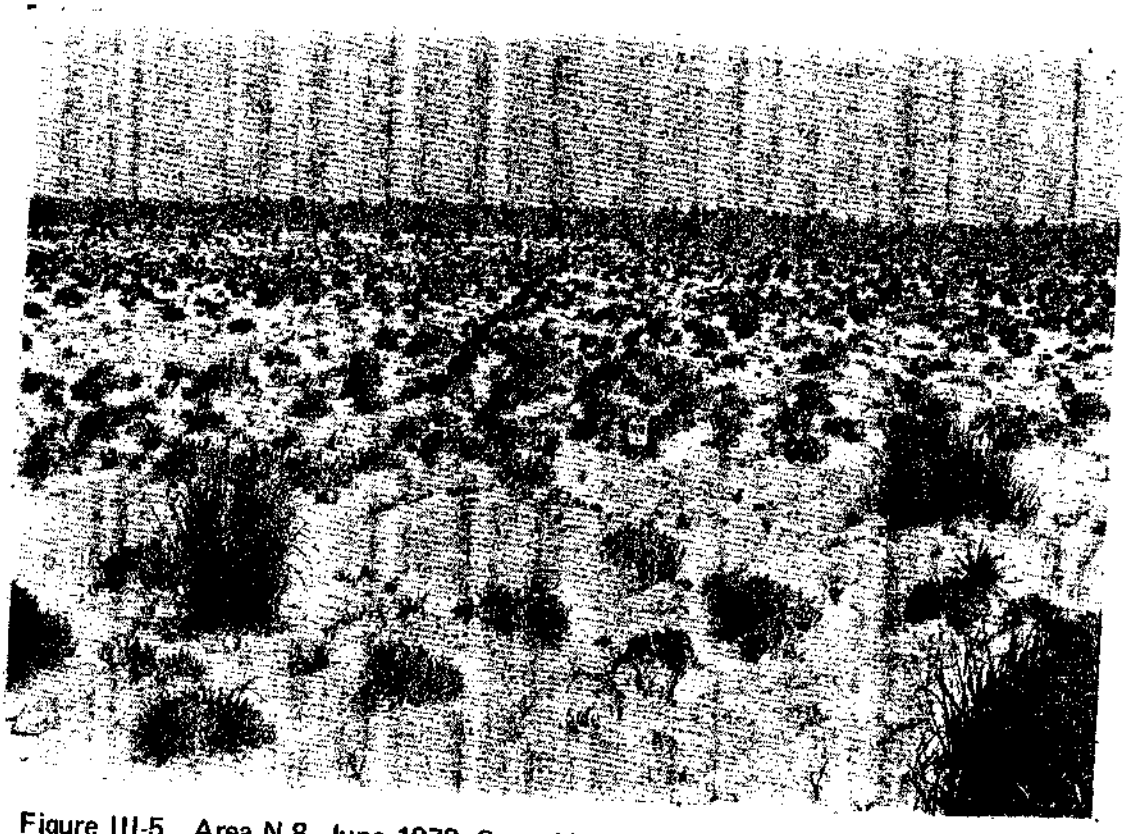

Figure III-5. Area N-8, June 1973, Same View as Figure III-4 After 2 Years

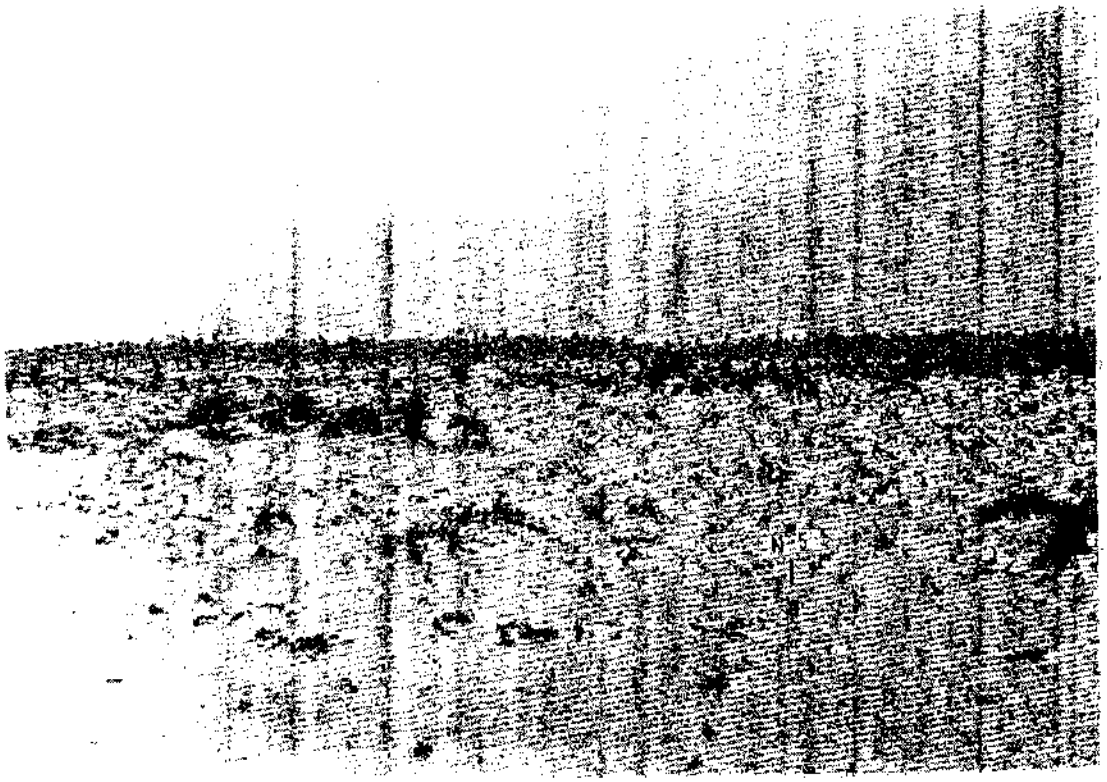

Figure III-6. Area N-5, June 1971, Looking SE from Sampler N-5

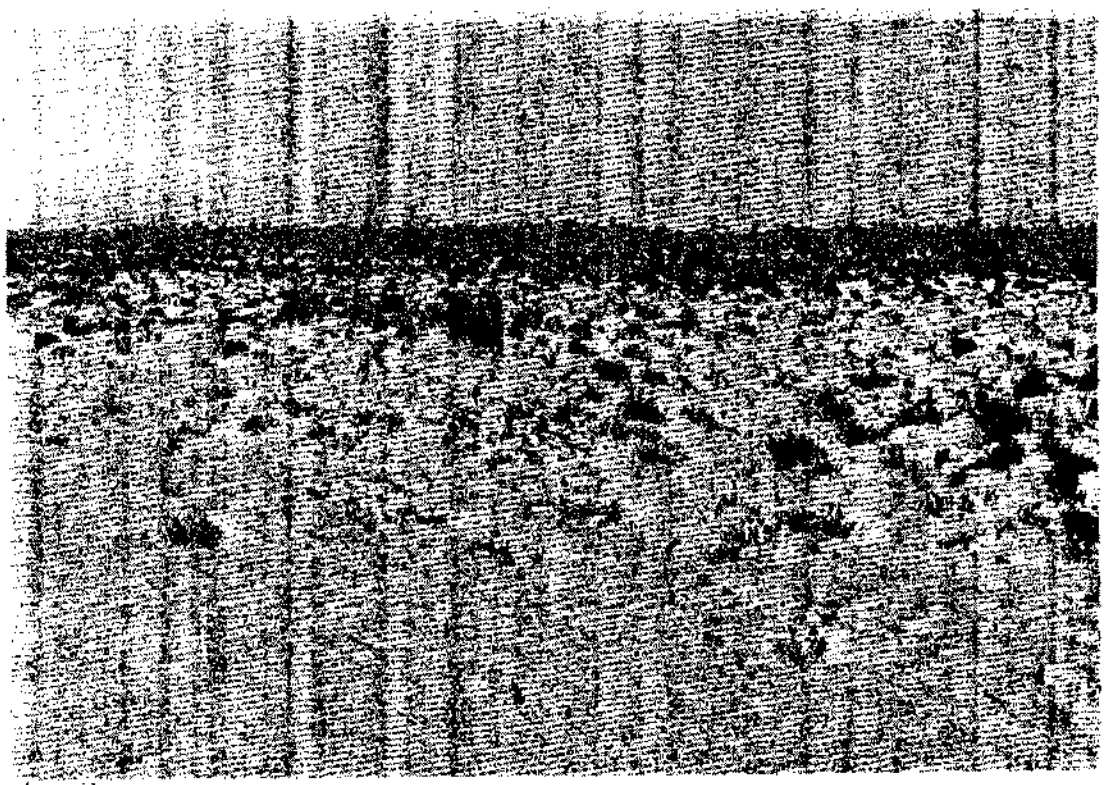

Figure III-7. Area N-5, June 1973, Same View as Figure III-6 After 2 Years

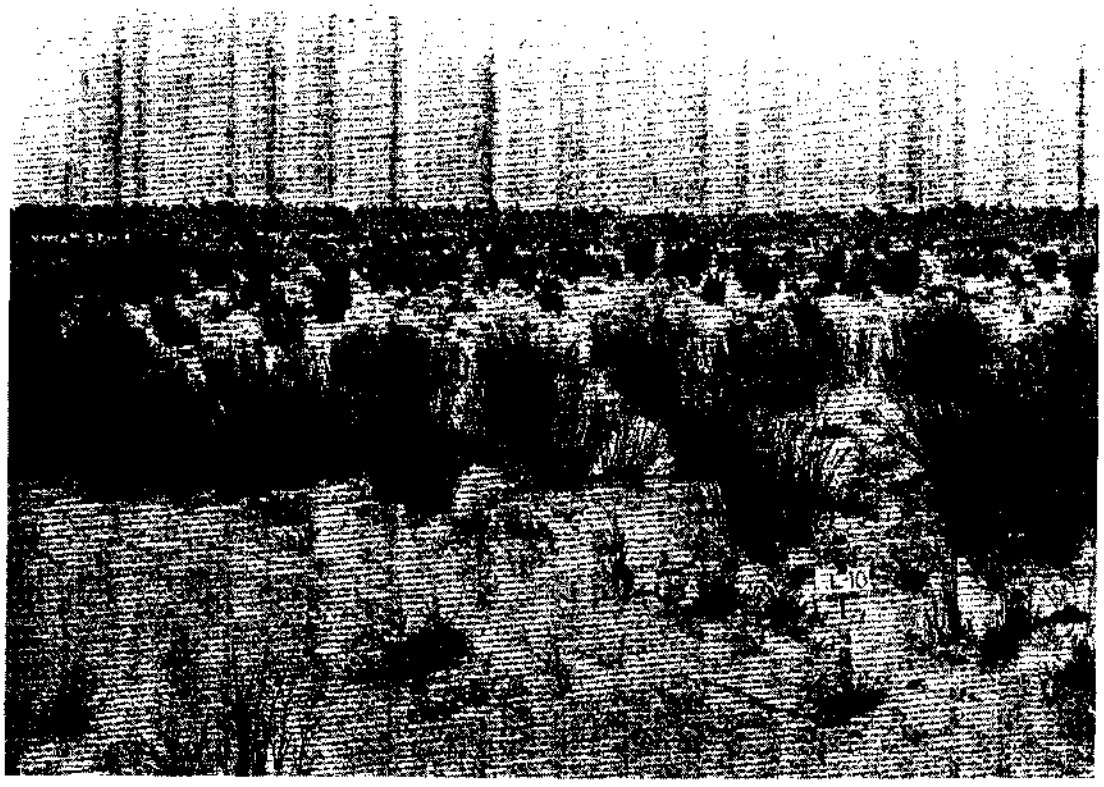

Figure III-8. Area L-10, June 1971, Looking SE from Sampler L-10

Figure III-9. Area L-10, June 1973, Same View as III-8 After 2 Years

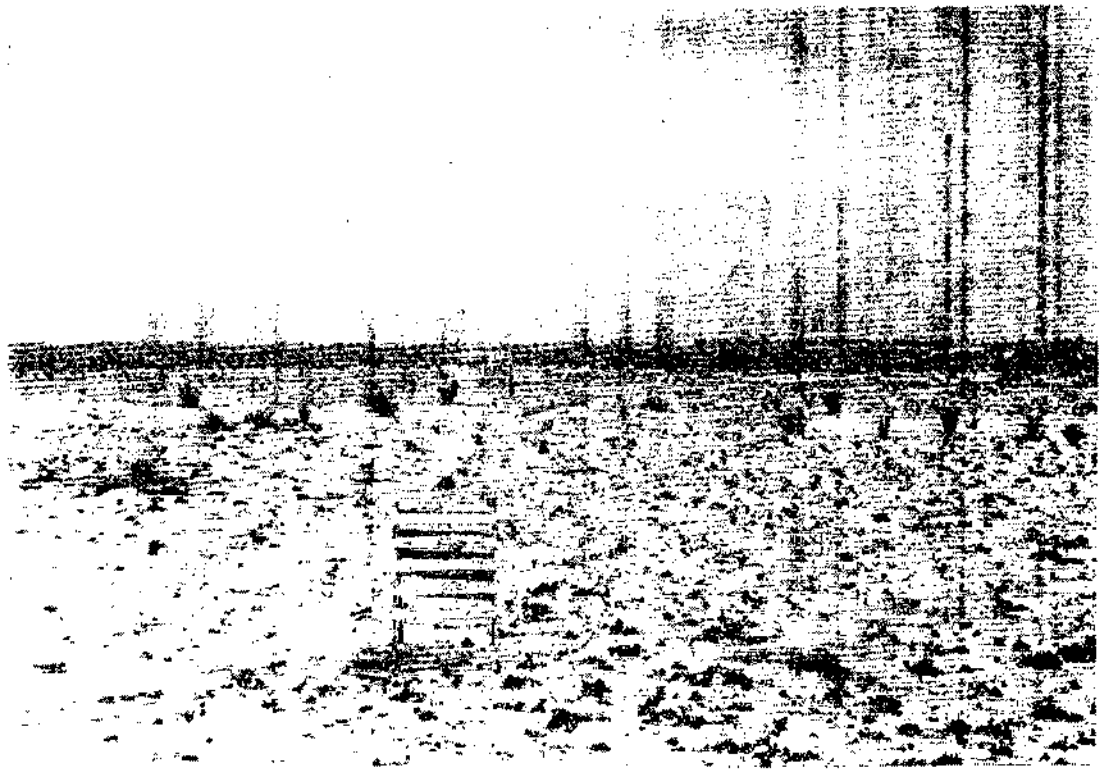

Figure III-10. September 1971 View of North-South Flightpath on One Square Mile Grid from Sampler A-9

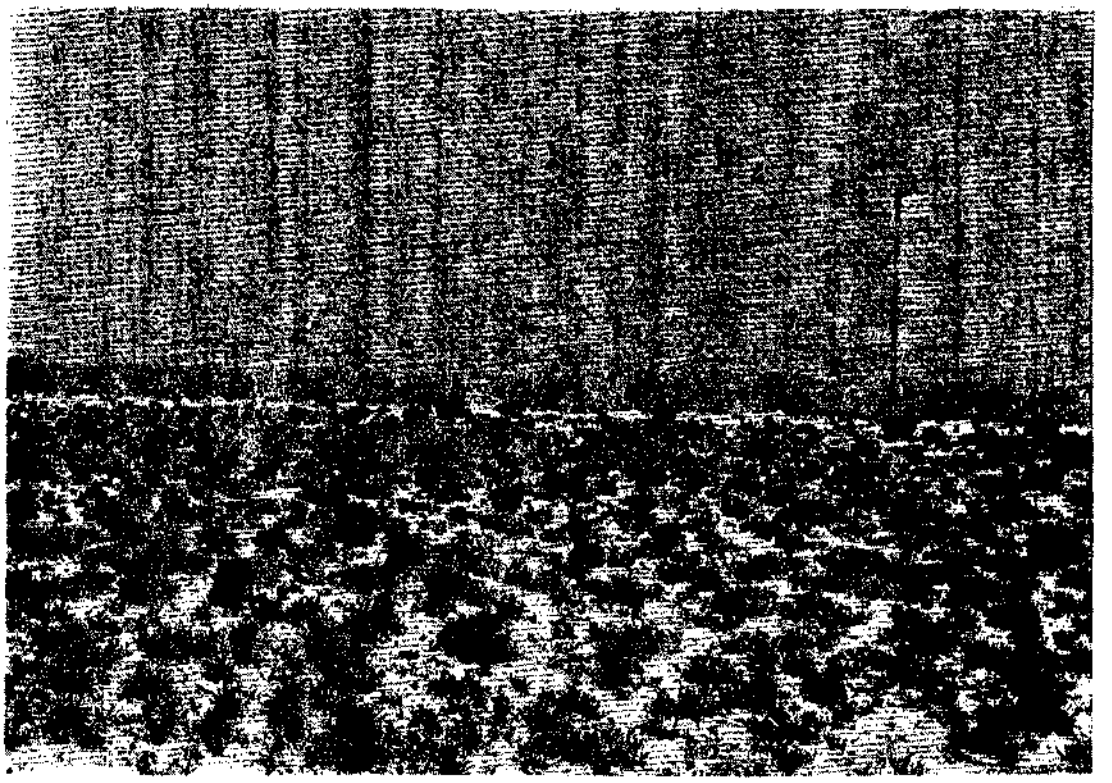

Figure III-11. June 1973 View from Same Position as Figure III-10 After 2 Years

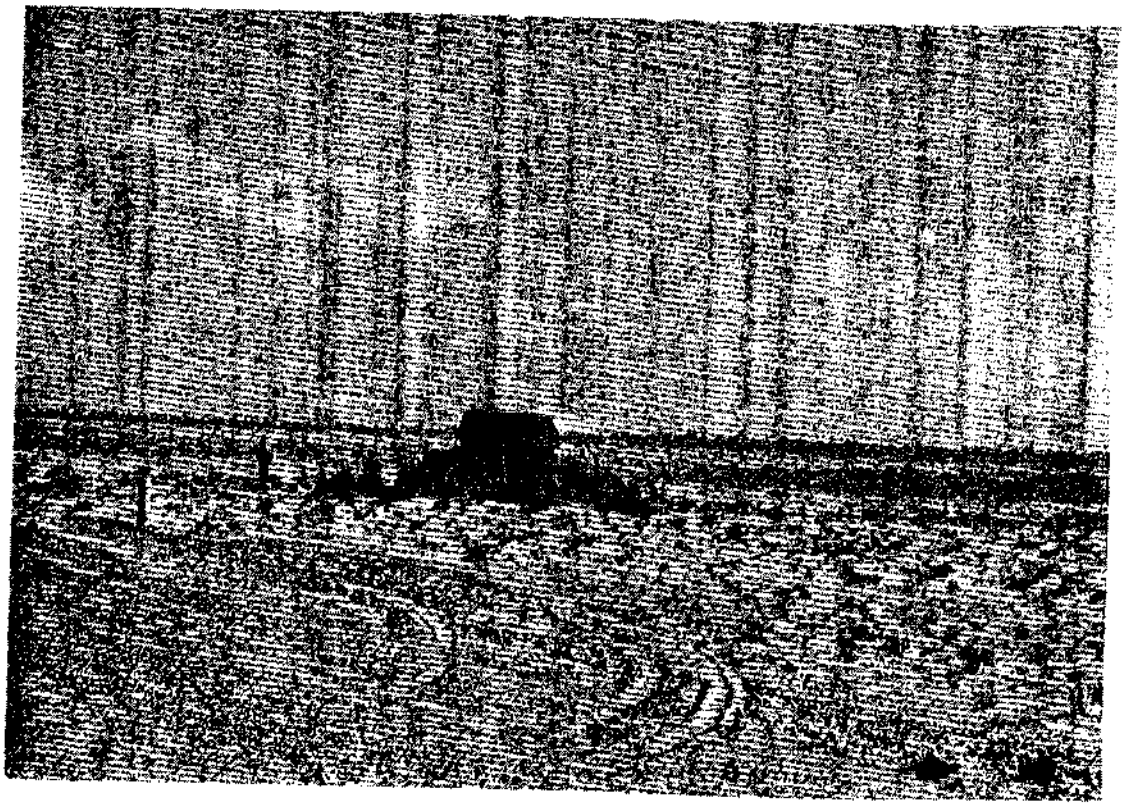

Figure III-12. April 1969 Looking South Near Sampler G-5

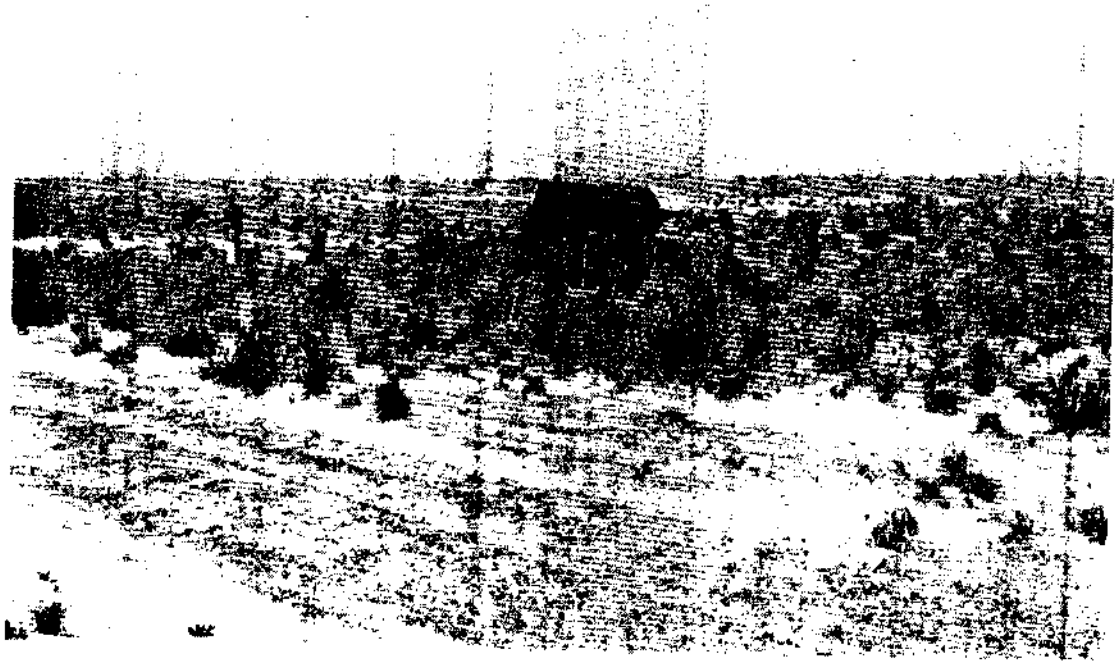

Figure III-13. July 1973, Same View as Figure III-12 After 4 Years

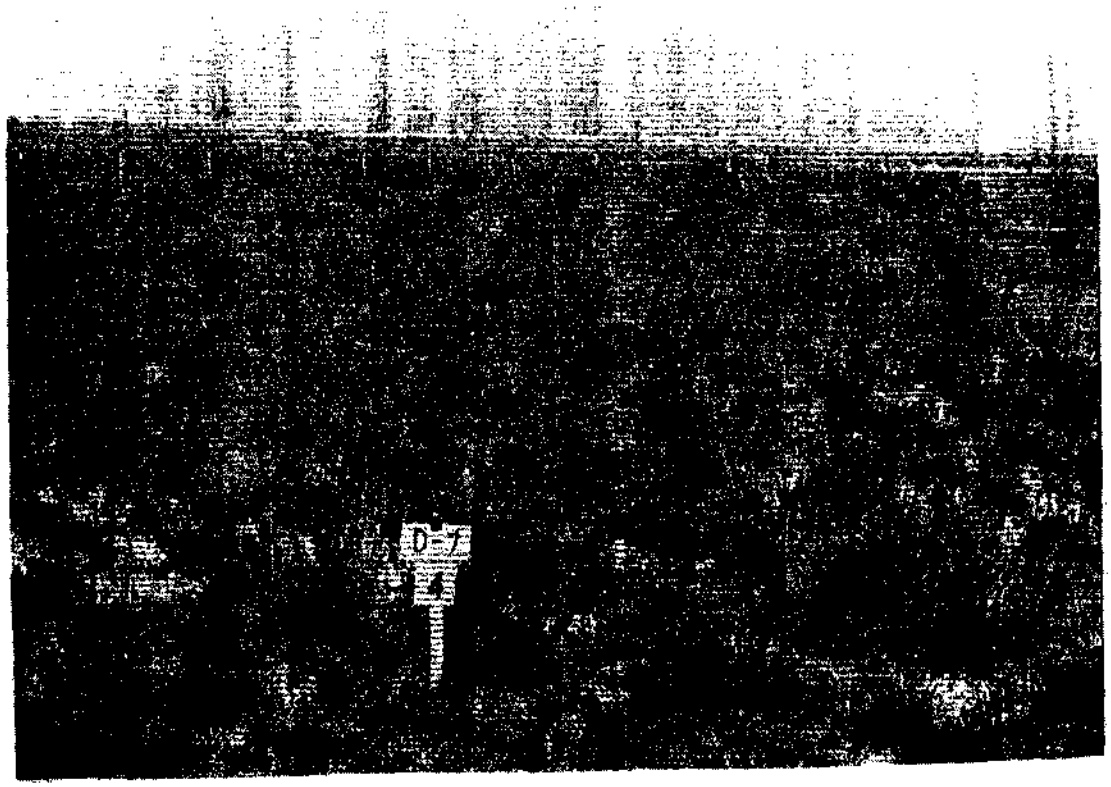

Figure III-14. Area D-7, June 1971, Looking SE From Sampler D-7

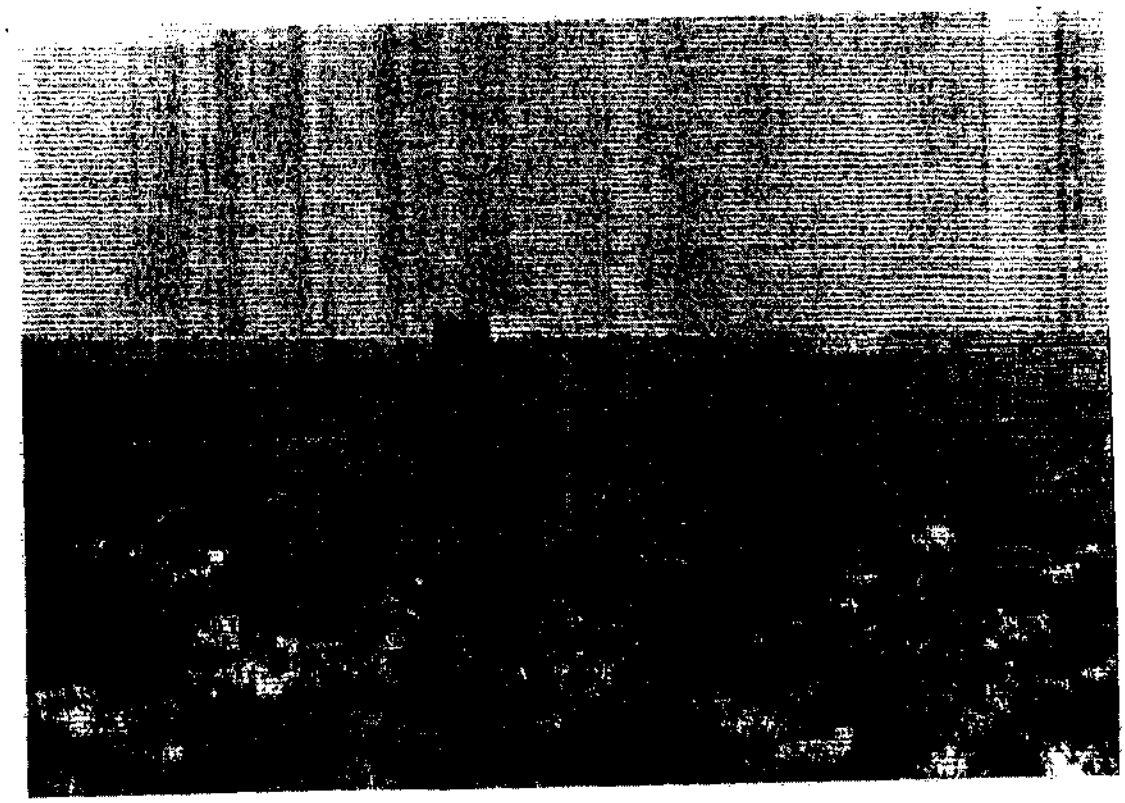

Figure III-15. Area D-7, July 1973, Same View as Figure III-14 After 2 Years (Notice increase in number of shrubs after 2 years)

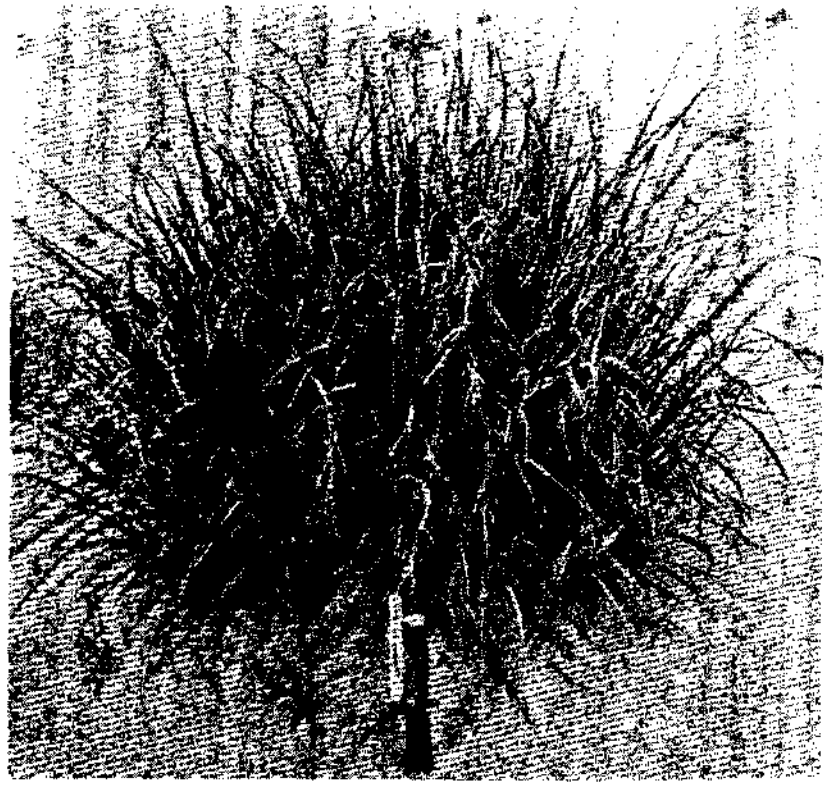

Figure III-16. Switchgrass (Panicum virgatum)

Figure III-17. Woolly panicum (Panicum lanuginosum)

Figure III-18. Poorjoe or Rough Buttonweed (Diodia teres)

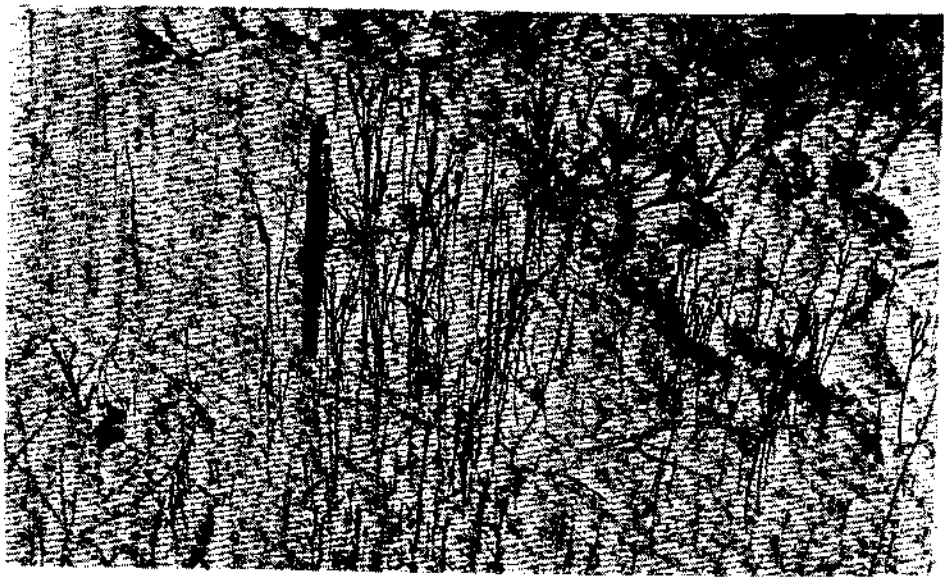

Figure III-19. Poverty Weed (Hypericum gentianoides)

TABLE III-2. VEGETATIVE COVER CHANGES AND DICOTYLEDONOUS PLANT SPECIES CHANGES BETWEEN JUNE 1971 AND JUNE 1973

400 BY 400 FT AREAS SURVEYED	CLASS RANKING		NUMBER OF DICOTYLEDONS COLLECTED		PERCENT INCREASE 1971 TO 1973
	1971	1973	1971	1973	
B-8, M-7, N-8	O	I, II	[a]5	[a]10	100
L-10, N-5, M-2	I	I,II,III	[a]6	[a]13	116
D-12, A-11, C-11	II	IV	[a]13	[a]23	76
D-9, K-13, J-1	III	IV	[a]17	[a]27	59
D-7, C-6, E-3	IV	V	[a]19	[a]37	94
E-6, F-12, H-11	V	V	[a]24	[a]37	54
Grid I Area	IV	IV	17	27	59
Control Area 1	V	V	28	39	39
Control Area 2	ND	IV	ND	44	ND

[a]Average of the three 400 by 400 ft areas

TABLE III-3. SPECIES OF DICOTYLEDONOUS SHRUBS AND HERBS COLLECTED ON TA C-52A ONE SQUARE MILE GRID IN SUMMER 1971 AND 1973

SPECIES	COMMON NAME	VEGETATIVE CLASS AND FREQUENCY OF OCCURENCE
SHRUBS		
*Baptisia ellipitica	wild indigo	V; rare
*Baptisia hirsuta	wild indigo	V; rare
Callicarpa americana	American beautyberry	IV; rare
Diospyros virginiana	common persimmon	IV; infrequent
Ilex glabra	gallberry	V; infrequent
Ilex opaca	American holly	IV; infrequent
Lespedeza sp.		III; rare
Pinus clausa	sand pine	IV; rare
Pinus palustris	longleaf pine	IV; rare
Quercus laevis	turkey oak	III, IV, V; frequent
Quercus sp.		V; infrequent
Quercus sp.		IV; infrequent
Quercus sp.		III, IV; infrequent
Quercus sp.		IV, V; infrequent
HERBS		
Acanthospermum australe	paraquay bur	III; frequent
Achillea millefolium	common yarrow	III; infrequent
Agaloma discoidalis		II, III; frequent
*Agalinis divaricata	foxclove	IV, V; infrequent
Ambrosia artemisiifolia	common ragweed	II, III, V; frequent
*Arenaria caroliniana	Carolina sandwort	II, IV, V; rare
Asclepia humistrata	common milkweed	IV; rare
*Asclepias tuberosa	butterfly weed	IV; rare
*Asyrum hypericoides	St Andrews cross	IV; rare
*Baccharis halimifolia	groundsel baccharis	
*Balduina angustifolia		IV, V; rare
Bigelowis nudata		V; infrequent
Cassia fasciculata	partridgepea senna	0,I,II,III; frequent
Centella asiatica		V; rare
*Chenopodium sp.	goosefoot	V; rare
Chrysobalanus oblongifolius	gopher apple	III, V; frequent
*Chrysoma pauciflosculosa		V; rare
*Chrysopsis aspera	golden-aster	
Chrysopsis graminifolia	grassleaf golden-aster	II; infrequent
Chrysopsis mixta	golden-aster	II; infrequent
*Clitoria fragrans	pigeonwings	V; rare
Cnidoscolus stimulosus	risky treadsoftly	III, IV; infrequent
Crotalaria maritima	rattlebox	III, IV, V; infrequent
Crotalaria sagittalis	arrow crotalaria	V; rare

*Collected only in June 1973

TABLE III-3. CONTINUED

SPECIES	COMMON NAME	VEGETATIVE CLASS AND FREQUENCY OF OCCURENCE
HERBS Continued		
*Croton argyranthemus		V; rare
Croton glandulosus	tropic croton	III; rare
*Cuphea sp.	cuphea	IV; rare
Diodia teres	rough buttonweed	all classes; common
Erechtites hieracifolia	fireweed	V; infrequent
*Erigeron annuus	annual fleabane	IV,V; frequent
Eriogonum tomentosum	wild buckwheat	II,IV; infrequent
Eupatorium capilifolium	dogfennel	II,III,IV; frequent
*Euphorbia maculata	spotted spurge	I,II,IV,V; frequent
Euphorbia supina Raf.	prostrate spurge	I; infrequent
Froelichia floridana	Florida snakecotton	I; infrequent
Galactia microphylla	milkpea	II,III; infrequent
Gnaphalium falcatum	cudweed	IV; infrequent
Gnaphalium obtusifolium	fragrant cudweed	IV,V; frequent
Gnaphalium purpurem	purple cudweed	III,IV; infrequent
Hedyotis procumbens		V; rare
Hedyotis uniflora		V; rare
*Heterotheca subaxillaris	camphor telegraph plant	IV; rare
Hypericum gentianoides	poverty weed	II,III,IV; frequent
*Hypericum myrtifolium	St Johns-wort	V; infrequent
*Kalmia harsuta	sandhill kalmia	V; rare
*Kragia virginica		IV; rare
Lechea patula	pinweed	III,IV; frequent
*Lechea villosa	hairy pinweed	IV; rare
*Liatris secunda	pinkscale gayfeather	V; infrequent
*Liatris gracilis	slender gayfeather	V; infrequent
Lithospermum caroliniense	Carolina gromwell	I,III; infrequent
Lobelia brevifolia	lobelia	V; frequent
Ludwigia virgata	false loosestrife	V; rare
Lupinus diffusus		0,I,III; infrequent
Lupinus nuttallii	sandhills lupine	I; rare
Mollugo verticillata	carpetweed	I; rare
Oxalis stricta	yellow woodsorrel	III; rare
Paronychia patula	nailwort	I,II,III; frequent
Petalostemon caroliniense	prarie-clover	I; infrequent
Phlox floridana	Florida phlox	II; infrequent
*Physalis heterophylla	clammy groundcherry	V; rare
Pluchea rosea		V; rare
Polygala nana	bachelor button	IV,V; infrequent
Polygala polygama	bitter polygala	II,III,IV,V; frequent
Polygala sp.	polygala	III; infrequent
*Polygonella gracilis	jointweed	I,II,IV; frequent

*Collected only in June 1973

TABLE III-3. CONCLUDED

SPECIES	COMMON NAME	VEGETATIVE CLASS AND FREQUENCY OF OCCURENCE
HERBS Concluded		
Polypremum procumbens	common polypremum	IV; infrequent
*Pterocaulon undalatum		V; rare
Rhexia alifanus	meadowbeauty	IV; frequent
*Rhexia lutea	yellow meadowbeauty	V; frequent
*Rhexia mariana	Maryland meadowbeauty	V; rare
*Rhexia salicifolius		V; rare
*Rhexia virginiana	common meadowbeauty	V; rare
Rhynchosia galactioides	pinebarrenpea	I,II,IV; frequent
Rhynchosia reniformis	dollarleaf rhynchosia	V; rare
Rubus sp.	blackberry	III; infrequent
*Rudbechia hirta	blackeyed coneflower	IV,V; rare
Rumex acetosella	red sorrel	II,III,IV; frequent
*Sabatia angularis	rosegentian	V; rare
Schrankia microphylla	littleleaf sensitive brier	IV,V; infrequent
*Smilax sp.	greenbrier	II,IV,V; frequent
Sophronanthe hispida		IV,V; frequent
Stylisma villosa		0,I; rare
Stylosanthes biflora	twin pencilflower	III; rare
Tephrosia sp.		III; rare
Tithymalus spaerospermus	common euphorbia	all classes; common
Tragia linearifolia	noseburn	IV; rare
Tragia smallii	noseburn	V; rare
*Tragia sp.	noseburn	V; rare
*Verbena carnea	verbena	V; rare
Vernonia angustifolia	ironweed	IV; infrequent
Wahlenbergia marginata	rockbell	III,IV; infrequent
Warea sessilifolia		III,IV; infrequent

*Collected only in June 1973

TABLE III-4. DATA COMPARISON OF PERCENT VEGETATIVE COVER BY VISUAL OBSERVATION OF OVERALL PLOT VERSUS THE SQUARE-FOOT TRANSECT METHOD, 1973 DATA

VEGETATIVE CLASS/SITE	VEGETATIVE CLASS CRITERIA	VISUAL ESTIMATE[a]	SQUARE-FOOT TRANSECT[b]
Class I	5 to 20%	19	11
Class II	20 to 40%	29	19
Class III	40 to 60%	60	41
Class IV	60 to 80%	76	67
Class V	80 to 100%	89	80
Control Site 2[c]		75	45
Grid 1[d]		75	47

[a]Average of 12 estimates

[b]Average of 27 transects

[c]Located 0.1 mile west of Sampler E-1

[d]Center section of Grid 1 located 1000 feet south of Sampler 0-7

e. Conclusions

A comparison of vegetative coverage and occurrence of plant species on the one square mile grid between June 1971 and June 1973 has shown that areas with 0 to 60% vegetative cover in 1971 have a coverage of 15% to 85% in 1973. Those areas having 0 to 5% coverage in 1971 (areas adjacent to or under flightpaths used during herbicide equipment testing) now have 15% to 54% coverage. The rate of change in the coverage seems to depend on soil type, soil moisture, and wind; there is no evidence to indicate that existing vegetative coverage is in any way related to herbicide residue in the soil. Dicotyledonous or broadleaf plants that are normally susceptible to damage from herbicide residues presently occur throughout the entire one square mile grid.

SECTION IV

STUDIES OF THE ANIMALS OF TEST AREA C-52A

In May 1970, a survey was initiated to determine the animal species composition of the spray-equipment testing grid on TA C-52A and within the adjacent 11 square mile area. The purpose of this survey was to determine the extent of faunal ecological alteration that occurred in the test area due to repetitive applications of military herbicides.

It was expected that application of military herbicides would temporarily alter the faunal ecology of an area, primarily due to the changes in the vegetation. It had been postulated that the animals living in a sprayed area would either be killed outright by herbicides or would receive doses via water or food that would affect their reproductive processes. Laboratory studies dealing with the teratogenic and embryotoxic effects of TCDD, a contaminant found in 2,4,5-T, have been reported (Reference IV-1). It had also been suggested that animals would totally avoid a sprayed area either due to the lack of food, the offensive appearance or taste of the vegetation, or odors produced by the herbicides or their degradation products.

The objectives of this animal survey were to determine species variation, distribution patterns, migration, and relative population sizes as found on the test grid or immediately adjacent to it. Methods of study included early morning, midday, and night field trips for identification and collection of mammals, birds, reptiles, and amphibians. Many species collected were brought into the laboratory where they were photographed and either preserved or mounted, and these now serve as a reference collection to facilitate identification for subsequent studies.

The results of the 1970 animal survey were reported in Reference IV-2. A synopsis of the 1972 report and comments concerning its correlation to the 1973 studies are included in this report.

1. SYNOPSIS OF QUALITATIVE ANIMAL SURVEYS, 1970 - 1973

A total of 18 mammal species were observed off the test grid with 12 of these species also being found on the grid. All of the animals sighted on the grid used the area for foraging or as a source of drinking water except the beach mouse and the hispid cotton rat, which were using the area as their habitat. The hispid cotton rat was first seen on the grid during the 1973 study. Table IV-1 lists the mammals observed both on and off the grid. The most important economic population in the area was the deer herd. Night field trips yielded average counts of from 24 to 36 deer on the grid and within the immediate area. Close inspection of aquatic areas on the grid during early morning field trips revealed extensive activity the previous nights. In addition to the deer herd a sizable herd of feral hogs earlier crossed with Russian Boars, also inhabited the area. The hogs frequented the marshy areas, drinking and rooting for food.

During the spring of 1970, a red fox was frequently observed close to the grid and its den was found approximately 100 yards from the edge of the grid. Five kits were found in the den and based upon gross observations, they appeared healthy and normal.

The most common rodents found off the grid in 1970 along the streams that drain the area were the cotton mouse and the hispid cotton rat. In the fields surrounding the grid, the eastern

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References:

IV-1. Report of the Advisory Committee on 2,4,5-T to the Administrator of the Environmental Protection Agency, 7 May 1971

IV-2. Pate, B. D., R. C. Voigt, P. J. Lehn, and J. H. Hunter: Animal Survey Studies of Test Area C-52A, Eglin AFB Reservation, Florida. AFATL-TR-72-72, Air Force Armament Laboratory, Eglin AFB, Florida, April 1972. Unclassified.

TABLE IV-1. MAMMALS FOUND ON THE 1 SQUARE MILE GRID AND WITHIN THE ADJACENT 11 SQUARE MILE AREA

SPECIES	COMMON NAME	AREA WHERE OBSERVED	
		ON GRID	OFF GRID
Canis familiaris	wild dog	[a]+	+
Dasypus novemcinctus	armadillo	+	+
Didelphis marsupialis	opossum	+	+
Geomys pinetis	southeastern pocket gopher	–	+
Lynx rufus	bobcat	+	+
Mephitis mephitis	striped skunk	+	+
Odocoileus virginianus	whitetail deer	[b,c] +	[b,c] +
Oryzomys palustris	rice rat	–	+
Peromyscus gossypinus	cotton mouse	–	[c] +
Peromyscus polionotus	beach mouse	[b,c] +	+
Reithrodontomys humulis	eastern harvest mouse	+	[c] +
Procyon lotor	racoon	+	+
Sciurus carolinensis	eastern gray squirrel	–	+
Sciurus niger	eastern fox squirrel	–	+
Sigmodon hispidus	hispid cotton rat	[a] +	[b,c] +
Sus scrofa	wild pig	+	+
Sylvilagus floridanus	eastern cottontail rabbit	+	+
Vulpes fulva	red fox	–	+

[a]Species found on or off grid for first time, 1973 data

[b]Dominant species; sighted during 80% of the field trips, 1973 data

[c]Dominant species; sighted during 80% of the field trips, 1970 data

harvest mouse was common. Eight pairs of the eastern harvest mouse were taken into the laboratory and allowed to breed. Six of the eight pairs had litters totalling 24 offspring which were normal in size and free from any apparent birth defects.

During the 1973 study, only two cotton mice and two eastern harvest mice were found. The cotton mice were caught near a stream draining the grid, and the eastern harvest mice were captured on the grid. There were eight beach mice captured off the grid in areas along streams and in open fields.

The most common rodent species on the grid was the beach mouse. Trapping studies during the summer of 1970 showed that this species was widely distributed throughout the grid, except in areas with less than 5% vegetative cover. In the 1973 study, the beach mouse was found predominantly in the areas of 5% to 60% vegetative cover.

At least 25 species of birds were observed in the area immediately adjacent to the grid or feeding within its boundaries. Many more species than those listed in Table IV-2 are found in the more densely forested areas near the outer limits of the 2 mile radius.

In 1970, seven species of water birds and waders were sighted repeatedly in the aquatic areas on or off the grid. The most common birds on the grid were the meadow lark and the mourning dove. It seems significant that all birds sighted, with the single exception of a grasshopper sparrow (caught in a live animal trap) were medium to large species.

In 1973, the first sightings of red-wing blackbirds and little blue heron occurred on the grid. In 1970, the meadow lark was the predominant species of bird found, while in 1973, frequent and repeated sightings of night hawks, bobwhite quail, Mississippi kites, mourning doves and meadow larks were reported.

Eighteen species of reptiles were collected or observed, with 10 species recorded on the grid and 12 species from the surrounding area (Table IV-3). Differences in faunal species composition on and off the grid due to vegetation differences can best be illustrated with the reptiles. Those species that are adaptable and occupy a variety of niches were found both on and off the grid in large numbers. The dominant species on the grid was the six-lined racerunner, and it was also one of the dominant species in the wooded area surrounding the grid. Those species whose habitat is characterized by definite vegetative type cannot adapt to the open habitat of the grid. The green anole and southern fence lizard are two of these. There are also species which occur in the forest areas but are more plentiful in the open areas, such as the eastern coachwhip. In 1973, the first softshelled turtle was seen on the grid.

Twelve species of amphibians were collected (Table IV-4). The amphibian population on the grid centered mainly around the aquatic areas with the exception of the two toad species, which were also found in the dry areas. There were breeding populations throughout most of the year in the aquatic areas on the grid: the southern cricket frog, the southern toad, the oak toad, the barking tree frog, the southern leopard frog, and the squirrel tree frog. The slimy salamander is one of the dominant species in the surrounding forest but does not occur on the grid, presumably because of its need for sufficient moist ground cover. The squirrel tree frog and the hog-nosed waterdog were first reported on the grid in the 1973 study.

2. CURRENT STUDIES ON ANIMALS

In the 1970 animal survey, 73 species of vertebrates were observed on and off the test grid. The most frequently observed species on the grid was the beach mouse Peromyscus polionotus

TABLE IV-2. BIRDS FOUND ON THE 1 SQUARE MILE GRID AND WITHIN THE ADJACENT 11 SQUARE MILE GRID

SPECIES	COMMON NAME	AREA WHERE OBSERVED	
		ON GRID	OFF GRID
Accipiter striatus velox	sharp-shinned hawk	+	+
Agelaius phoeniceus	red-wing blackbird	[a]+	+
Ammodramus savannarum	grasshopper sparrow	+	+
Bubulcis ibis	cattle egret	+	+
Botaurus lentiginosus	American bittern	+	+
Buteo jamaicensis	red-tailed hawk	–	+
Buteo liniatus	red-shouldered hawk	–	+
Butorides virescens virescens	eastern green heron	+	–
Caprimulgus vociferus	eastern whippoorwill	–	+
Casmerodius acbus egretta	American egret	+	+
Cathartes aura	turkey vulture	+	+
Chordeiles minor	night hawk	[b]+	[b]+
Colinus virginianus	bobwhite quail	[b]+	[b]+
Coragyps atratus	black vulture	+	+
Corvus brachyrhynchos	American crow	+	+
Florida caerulea	little blue heron	[a]+	+
Elanoides forficatus forficatus	swallowtail kite	+	+
Falco sparverius	sparrow hawk	–	+
Ictinia misisippiensis	Mississippi kite	[b]+	+
Sturnella magna	meadow lark	[b,c]+	[b]+
Turdus migratorius	robin	+	+
Zenaidura macroura	mourning dove	[b]+	[b]+
Unidentified Duck		+	+
Unidentified Goose		+	+
Unidentified Grebe		+	+

[a]Species found on grid for the first time, 1973 data

[b]Dominant species; sighted during 80% of the field trips, 1973 data

[c]Dominant species; sighted during 80% of the field trips, 1970 data

TABLE IV-3. REPTILES FOUND ON THE 1 SQUARE MILE GRID AND WITHIN THE ADJACENT 11 SQUARE MILE AREA

SPECIES	COMMON NAME	AREA WHERE OBSERVED	
		ON GRID	OFF GRID
Agkistrodon piscivorus	eastern cottonmouth	+	+
Alligator mississippiensis	American alligator	[a]+	+
Anolis carolinensis carolinensis	green anole	–	+
Cnemidophorus sexlineatus	six-lined racerunner	[b,c]+	[b,c]+
Coluber constrictor priapus	southern black racer	+	+
Crotalus adamanteus	eastern diamondback rattlesnake	+	–
Elphae guttata guttata	corn snake	–	+
Ferox sp.	soft-shelled turtle	[a]+	–
Heterodon platyrhinos	eastern hognose	+	–
Lampropeltis doliata doliata	scarlet kingsnake	+	–
Lygosoma laterale	ground skink	–	+
Masticophis flagellum flagellum	eastern coachwhip	+	+
Natrix sipedon pictiventris	Florida water snake	–	+
Pituophis melanoleucus mugitus	Florida pine snake	+	–
Pseudemys scripta scripta	yellow-bellied turtle	+	–
Sceloporus undulatus undulatus	southern fence lizard	–	+
Sistrurus miliarius barbouri	dusky pigmy rattlesnake	–	+
Sternotherus minor minor	loggerhead musk turtle	–	+

[a]Species found on grid for the first time, 1973 data

[b]Dominant species; sighted during 80% of the field trips, 1970 data

[c]Dominant species; sighted during 80% of the field trips, 1973 data

TABLE IV-4. AMPHIBIANS FOUND ON THE 1 SQUARE MILE GRID AND WITHIN THE ADJACENT 11 SQUARE MILE AREA

SPECIES	COMMON NAME	AREA WHERE OBSERVED	
		ON GRID	OFF GRID
Acris sryllus gryllus	southern cricket frog	b+	b+
Bufo quercicus	oak toad	b+	–
Bufo terrestris	southern toad	b+	b+
Eurycea bislineata cirrigera	southern two-lined salamander	–	+
Gastrophryne carolinensis	eastern narrow-mouthed toad	–	+
Hermidactylium sccutatum	four-toed salamander	–	+
Hyla gratiosa	barking tree frog	b+	+
Hyla squirella	squirrel tree frog	a,b+	–
Necturus beyeri	hog-nosed waterdog	a+	–
Plethodon glutinosus glutinosus	slimy salamander	–	+
Rana clamitans clamitans	bronze frog	–	+
Rana pipiens/ sphenocephala	southern leopard frog	b+	b+

[a]Species found on grid for first time, 1973 data

[b]A breeding population

and the six-lined racerunner Cnemidophorus sexlineatus. These two species were suggested as candidates for future studies of population distribution. During the months of February, March, and May 1971 a trapping study was performed on the test area for three, 4-day periods in each month (Reference IV-3). A total of 38 beach mice were captured during the three trapping periods. Thirty beach mice were captured during the February-March periods, and of these, six from the test grid and three from a control area were examined for gross deformaties. Sections of liver, kidney, and gonads were free of abnormalities, and no cleft palates were observed.

The primary purpose of the present study was two fold. First (Test Program I), animals were to be obtained for examination of gross and microscopic lesions, since it has been reported (Reference IV-1) that TCDD produces teratogenic and embryotoxic effects under certain experimental conditions. Second (Test Program II), the trapping survey discussed in the previous paragraph was to be expanded in an attempt to correlate habitat preference for the most prevalent mammal observed on the grid in order to determine if the population distribution is related to vegetative cover.

3. MATERIALS AND METHODS

Traps used for this study were Havahart traps (Havahart Traps, Department 1, P.O. Box 551, Ossining, N.Y. 10562) numbers 0 and 1 for small mammals. Traps were baited with peanut butter and oatmeal.

In June 1973, Test Program I was initiated by placement of traps on the square mile grid in two patterns. At first, one trap was placed in every other plot (400 by 400 foot areas) in every other row. For example, Row A had one trap each in plots 1, 3, 5, 7, 9, 11, and 13. Row C had one trap each in plots 2, 4, 6, 8, 10, and 12. Four to eight traps were placed in these areas. A total of 90 traps were emplaced. Traps were checked daily and the trapping duration was one week. A second portion of Test Program I utilized four sampling plots with distinct physical characteristics and involved the placement within each plot of 25 traps in five rows of five traps each, 20 paces apart, in each plot. Historically, these areas were exposed to low or high concentrations of herbicide[3]. Traps were checked daily, and the trapping was carried out for 7 days. In October 1973, a third portion of Test Program I was conducted on Grid 1 exclusively (Figure I-5). Grid 1 was divided into equal quadrants North-South and East-West, and was numbered upper left (Area 1), lower left (Area 2), lower right (Area 3), and upper right (Area 4). Twenty-five traps were placed in each area in two rows of 10 traps per row and one row of five traps with 15 paces between traps. The rows were located at 250, 500, and 750 feet from the center of the grid on the ordinate. Traps were checked daily for 7 days.

Mice, rats, and reptiles were taken to the laboratory for gross examination and prepared for histologic examination. The majority of the animals were alive on arrival but some had succumbed to the intense heat and confinement in the trap.

Live animals were subjected to a euthanasic procedure using ether. All animals were photographed, weighed, measured, and examined for developmental defects such as cleft palate, cleft lip, polydactyly, and micro-ophthalmia. All internal organs were examined for gross lesions, and individual organ weights were recorded. Representative sections of each tissue were placed in

[3]Personal communication with Donald King, Department of Zoology, University of Minnesota, Minneapolis, Minnesota.

neutral 10% buffered formalin and processed for microscopic study by the Veterinary Pathology, Washington, D.C. 20305. All remaining control and grid rat liver tissue and mouse fat and liver tissues were collected, placed in clean glass jars, frozen, and sent to the Interpretive Analytical Services Laboratory, Dow Chemical U.S.A., for TCDD analysis. The method of analysis was as described in Section II with the following exceptions: Ten grams of tissue were added to 10 ml ethanol and 20 ml 40% aqueous KOH and refluxed 2 hours. The resulting mixture was extracted with four 10 ml portions of hexane. The hexane extracts were combined, subjected to H_2SO_4 extraction and the same subsequent steps as in analysis of the soil samples.

Recovery studies using blank fish, beef liver, and soil averaged 70+% at the 10 to 25 part per trillion level.

In Test Program II, eight mouse traps were placed by computer randomization in areas which were classified according to vegetative coverage. Five vegetative areas were classified as follows: 5% to 20% coverage; 20% to 40% coverage; 40% to 60% coverage; 60% to 80% coverage; 80% to 100% coverage (see Section III, Figure III-3). Three of the 400 by 400 foot plots for each of Classes 1 to 5 were chosen at random. Eight pairs of coordinates were generated using the library random number generator for the Wang 720C programmable calculator. Each trap was placed in accordance with these coordinates. A total of 120 traps were utilized. Animals thus trapped were ear tagged with size 1, sequentially numbered, fingerling tags (National Band and Tag Company, Newport, Kentucky, 41071) and species, sex, and trap location was recorded. They were examined for external abnormalities and released. Traps were checked daily during the 8 days this study was conducted, and records were kept of original capture and the recapture of individual animals.

4. RESULTS AND DISCUSSION

During Test Program I, several different species of animals were caught, both on and off the test grid (Table IV-5). The sex distribution of the trapped animals was 23 male and 14 female beach mice, eight male and eight female cotton rats, two female eastern harvest mice, one male and one female cotton mice, ten male and seven female six-lined racerunners, one male eastern cotton mouth, and one male toad.

TABLE IV-5. TOTAL NUMBER AND LOCATION OF ANIMALS COLLECTED DURING 1973 TEST PROGRAM I

CLASS	COMMON NAME	OFF GRID	ON GRID
Mammalia			
	beach mouse	8	42
	cotton rat	10	6
	eastern harvest mouse	0	2
	cotton mouse	2	0
Reptilia			
	six-lined racerunners	4	13
	eastern cottonmouth	1	0
Amphibia			
	toad	1	0

The age of the rodents was determined by histological examination of the gonads based on the presence or absence of sperm or ova (gametes) in the gonads. Animals with gonads showing gametogenesis were classified as adults and those with gonads showing no gametogenesis were classified as immature. The age of the animals varied, but adults predominated in the sample, 55 adults, 33 immature. Nine pregnant mice and five pregnant rats were found in the adult female animals. The stage of gestation varied considerably from early pregnancy to near term. Fifty-four embryos and fetuses were examined grossly and microscopically. No developmental defects or other lesions were seen.

Gross necropsy lesions were relatively infrequent in the test population and consisted primarily of lung congestion in those animals that died prior to being brought to the laboratory. No developmental defects were seen in any of the adult animals.

Histologically, the tissues of 13 of the 26 control animals and 40 of the 63 animals from the test grid were considered normal. Microscopic lesions were noted in some animals from both groups. For the most part, these were minor changes of a type that would be expected in any animal population. One of the most common findings was parasites. A total of 11 controls and 9 grid animals were affected with one or more classes of parasites. These are summarized in Table IV-6.

Parasites may be observed in any species, and those in this population were for the most part incidental findings that were apparently not harmful to the animal. There were exceptions however. Protozoan organisms had produced focal myositis in one rat and were also responsible for hypertrophy of the bile duct epithelium in a six-lined racerunner.

Moderate to severe pulmonary congestion and edema were seen in several rats and mice. All of these animals were found dead in the traps before reaching the laboratory, and the lung lesions were probably the result of heat stroke. The remainder of the lesions in both groups consisted principally of inflammatory cell infiltrates of various organs and tissues. They were usually mild in extent, and although the etiology was not readily apparent, the cause was not interpreted as toxic.

It was highly improbable that any of the mice trapped during this study were alive during the final phase of herbicide dissemination (September 1970), although the life span of the beach mouse has been reported to be 5 years in captivity (Reference IV-3). A portion of the grid population was certainly made up of offspring of these animals present in 1970. Emigration from, or immigration to, the test grid could occur, especially on the fringe areas, since it has been reported that the area traveled by an individual beach mouse during its daily activities may extend to 5 acres (Reference IV-4).

An analysis of the ratios of organ weight to body weight, and organ weight to body length for mice captured off the grid versus mice captured on the grid was conducted within the severe constraints of limited data (June 1973 data). Female mice were not considered due to the fact all

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References:
IV-3. Benton, A.H. and W. E. Werner, Jr. Field Biology and Ecology. McGraw Hill, New York, 1966.
IV-4. Andrewartha, H.G. Introduction to the Study of Animal Populations. University of Chicago Press, 1961.

control females were pregnant and showed large individual body weight and organ weight variations. Only two of 11 female mice from the grid were pregnant. There were five control males (three mature and two immature) and 18 males (ten mature and eight immature) captured on the grid. Complete organ data were available only for 13 of the 18 grid males (nine mature and 4 immature).

It is recognized that the mature and immature mice will likely show different characteristics; however, combination of these two groups was necessary to produce any reasonable sample size. The t test for unpaired samples was used on 16 different factors (Table IV-7). These factors are as designated below:

FACTOR		DESCRIPTION
A	=	Total organ weight/body length
B	=	Total organ weight/body length
C	=	Sum of lung, heart, kidney, and brain/body weight
D	=	Sum of lung, heart, kidney, and brain/body weight
E	=	Lung weight/body weight
F	=	Heart weight/body weight
G	=	Spleen weight/body weight
H	=	Liver weight/body weight
I	=	Kidney weight/body weight
J	=	Brain weight/body weight
K	=	Lung weight/body length
L	=	Heart weight/body length
M	=	Spleen weight/body length
N	=	Liver weight/body length
O	=	Kidney weight/body length
P	=	Brain weight/body length

Formula for the procedure used:

$$\text{"t"} = \frac{\left| \frac{\Sigma X_2}{N_2} - \frac{\Sigma X_1}{N_1} \right|}{\sqrt{\left[\Sigma X_1^2 - \frac{(\Sigma X_1)^2}{N_1} + \Sigma X_2^2 - \frac{(\Sigma X_2)^2}{N_2} \right] \left[\frac{N_1 + N_2}{N_1 N_2} \right]}{N_1 + N_2 - 2}}$$

Where

$$N_1 + N_2 \neq 2$$

TABLE IV-6. PARASITES FOUND IN RODENTS COLLECTED FROM CONTROL AND TEST AREA SITES, JUNE 1973

LOCATION	NUMBER OF ANIMALS EXAMINED	NUMBER OF ANIMALS EFFECTED	PARASITES		
			NEMATODES	CESTODES	PROTOZOANS
Control	20	11	9	1	5
Test Area[a]	50	9	4	0	7

[a]Animals trapped on Grids 2, 3, and 4

TABLE IV-7. SUMMARY OF RESULTS OF ORGAN WEIGHT TO BODY WEIGHT, AND ORGAN WEIGHT TO BODY LENGTH FOR MICE OFF THE GRID VERSUS MICE ON THE GRID (H ≡ On-Grid and Off-Grid Samples are from the Same Population)

$\nu = 16$

FACTOR	t	P (exceeding t, Given H)
A	0.1863	0.43
B	0.8859	0.19
C	0.9750	0.17
D	0.1025	0.45
E	1.6618	[a]0.06 [b](0.05)
F	0.2750	0.38
G	1.1025	0.14
H	0.7077	0.25
I	2.2228	[a]0.02 [b](0.10)
J	0.2363	0.41
K	0.6500	0.27
L	0.5659	0.29
M	0.4979	0.32
N	1.0214	0.16
O	1.1034	0.14
P	1.1647	0.13

[a]Significant at $p \leq 0.05$

[b]Value when control animal with lung and kidney lesions is removed from sample

When the ratios of average body weight to average organ weight of various visceral organs were compared between the male mice captured on the grid and the male mice captured off the grid it was found that on the average, the control animals had lung and kidney weights that varied significantly at the 95% confidence level. The lung variation just being significant. When this information was compared to the pathological work-up, it was found that one male control animal had multifocal subacute pneumonitis and multifocal subacute nephritis. When this animal was removed from the sample, the ratio of kidney weight/body weight between the control and grid animals no longer varied significantly. The lung weight/body weight variation became slightly more significant. It is felt this variance is due to the difference in ratio of mature to immature animals between the two groups, i.e., controls 3:2 compared to 9:4 for the grid animals.

The analyses for TCDD from rodents collected in June and October 1973 are shown in Table IV-8. An initial interpretation would be that TCDD does in fact accumulate in liver and fat of tissue from rodents living on the test grid. Data from soil analysis (Table II-10, Section II) confirm the presence of TCDD in soils of the test area. Discrepancy of levels of TCDD between soils and tissues suggest the potential for bio-magnification of this compound. These data do not correlate with previously published research (Reference IV-5). Such levels encounted in the animals reported herein would be suspect of teratogenic or pathologic abnormalities. Such abnormalities, however, were not encountered in this study. It would appear that analytically, via mass spectrometry, the chemical detected is of a very similar nature to TCDD, but biologically does not behave in the manner characterized for TCDD (Reference IV-5).

TABLE IV-8. CONCENTRATION (PARTS PER TRILLION) OF 2,3,7,8-TETRACHLORO-DIBENZO-p-DIOXIN (TCDD) IN LIVER AND FAT SAMPLES FROM RODENTS COLLECTED FROM CONTROL AND TEST SITES ON TA C-52A, JUNE OR OCTOBER 1973[a]

RODENT	TISSUES	CONTROL	LOCATION	
			GRIDS 2, 3, 4	GRID 1
Rats	Liver, Fat[b]	< 20	210	No Sample[c]
Mice	Liver, Fat[b]	< 20	300	540[d]

[a]Analysis for TCDD was performed by the Interpretive Analytical Services, Dow Chemical U.S.A., Midland, Michigan
[b]Tissues represent a composite from all animals collected at the respective location
[c]Rats do not frequent dry areas.
[d]Sample collected in October 1973.

Reference:

IV-5. Conference on Dibenzodioxins and Dibenzofurans, Environmental Health Perspectives, Experimental Issue Number Five, September 1973, National Institute of Environmental Health Science, Research Triangle Park, North Carolina.

The beach mouse was reported in Reference IV-2 as the most common rodent species on the grid in 1970. Observations in the field indicate that the beach mouse remains the most common rodent on the grid.

The 1970 study (Reference IV-2) also indicated that the beach mouse was widely distributed throughout the grid except in areas of less than 5% cover. In an attempt to correlate distribution of the beach mouse with vegetative cover, a second test program (Test Program II) was initiated with a total of 83 animals being trapped during an 8 day period, 28 June to 3 July 1973. The majority of animals (63) were found in areas with 5% to 60% vegetative cover; within this range, the greatest number of animals trapped (28) was from an area with 40% to 60% cover (Figure IV-1). A similar habitat preference has been observed along the beaches of the Gulf Coast (Reference IV-5). In this study, it appears that the beach mouse utilizes the seeds of switchgrass, (Panicim virgatum) and woolly panicum (Panicum lanuginosum) for a food source, and these are two of the most dominant plants on the grid (see photographs in Section III). Seed husks of these plants have been observed in areas of mouse activity. It is possible that another prominant plant, broomsedge bluestem (Andropogon virginicus), also provides food for the beach mouse.

In an attempt to compare the trapping data from 1971 with those data obtained in the 1973 study and, hence, to determine whether an increase in the population of beach mice has occurred, the following assumptions were made:

a. It was assumed that the traps, the bait, and the methods employed for setting and placing the traps were equally as effective and similar in the 1971 and 1973 studies.

b. It was assumed that the density of trap placement was equally as effective and similar in the 1971 and 1973 studies.

c. It was assumed that traps should be no further apart that approximately 1-1/2 times the mean random travel distance of the animal being trapped. For beach mice, this distance is approximately 300 feet (Reference IV-5).

d. On the other extreme, it was assumed that the traps should not be so dense as to impede animal movement nor disrupt animal habits.

All trapping experiments involved in this study were conducted within these extremes.

In order to produce an estimate of the population density of the beach mouse in the herbicide treated area, it was necessary to determine what portion of that area was effectively surveyed. It was also necessary to normalize the areas sampled for comparison based on the mean random travel of the beach mouse from the 1973 recapture data. A tabulation was made of the distances between the trap where initial capture occurred and the trap location of the recapture farthest from the initial capture point. These distances represent the distances that the mice from the sample were known to have traveled and were assumed to be random samples from the population of habitat radii. The longest radius observed was 3,200 feet, the next longest was 285 feet, and the shortest was 45 feet. The 3,200-foot distance was disregarded as a freak occurence because such a number appeared only once out of a sample

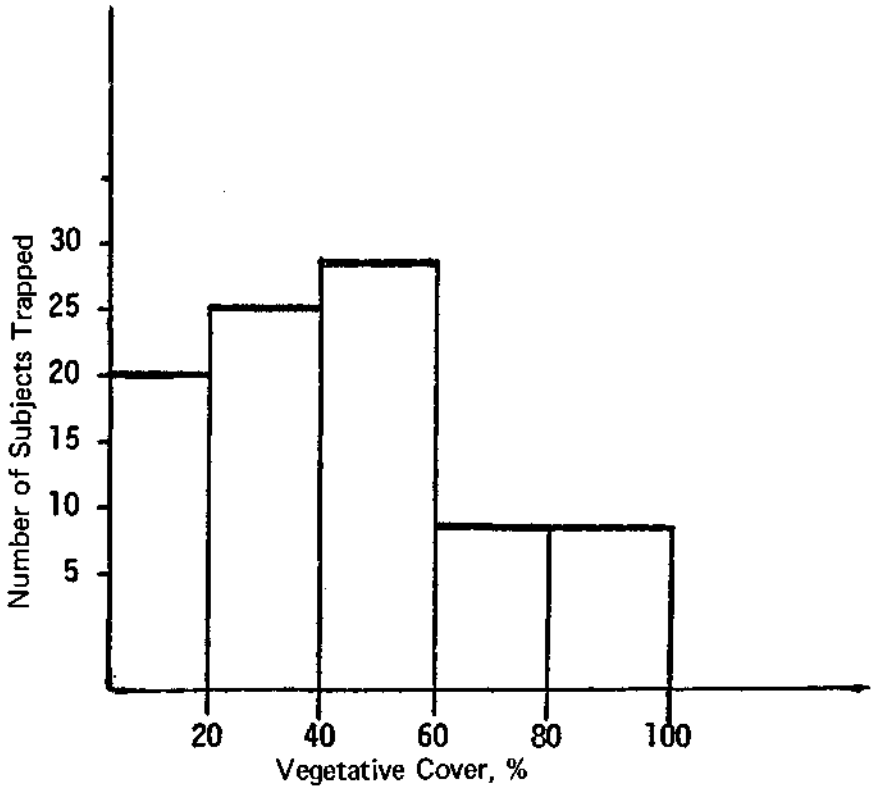

Figure IV-1. Relationship of Animals Trapped to Vegetative Cover

of 18 and it is more than 11 times as large as the next largest distance which is, itself, only 6.3 times as large as the smallest distance observed. The mean distance, 194 feet, of the remaining 17 distances was used as the average habitat radius. Circles with 194-foot radii were drawn using the traps of 12 randomly selected 400 by 400 foot plots as centers. The envelope area around the traps was estimated using a triangle, rectangle, or circle according to the fit which, by inspection, appeared to be the best. The average of the 12 areas measured was 8.3 acres with a standard deviation of 1.69, a maximum of 10.45, and a minimum of 5.17. The estimate, 8.3 acres, was used as the effective survey area for each group of eight traps.

a. Variation of the Lincoln Index

The Lincoln Index may be stated mathematically as:

$$P_1 = \frac{N_1(N_2 - R)}{R} + N_1$$

Where

P_1 = first population estimate

N_1 = pre-census sample

N_2 = total census sample

R = number of recaptures

Using the catch of 26 June and considering only the tagged animals, $N_1 = 13$. Using the catch of 27 June, $N_2 = 18$ and $R = 1$. According to this information, the first estimate is $P_1 = 222$. Enough uncertainty is introduced, however, such as how a dead animal should be counted in the pre-census sample, that an alternative method of estimation was desirable. The following equation was used to calculate the second estimate of the population.

$$P_2 = \frac{\sum_{i=1}^{8} \frac{M1_i\,(N2_{i+i} - R_{i+1}) + N1i}{R_i + 1}}{i_{max} - 1}$$

Where

N_1i = the catch from the ith day

$N_2 + 1$ = the catch from the (i + 1)th day

$R_i + 1$ = the recaptures from the (i + 1)th day which were captured on the (ith)day also

The values for $R_i + 1$ were corrected for the influence of mice no. 18, recaptured six times; no. 27. recaptured four times; no. 28, recaptured four times; no. 41, recaptured four times; no. 45 recaptured three times; and no. 50, recaptured three times. These mice were considered to be trap addicts. From the data available, $P_2 = 152$.

A third method was used for calculating P_3 in which the first catch was considered to be the pre-census and the second catch the census. A computer program to simulate the trapping of mice was developed with inputs of average sample size, Sa, and true population size, Pt. Twenty-five runs were made at specific values of Sa and Pt, and the estimated population was calculated with the Lincoln Index and averaged over the 25 trials. The average recapture number, Ra was also calculated. Pt was changed until $\bar{R}a$ was equal to the observed recapture rate and $\bar{S}a$ was equal to the observed sample size. $\bar{P}t$ at these values is considered to be the best estimate of the population, i.e., $\bar{P}t$ is the number most likely to produce the results which were observed in the real case. P_3 by this method was 191. This is not grossly different from the 222 and 152 estimated from P_1 and P_2. The average of P_1, P_2, and P_3 is probably the best overall estimate of the population. This average is approximately 189 total population - approximately 1.64 mice per acre.

5. CONCLUSIONS

Based on the pathologic findings of the Test Program I study, it was concluded that there was no evidence that the herbicide contaminant in question (TCDD) had produced any developmental defects or other specific lesions in the animals sampled or in the progeny of those that were pregnant. The lesions found were interpreted to be of a naturally occurring type and were not considered related to any specific chemical toxicity. The organ to body weight and organ to body length comparisons for the grid versus the control animals did not vary significantly when age and pathological lesions were considered. Chemical analysis of composite rodent liver and fat tissue indicated that there was an accumulation of TCDD-like chemical in tissue. If these data are valid (an assumption that may be challenged), what is the source of the TCDD? Seeds of switchgrass (Panicum virgatum) were found in abundance in the stomachs of beach mice. Samples of such seed collected from the test area were analyzed for TCDD. Results indicated no residue of TCDD at a minimum detection limit of less than 10 parts per trillion.

Based on information provided by the Test Program II study, it was concluded that the beach mouse forms a natural, integral part of the ecosystem of the Lakeland Sand Complex utilizing the dominant plants on the grid for food. The beach mouse continues to inhabit areas of 5% to 60% cover, with a preference for areas of 40% to 60% vegetative cover. This is indeed similar to the habitat preference of the beach mouse in other locations.

The statistical evidence derived from the Test Program II study shows that the 1.64 beach mice per acre population (based on the Lincoln Index for 1973) is slightly higher than the 0.8 and 1.4 mice per acre found on Santa Rosa Island (Reference IV-6). It was also concluded that the population of beach mice was higher in 1973 than in 1971 in the area of the test grid. Even though the first trial of the 1971 data reflected a higher count of mice per acre trapped, the low capture count on the second trial in 1971 indicates a lower actual population based on the Lincoln Index assumptions than the 1973 data. The apparent increase in beach mouse population on the grid in 1973 over 1971 was probably due to the natural recovery phenomenon of a previously disturbed area. Some areas of the test grid have already exceeded the preferred percentage of vegetative coverage of the beach mouse habitat, and other areas are either ideal or fast developing into an ideal habitat. If the test grid remains undisturbed and continues toward the climax species, a decline in the number of beach mice will probably occur simply due to his habitat preference.

Reference:
IV-6. Blair, W. F., Population Structure, Social Behavior, and Environmental Relations in Natural Populations of the Beach Mouse (Peromyscus polionotus leucephalus). Contribution from the Laboratory of Vertebrate Biological, University of Michigan, Number 48:1-47, June 1951.

SECTION V

INSECT DENSITY AND DIVERSITY STUDIES ON TEST AREA C-52A

During 1970 and 1971, an initial survey of the arthropod populations of Test Area C-52A, Eglin Air Force Base, Florida, was accomplished, and the results were published in Reference V-1.

1. SYNOPSIS OF PREVIOUS RESEARCH, MAY - JUNE 1971 (Reference V-1)

A sweep net survey of the insects on a 1 mile linear transect of Test Area C-52A resulted in the collection of more than 1,800 specimens belonging to 74 insect families and two non-insect arthropod orders. Eighteen of the taxa collected accounted for 97 percent of the collection, and of these, six taxa accounted for 72 percent of the collection: order Araneida (spiders), insect families Cicadellidae (leafhoppers), Elateridae (click beetles), Asilidae (robber flies), Hygaeidae (lygaeid plant bugs), and Pentatomidae (stink bugs). Spiders and robber flies are carnivores, stink bugs are carnivores or herbivores, and the other families are herbivores.

As plants were eliminated by the herbicides, those insects which fed specifically upon those plants disappeared; however, no direct effects of residue on the insects were observed.

The objective of the present study was to duplicate the techniques of the 1971 study as closely as possible in order to evaluate populations along the same grid line 2 years later. Qualitative and quantitative comparisons were drawn to indicate the changes in variety and number of arthropods (especially insects) that had become established on the grid since the aerial dispersal tests were terminated in 1970.

2. MATERIALS AND METHODS

The techniques used in the study discussed in this report were the same as those outlined in Reference V-1 except as discussed in the following paragraphs.

Time and manpower limitations precluded general, non-systematic sampling of the grid, therefore the bulk of this comparative study was based on sweep net surveys along sampler row 8 of the test area. This allowed a quantitative comparison of results while non-systematic net sampling of grid areas did not lend itself to such analysis. A total of five paired sweep net surveys were performed on the mornings of June 14, 16, and 18; these dates being approximately 2 years and 2 weeks after the study discussed in Reference V-1. A given "paired sweep" (200 sweeps made by 2 individuals using 15-inch diameter nets) was taken across the grid and then back to the starting point. The simultaneous sweeps were 20 feet apart. At the end of each 400 foot transect, rather than using killing jars, the net contents were emptied into a paper bag into which a vial of ethyl acetate had been placed. These bags were then tightly folded and placed in a large sack that was carried out to the same taxonomic level as in the 1971 study. Exceptions to this classification scheme included the listing of Acalypterate muscoid flies as a group and the listing of certain

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Reference:

V-1. Valder, S. M.: Insect Density and Diversity Studies on Test Area C-52A, Eglin AFB Reservation Florida. AFATL-TN-72-4, Air Force Armament Laboratory, Eglin AFB, Florida. January 1972. Unclassified.

undetermined insects only to order. Table V-1 represents a full listing of the arthropods found in the sweep net survey. The undetermined insects (112 specimens) for the most part were either immatures or only partial specimens. The identifications were based on information in Reference V-2.

Finally, due to time limitations, only one full set of sweep net survey samples had been identified at the time of this report. Therefore, while the 1971 report discussed the results of five 2-mile sweeps of sampler row 8 (essentially 10 replicates), the present study considers data only from one 2-mile sweep of the same sampler row (essentially two replicates of each 400 foot transect of the row). Representative specimens of the identified samples are in the reference collection of the Biological Studies Branch, USAF Environmental Health Laboratory, Kelly AFB, Texas.

3. RESULTS AND DISCUSSION

Those taxa or arthropods that were collected in numbers exceeding one percent of the total number of specimens collected are listed in Table V-2. This table was formatted for comparison with Table V-3 (from Reference V-1). (The various taxa are treated as families for simplicity.) Such a comparison indicated that even though the 1973 data are based on only one sweep of the grid, the total number of identified arthropods equaled 1,614 as compared to a total of 1,803 specimens from five sweeps in 1971. These data would then indicate that if the one 1973 sweep were representative of all five sweeps taken during this second study, the total number of insects caught would likely be greater than four times the number taken during the 1971 study. Further analysis of the data indicates that the 1973 survey found great numbers of very small insects as compared to the 1971 study. The majority of the Chrysomelid beetles were very small insects, and the Ocalyptrate muscoids, Psocoptera, Thysamoptera, Sminthuridae, Ocarine, and Chalcidoidea also fall into this small to minute category. Table V-1 represents a listing of all arthropods collected during the 1973 survey, and is compared to Table V-4 (from Reference V-1). Reference V-1, however, lists not only those arthropods collected in 1971, but also those groups only observed in 1970 and 1971. Therefore, the tables are not fully comparable either in taxa listed or in the number of specimens reported. Comparison of this second set of tables again shows a relatively large number of small insects found in the 1973 study. This discrepancy may simply represent a difference in sampling/separation techniques or it may indicate an influx of populations of these smaller arthropods as the vegetation and other environmental characteristics of the transects have developed since the spray program was terminated.

Figures V-1 and V-2 represent arthropod/vegetation comparisons on Test Area C-52A for both the 1971 and 1973 surveys. There exists a similarly vegetative distribution, and a slightly greater plant coverage is indicated in 1973. The extreme differences in the numbers of arthropods found on the transects during the 1971 study are shown as being reduced in 1973, and further replication as well as time would likely reduce these differences more. Comparisons of the arthropod populations have to take into consideration the fact that Figure V-1 is based on the total observed and collected specimens from Table V-4, while Figure V-2 is derived from only the identified specimens of a single sweep of the 1973 study. Therefore, although the graphs representing the number of arthropod "families" in both figures are relatively similar, and there is a tendency toward reduction of extreme differences in the 1973 transect data, further discussion and comparison might be spurious. Similar comments pertain to comparisons of the arthropod diversity graphs, even though basic

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Reference:

V-2. Pate, B. D., P. J. Lehn, R. C. Voigt, and J. H. Hunter: Animal Survey Studies on Test Area C-52A, Eglin AFB Reservation, Florida. AFATL-TR-72-72, Air Force Armament Laboratory, Eglin AFB, Florida. April 1972. Unclassified.

TABLE V-1. ANTHROPODS COLLECTED ON TEST AREA C-52A, EGLIN AFB RESERVATION, FLORIDA, JUNE 1973

TAXON	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
CLASS: ARACHNIDA															
ORDER: Araneida (Spiders)		144	4	9	9	16	18	36	18	7	6	6	8	4	3
ORDER: Acarine (Mites)		46			2	2	3	27	7			2	3		
CLASS: INSECTA															
ORDER: Coleoptera (Beetles) 275 Specimens collected															
Anthicidae	Antlike Flower Beetles	3						1	1		1				
Carabidae	Ground Beetles	1								1					
Chrysomelidae	Leaf Beetles	219	1	1	84	35	5	69	18			2	1		2
Cicindellidae	Tiger Beetles	7		2	1	1				1		1	1		
Coccinellidae	Lady Beetles	1											1		
Curculionidae	Snout Beetles	5		1			1		2				1		
Elateridae	Click Beetles	12		1	1	1		2		1	1	2	1	1	1
Meloidae	Blister Beetles	1							1						
Mycetaeidae	Mycetaeid Fungus Beetles	1							1						
Phalacridae	Shining Flower Beetles	12			11	1									
Tenebrionidae	Darkling Beetles	7	2	2			1	1							1
	Undetermined larvae and adults	6						2	3			1			
ORDER: Collembola (Springtails) 53 Specimens collected															
Sminthuridae		53			7	3	7	14	12	9		1			

TABLE V-1. ARTHROPODS COLLECTED ON TEST AREA C-52A, EGLIN AFB RESERVATION, FLORIDA, JUNE 1973 (Continued)

TAXON	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: Diptera (Flies) 165 Specimens collected															
Acalyptrate	Muscoids	86	9	2	2	6	6	21	14	7	7	1	8	3	
Asilidae	Robber Flies	3									3				
Bombiliidae	Bee Flies	1							1						
Cecidomyiidae	Gall Midges	3			1	1							1		
Ceratopogonidae	Biting Midges	1					1								
Chironomidae	Midges	14			2	1	5	5	1						
Culicidae	Mosquitoes	2						1	1						
Muscidae	Muscid Flies	2						1	1						
Pipunculidae	Bigheaded Flies	3						1	1				1		
Sarcophagidae	Flesh Flies	4	1		2	1									
Syrphidae	Flower Flies	2			1				1						
Tachinidae	Tachina Flies	10		2	4	1	1		1			1			
Tipulidae	Crane Flies	1						1							
	Undetermined Adults	33		1	2	2	4	4	6	2	2	4	4	2	
ORDER: Ephemeroptera (Mayflies) 1 Specimen Collected															
Epnemeridae	Burrowing Mayflies	1				1									
ORDER: Hemiptera (True Bugs) 183 Specimens collected															
Corimelaenidae	Corimelaenid Bugs	6						1	5						
Corizidae	Grass Bugs	3	1	1			1								

TABLE V-1. ARTHROPODS COLLECTED ON TEST AREA C-52A, EGLIN AFB RESERVATION, FLORIDA, JUNE 1973 (Continued)

TAXON	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: Hemiptera (True Bugs) Continued															
Lygaeidae	Lygaeid Bugs	6			1			4					1		
Miridae	Plant Bugs	33			2	1	1	13	5	3				6	2
Nabidae	Damsel Bugs	33	2	7		3		3	1	2	5	1	7	2	
Neididae	Neidid Bugs	1		1											
Pentatomidae	Stink Bugs	1				1									
Reduviidae	Assassom Bugs	19			3	1		4	3	1	1	2	3	1	
Scutelleridae	Scutellerid Bugs	13	2	2	1		6			1			1		
	Undetermined Nymphs	68	2	10	3	3	1	10	2	4	12	6	11	1	3
ORDER: Homoptera (True Bugs) 454 Specimens collected															
Aleyrodidae	Whiteflies	1							1						
Aphidae	Plantlice	5				1		3				1			
Cercopidae	Spittlebugs	43	1		3	25	1		4		1	4	3		1
Cicadellidae	Leafhoppers	400	21	27	81	41	32	28	27	36	28	31	22	8	18
Coccoidea	Scale Insects	1							1						
Fulgoridae	Fulgorid Planthoppers	3			2					1					
Membracidae	Treehoppers	1			1										

TABLE V-1. ARTHROPODS COLLECTED ON TEST AREA C-52A, EGLIN AFB RESERVATION, FLORIDA, JUNE 1973 (Continued)

TAXON	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: Hymenoptera (Bees, Wasps, Ants) 167 Specimens collected															
Andrenidae	Mining Bees	2			1				1						
Bethylidae	Bethylids	2					1		1						
Braconidae	Braconid Wasps	2			1				1						
Chalcidoidea	Chalcids	40	2	3	7	3	3	9	6	3	1	2	1		
Cynipoidea	Gall Wasps	1					1								
Dryinidae	Dryinids	1					1								
Formicidae	Ants	99	3	4	2	4	10	28	8	10	9	4	15	2	
Halictidae	Sweat Bees	9				1		4	2	1			1		
Ichneumonidae	Ichneumon Wasps	1	1												
Mutillidae	Velvet Ants	3				1							1		1
Pompilidae	Spider Wasps	2		1								1			
	Undetermined Adults	5						2	1		1			1	
ORDER: Lepidoptera (Butterflies and Moths) 13 Specimens collected															
Microlepidoptera	Several Families	12	1				1	4	2		1	1	2		
Noctuidae	Owl Moths	1								1					
ORDER: Neuroptera (Nerve Winged Insects) 1 Specimen collected															
Myrmeleonidae	Antlions	1											1		

TABLE V-1. ARTHROPODS COLLECTED ON TEST AREA C-52A, EGLIN AFB RESERVATION, FLORIDA, JUNE 1973 (Concluded

TAXON	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: Odonata (Dragonflies and Damselflies) 12 Specimens collected															
Coenagrionidae	Damselflies	12				2	3	7							
ORDER: Psocoptera (Psocids) 66 Specimens collected															
	Family not determined	66		2	7	5	1	13	3	1	10	15	6	3	
ORDER: Orthoptera (Grasshoppers and Crickets) 92 Specimens collected															
Acrididae	Grasshoppers	36			6	7	3	11	3	3		1	2		
Gryllidae	Crickets	34	2	1	2	3	7	1	7	5	2	1	2	1	
Mantidae	Mantids	14	1		2			6	1	3	1				
Phasmidae	Walkingsticks	1	1												
Tettigoniidae	Katykids	7			1				1				5		
ORDER: Thysanoptera (Thrips) 54 Specimens collected															
	Family not determined	54		1	9	6	2	19	9	1		1	4		2
	TOTAL ARTHROPODS	1726	57	81	253	190	128	357	184	104	92	92	118	37	33
	TOTAL IDENTIFIED ARTHROPODS	1614	55	70	248	185	123	339	172	98	77	81	103	33	30

TABLE V-2. TAXA COLLECTED IN NUMBERS EXCEEDING ONE PERCENT OF THE TOTAL SPECIMENS COLLECTED[a], JUNE 1973

FAMILY	COMMON NAME	NUMBER COLLECTED	PERCENT OF TOTAL	CUMULATIVE PERCENT OF TOTAL[c]
Cicadellidae	Leafhoppers	400	24.8	24.8
Chrysomelidae	Leaf Beetles	219	13.6	38.4
Araneida	Spiders	144	8.9	47.3
Formicidae	Ants	99	6.1	53.4
Acalyptrate Muscoid	Flies	86	5.3	58.7
Psocoptera	Psocids	66	4.1	62.8
Thysanoptera	Thrips	54	3.3	69.4
Sminthuraidae	Springtails	53	3.3	72.3
Acarina	Mites	46	2.9	72.3
Cercopidae	Spittlebugs	43	2.7	75.0
Chalcidoidea	Chalcid Wasps	40	2.5	77.5
Acrididae	Grasshoppers	36	2.2	79.7
Gryllidae	Crickets	34	2.1	81.8
Miridae	Plant Bugs	33	2.0	83.8
Nabidae	Damsel Bugs	33	2.0	85.8
Reduviidae	Assassin Bugs	19	1.2	87.0

[a]Total equals 1,614 identified specimens: 1 percent of the total equals 16 specimens

[b]As discussed in the text, several of the taxa represent ordinal or super family levels of classification rather than family.

[c]Cumulated percent of total is derived by the progressive summation of the figures in the percent of total column.

TABLE V-3. TAXA COLLECTED IN NUMBERS EXCEEDING ONE PERCENT OF THE TOTAL SPECIMENS COLLECTED[a], JUNE 1971

FAMILY-COMMON NAME	PERCENT OF TOTAL	CUMULATIVE PERCENT OF TOTAL[b]
Cicadellidae - leafhoppers	31.7	31.7
Araneida - spiders (order)	18.6	50.3
Lygaeidae - lygaeid bugs	7.7	58.0
Elateridae - click beetles	4.7	62.7
Pentatomidae - stink bugs	4.5	67.2
Asilidae - robber flies	4.2	71.4
Nabidae - damsel bugs	3.9	75.3
Acrididae - grasshoppers	3.2	78.5
Reduviidae - assassin bugs	2.7	81.2
Sphecidae - sand wasps	2.6	83.8
Tenebrionidae - darkling beetles	2.4	86.2
Chrysomelidae - leaf beetles	2.2	88.4
Scutelleridae - scutellerid bugs	2.1	90.5
Coenagrionidae - dragonflies	1.4	91.9
Halictidae - sweat bees	1.4	93.3
Mydaidae - mydas flies	1.3	94.6
Tettigoniidae - katydids	1.3	95.9
Mycetophilidae - mycetophilid flies	1.0	96.9

[a]Total equals 1803 specimens: 1 percent of the total equals 18 specimens

[b]Cumulated percent of total is derived by the progressive summation of the figures in the percent of total column

TABLE V-4. INSECTS AND ARACHNIDS COLLECTED OR OBSERVED ON TEST AREA C-52A, EGLIN AFB RESERVATION, FLORIDA, JUNE 1971

ARACHNIDS ORDER	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
Araneida	Spiders	355	4	3	25	8	30	188	26	28	7	4	8	2	2
Phalagida	Harvestmen	1													

FAMILY	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: COLEOPTERA (BEETLES) 206 Specimens Collected															
Anthicidae	Antlike Flower Beetles	1											1		
Bruchidae	Seed Beetles	1					1								
Buprestidae[a]	Metallic Wood Borers														
Carabidae	Ground Beetles	4						2	1	1					
Cerambycidae	Long Horned Beetles	1			1										
Chrysomelidae	Leaf Beetles	43	4	4	1	1	6	5	6	4	2	4	2	4	
Cicindellidae	Tiger Beetles	2				1		1							
Coccinellidae	Lady Beetles	8			1		1		4		2				
Curculionidae	Snout Beetles	10						10							
Dytiscidae[b]	Predacious Diving Beetles														
Elateridae	Click Beetles	84	12	10	15	5	13	10	2	5	1	1		7	3
Gyrinidae[a]	Whirligig Beetles														
Meloidae	Blister Beetles	3							2	1					
Mordellidae	Tumbling Flower Beetles	6						2	4						
Passalidae[b]	Passalid Beetles														

[a]Sighted but not collected in 1971

[b]Sighted or collected in 1970

TABLE V-4. CONTINUED

FAMILY	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: COLEOPTERA (Continued)															
Scarabaeidae	Scarab Beetles	2		1					1						
Staphylinidae	Rove Beetles	1										1			
Tenebrionidae	Darkling Beetles	43	2	2	10		2	3	3	4		1	7	3	6
ORDER: DERMAPTERA (EARWIGS) 1 Specimen Collected															
Forficulidae	Forficulid Earwigs	1													
ORDER: DIPTERA (FLIES) 211 Specimens Collected															
Anthomyiidae	Anthomyiidid Flies	14						9	1	2	1	1			
Asilidae	Robber Flies	76	1	4	4	8	11	10	14	7		3	9	3	2
Bibionidae	March Flies	4						4							
Bombiliidae[a,b]	Bee Flies														
Calliphoridae	Blow Flies	2						2							
Chironomidae	Midges	8					4		4						
Chloropidae[b]	Chloropid Flies														
Culicidae[a,b]	Mosquitoes														
Dolichopodidae	Long-Footed Flies	3						2	1						
Drosophilidae	Vinegar Flies	16	1		2	2		2	1				6	1	1
Mycetophilidae	Fungus Gnats	18						16	2						
Mycaidae	Mydas Flies	23	1	4	3	1	3	2		1	1	4	2	1	
Muscidae	Muscid Flies	17		1			2	9	3				1		1
Pipunculidae	Bigheaded Flies	3					1		2						

TABLE V-4. CONTINUED

FAMILY	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: DIPTERA (FLIES) (Continued)															
Sepsidae	Sepsid Flies	11						3	7	1					
Syrphidae	Flower Flies	9						9							
Tabanidae	Horse Flies, Deer Flies	1							1						
Tachinidae	Tachina Flies	1							1						
Tipulidae	Crane Flies	1				1									
Tripetidae	Trypetid Flies	4						3	1						
ORDER: HEMIPTERA (TRUE BUGS) 390 Specimens Collected															
Belastomatidae[b]	Giant Water Bugs														
Coreidae	Coreid Bugs	3			2										
Corimelaenidae	Corimelaenid Bugs	5			5										
Cydnidae	Cydnid Bugs	2							1	1					
Gerridae[a]	Water Striders														
Lygaeidae	Lygaeid Bugs	138		4	38	10	19	40	6	19	1		5	1	2
Miridae	Plant Bugs	2	1							1					
Nabidae	Damsel Bugs	71	2	7	8	15	3	5	9	14	2	2	4		
Neididae	Neidid Bugs	1		1											
Nepidae[b]	Water Scorpions														
Notonectidae[b]	Backswimmers														
Pentatomidae	Stink Bugs	82	2	7	22	13	7	5	4	18			4		
Reduviidae	Assassin Bugs	49	1	1	11		14		6	6	1	1	7	1	
Scutelleridae	Scutellerid Bugs	37		2	3	1	12	1	13	2			2		

TABLE V-4. CONTINUED

FAMILY	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: HOMOPTERA (TRUE BUGS) 360 Specimens Collected															
Aphidae	Plantlice	4				1		1		1	1				
Cercopidae	Spittlebugs	9			1	2	1	1	1	3					
Cicadellidae	Leafhoppers	343	10	30	46	54	41	80	21	29	10	10	10	2	2
Coccidae[a,b]	Scale Insects														
Fulgoridae	Fulgorid Planthoppers	1							1						
Membracidae	Treehoppers	3						2	1						
ORDER: HYMENOPTERA (BEES, WASPS, ANTS) 125 Specimens Collected															
Apidae	Apid Bees	1								1					
Bombidae[b]	Bumble Bees														
Braconidae	Braconid Wasps	11		1	1	1	2	1	3	2					
Chalcididae	Chalcids	2						1	1						
Chrysididae	Cuckoo Wasps	1						1							
Cynipidae	Gall Wasps	2								2					
Formicidae	Ants	12			1	2		6	2			1			
Halictidae	Sweat Bees	25	3	1	2	1		12	5	1					
Ichneumonidae	Ichneumon Wasps	3								1				2	
Megachilidae	Leafcutting Bees	2	1					1							
Mutillidae	Velvet Ants	4		3										1	
Pamphiliidae	Webspinning Sawflies	5	1		1									1	2
Pompilidae	Spider Wasps	6						1		1		1		2	1

TABLE V-4. CONTINUED

FAMILY	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: HYMENOPTERA (BEES, WASPS, ANTS) (Continued)															
Scoliidae	Scoliid Wasps	1						1							
Sphecidae	Sand Wasps	46	2	2		1	2	4	6	2		1		6	20
Tiphiidae	Tiphiid Wasps	4						4							
Xylocopidae[a]	Carpenter Bees														
ORDER: ISOPTERA (TERMITES) Observed Only															
Rhinotermitidae[b]	Subterranean Termites														
ORDER: LEPIDOPTERA (BUTTERFLIES AND MOTHS) 38 Specimens Collected															
Danaidae[b]	Milkweed Butterflies														
Geometridae[a,b]	Geometrid Moths														
Lycaenidae[a,b]	Blues and Coppers														
Hesperiidae[a,b]	Skippers														
Microlepidoptera[c]	Several Families	23				5	3								
Noctuidae	Owl Moths	1											1		
Nymphalidae[a,b]	Brushfooted Butterflies														
Papilionidae[a,b]	Swallowtail Butterflies														
Pieridae[a,b]	Sulfurs														
Psychidae[b]	Bagworm Moths														
Pyralidae	Pyralid Moths	14						3	1	2	1				

[c]Several families in this group, but identified no further

TABLE V-4. CONCLUDED

FAMILY	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: NEUROPTERA (NERVE WINGED INSECTS) 9 Specimens Collected															
Chrysopidae[a]	Green Lacewings														
Hemerobaeidae	Brown Lacewings	1					1								
Myrmeleonidae	Antlions	8		1								1	5		1
ORDER: ODONATA (DRAGONFLIES AND DAMSELFLIES) 40 Specimens Collected															
Aeshnidae[b]	Dragonflies														
Coenagrionidae	Damselflies	25					2	21	1		1				
Corduliidae[b]	Dragonflies														
Lestidae[b]	Damselflies														
Libellulidae	Dragonflies	15					1	14							
ORDER: ORTHOPTERA (GRASSHOPPERS AND CRICKETS) 74 Specimens Collected															
Acrididae	Grasshoppers	58	1	6	6	6	8	16	7	4	1	1		1	1
Gryllidae	Crickets	3			1				1	1					
Gryllotalpidae[a,b]	Mole Crickets														
Mantidae	Mantids	4				1	3								
Tettigoniidae	Katykids	23	1		6	4	2	4	5	1					
Trydactylidae	Pygmy Mole Crickets	7						7							
ORDER: TRICHOPTERA (CADDISFLIES) Observed Only															
Family not determined															

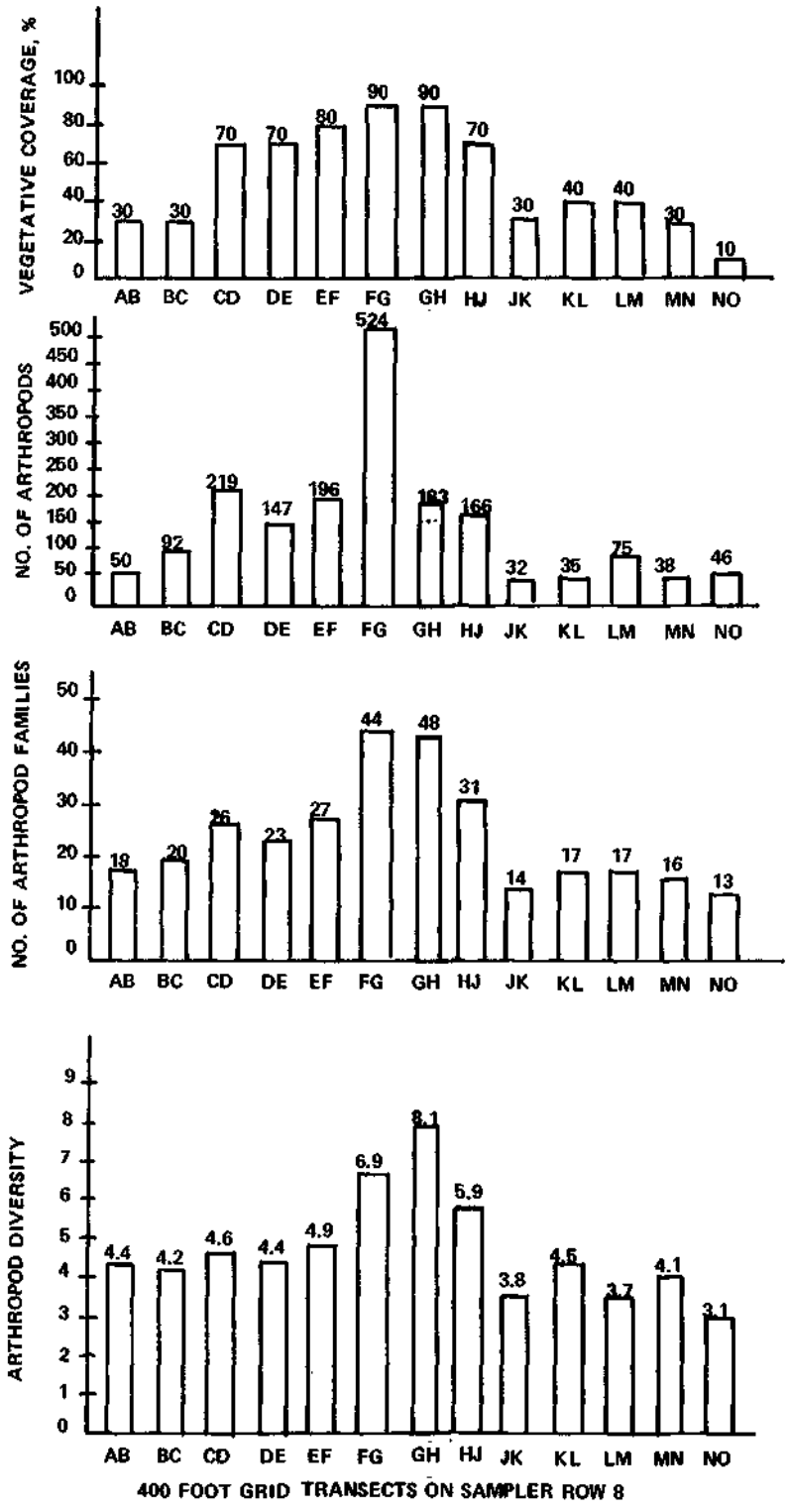

Figure V-1. Arthropod/Vegetation Comparisons on TA C-52A, 1971 Study

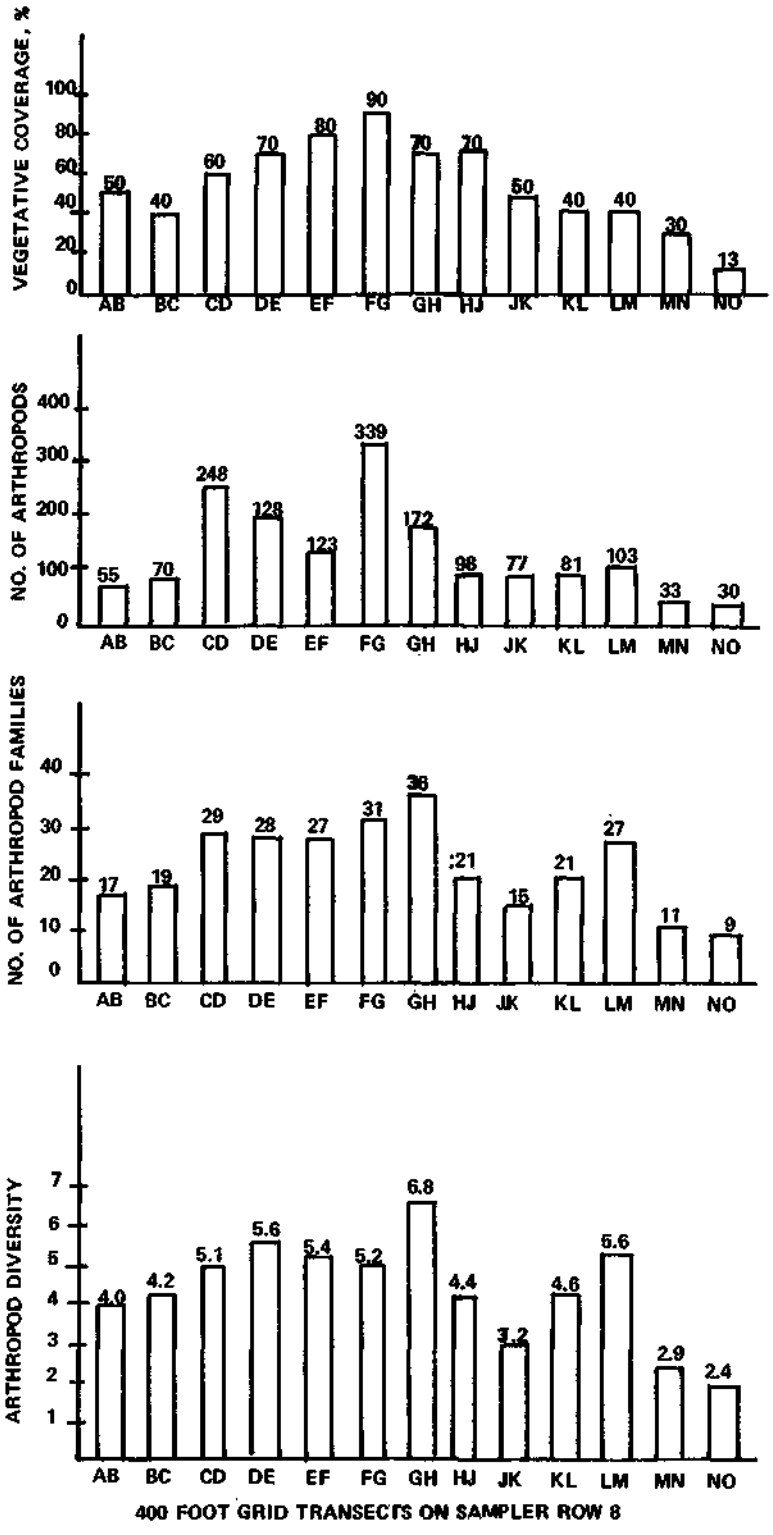

Figure V-2. Arthropod/Vegetation Comparisons on TA C-52A, 1973 Study

similarities exist. The diversity index used was that of Margalef:

$$d = \frac{S - 1}{\ln N}$$

Where

d = diversity
S = number of Taxa
N = number of speciments
ln = natural log

This formula has been used in previous diversity studies on Test Area C-52A (References V-3 and V-4), and it is used elsewhere in the present report. The greatest diversity of Arthropods as indicated by this formula was in transect GH in both surveys, even though the adjacent transect FG showed equal or greater amounts of vegetation (due to the presence of standing water in this area). The greater diversity in GH is due to a large number of taxa relative to the total number of Arthropods present. This large number of different organisms is likely due to the existence of a wider variety of available niches in transect GH, which includes influences of the adjacent aquatic area, dry sandy areas, and areas more disturbed by man. (The central sampling tower of the test area is located in this transect.)

Other factors would be of interest in comparisons of the 1971 and 1973 data, such as the relationship of Arthropod population biomass to vegetative cover. While this biomass-vegetation comparison would ideally show a close correlation, two factors make it impractical. First, the influence of randomly caught large insects (especially the grasshoppers) on biomass data would be quite confounding until a great deal of replication had produced a representative sampling of these animals. Second, the 1971 insect survey considered factors other than biomass, so there is no direct basis for comparisons. Many of the other topics that were discussed in the 1971 study hold equally true at this time. Experimental biases of the sweep net technique factors affecting plant distribution, and plant-insect relationships are discussed in Reference V-1.

4. SUMMARY AND CONCLUSIONS

A sweep net survey of the Arthropods of Test Area C-52A on the Eglin AFB Reservation resulted in the collection of over 1,700 specimens belonging to 66 insect families and Arachnid orders. These totals represent only one of five paired sweeps taken over a one-mile section of the test grid. A similar study performed in 1971 produced 1,803 specimens and 74 families from five paired sweeps of the same area using the same basic sampling techniques. A much greater number of small to minute insects were taken in the 1973 survey. Vegetative coverage of the test area had increased since 1971. The two studies showed similarities in distribution pattern of vegetative Arthropod numbers, number of Arthropod varieties, and Arthropod diversity. Generally, the present study showed a reduction of the extremes found in the above parameters in the 1971 study. This result is expected to continue as the test area stabilizes and develops further plant cover, thus allowing a succession of animal populations to invade the recovering habitat.

References:
V-3. Boror, D. J. and D. M. DeLong: An Introduction to the Study of Insects. New York, Rinehart. 1952.
V-4. Lehn, P. J., A. L. Young, N. A. Hamme, and B. C. Wolverton: Studies to Determine the Presence of Artificially Induced Arsenic Levels in Three Freshwater Streams and its Effects of Fish Species Diversity. AFATL-TR-70-81, Air Force Armament Laboratory, Eglin AFB, Florida. August 1970. Unclassified.

SECTION VI

AQUATIC STUDIES OF TEST AREA C-52A

One of the major parameters involved in the process of herbicide movement and/or persistence in soils is the adsorptive capacity of the soil. The adsorptive capacity, or the cation exchange capacity (i.e., the ability of a cation to be displaced or exchanged from the soil by another cation), is closely associated with the inorganic colloids (e.g., clay particles) and organic colloids (e.g., organic matter) of the soil. A soil with a large cation exchange capacity could bind within its colloidal system a large concentration of herbicide. Soils with a low cation exchange capacity do not retain cationic herbicides (e.g., cacodylic acid or sodium cacodylate), and thus, soil leaching of these herbicides would be expected. From June 1969 to October 1970, 4,395 gallons of military herbicide Blue were disseminated on TA C-52A (Table I-7). Approximately 13,624 pounds of cacodylic acid and sodium cacodylate were sprayed onto an area of less than one square mile. The soil of the test area has a low cation exchange capacity of approximately 0.8 mg exchangeable cation per 100 g of soil (Table I-4), while the annual precipitation of the area is high (Table I-1). Data from the analyses of soil cores for arsenic (Table II-9) confirm the movement and/or disappearance of arsenic from the test grid. Moreover, Table II-7 suggests that picloram, a component of the herbicide White, has moved within the soil profile and is apparently rather residual in nature.

Test Area C-52A is drained by five streams: Mullet, Trout, Basin, Grassy, and Rucker Creeks (Figure VI-1). The combined annual flow from these streams exceeds 24 billion gallons of water. However, only Mullet, Trout, and Basic Creeks are closely associated with the test grid. The mean daily flow rate for these three streams is shown in Table VI-1. As previously noted, studies on the movement of arsenicals and picloram indicated the possibility of herbicides contaminating the three freshwater stream communities draining the test grid. Since arsenical residues may concentrate in the tissue of fish, and particularly in the tissue of oysters, studies were conducted in 1969 and 1970 to determine (1) whether arsenic residues were entering the streams from the test grid and (2), if so, whether these residues were having adverse effects on the fish populations in the streams or were accumulating in oysters found at the mouth of streams adjoining Choctawhatcheee Bay. Synopses of these studies (Reference VI-1) are included in this report.

1. SYNOPSIS OF PREVIOUS RESEARCH, 1969

a. Fish Study

To assess the effects of possible arsenic residues, a diversity index study of the fish populations of Mullet, Trout, and Basin Creeks was initiated 3 months prior to the aerial spraying of Blue and continued for approximately 4 months after spraying.

Of the three streams under investigation, Trout Creek seemed the most likely to receive herbicide residues from the grid area. The headwaters of the stream are at the bottom of steep-

Reference:

VI-1. Lehn, P. Jeffery, A. L. Young, N. A. Hamme, and B. C. Wolverton: Studies to Determine the Presence of Artifically Induced Arsenic Levels in Three Freshwater Streams and its Effects on Fish Species Diversity. AFATL-TR-70-81, Air Force Armament Laboratory, Eglin Air Force Base, Florida, 1970. Unclassified.

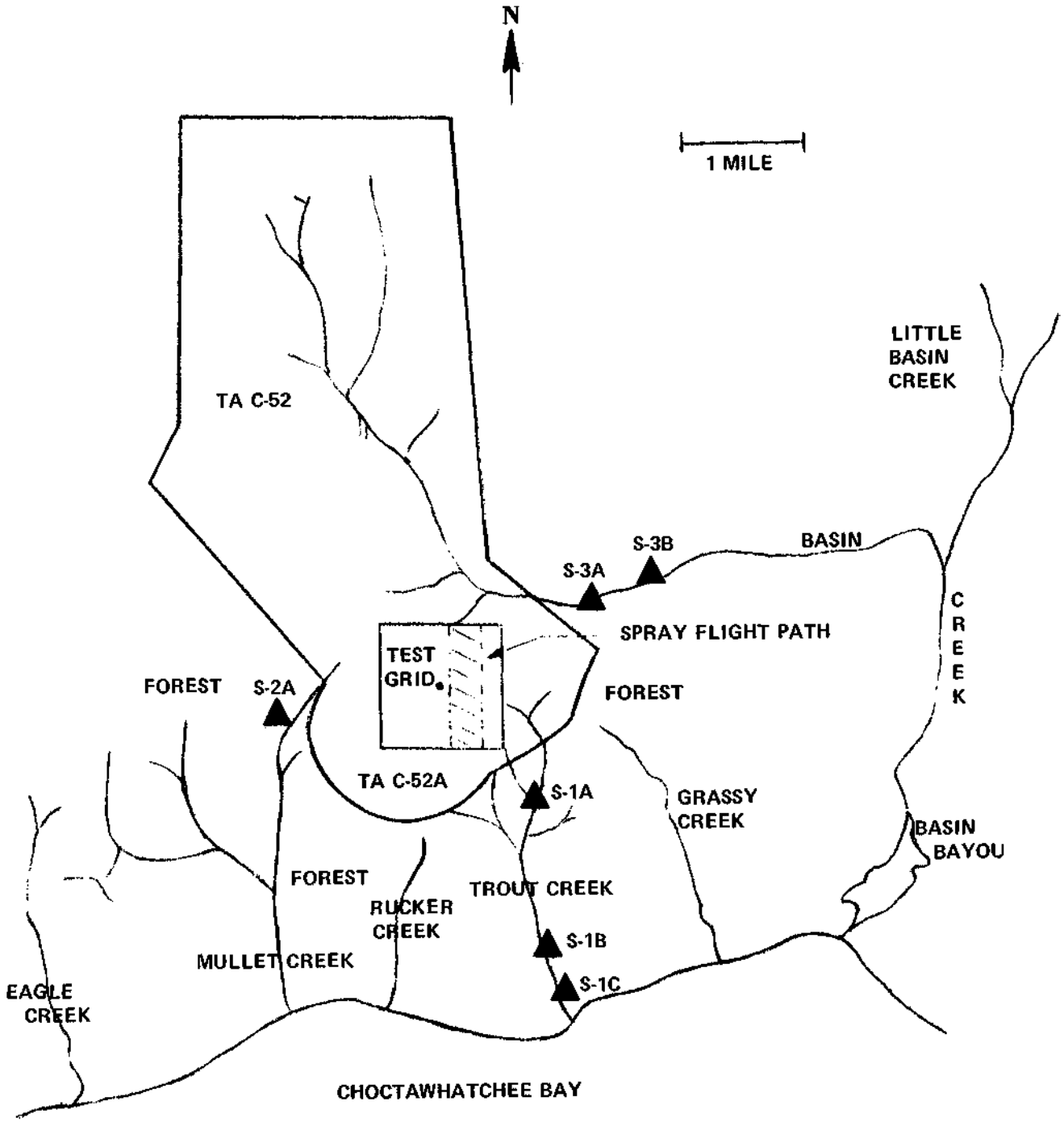

Figure VI-1. Map of Test Area Showing Streams in Relation to Test Grid and Location of Sampling Stations Used in Arsenic Monitoring Study

TABLE VI-1. CHARACTERISTICS OF SAMPLING SITES ON STREAMS DRAINING TEST AREA C-52A

NAME	Temperature, Range, ^{o}C	Mean pH	Width, Feet	Depth, Feet	Bottom Material	Mean Flow Rate, gal/day
Basin Creek	16-23.5	5.8	to 12	to 4	Sand	39,073,000
Mullet Creek	14-23.0	6.0	to 10	to 2	Sand	3,648,000
Trout Creek	13-23.5	6.1	to 15	to 2	Sand	5,870,000
Little Basin Creek[a] (Control Stream)	15-22.5	6.0	to 8	to 3.5	Sand	3,450,000

[a]Does NOT drain TA C-52A

sided bayheads adjacent to the edge of the grid and directly in line with the lower extremities of the repeatedly used spray flightpath (Figure VI-1). From its headwaters, the stream flows approximately 2 miles directly south into Choctawhatchee Bay. As the stream nears the bay, it deepens to several feet and has a heavy deposit of leaves and other organic matter on the bottom.

Mullet Creek has portions of its headwaters originating in steep-sided bayheads within 0.5 mile of the west boundary of the spray grid and flows south for approximately 2.5 miles into Choctawhatchee Bay, deepening near its mouth with a heavy deposit of leaves and other organic matter on the bottom (Figure VI-1).

The headwaters of Basin Creek originate several miles to the north of the spray grid. The stream flows southeast within 0.25 mile of the northeast corner of the grid and joins with a small tributary originating at the north margin of the grid, continues east for approximately 3.5 miles, and turns south for 2.25 miles emptying into Basin Bayou and Choctawhatchee Bay (Figure VI-1).

Six sampling stations were established on the three streams: One on Mullet Creek, two on Basin Creek, and three on Trout Creek (Figure VI-1). The selection of sampling station locations was determined mainly by their accessibility, variation of habitat within the station, and apparent fish populations. Because of the number of stations and time involved, they were not all sampled on the same day.

On each sampling date, observations were made in an effort to detect any gross changes in the population levels of the following selected benthic organisms: crayfish (Orconectes sp.), dragonfly naiad (Gomphus sp.), freshwater snail (Neritian sp.), and an unidentified immature freshwater clam. Observations were also made to detect any morphological effects that may have occurred to eelgrass (Vallisneria americana), the only species of vascular aquatic plant common to all stations.

Fish were collected with a variety of seines ranging in length from 4 to 15 feet and in mesh size from 1/8 to 1/4 inch. All represented habitats within each station were sampled randomly, and the time of day that the samples were taken was also varied. For the first several weeks of the survey, the fish were returned to the stream after the total catch was made and counted; however, for the remainder, and majority, of the survey, the fish were preserved in 10% formalin and counted in the laboratory. In conjunction with the stream sampling, two ponds on the test grid were sampled using dip nets.

b. Residue Sampling

Samples were routinely collected at 11 stations on the streams and in Choctawhatchee Bay after each rainfall following herbicide missions, or, if no missions had been flown, samples were collected monthly. Water from these streams was sent to the Regional Environmental Health Laboratory, Kelly AFB, Texas, where it was analyzed for arsenic. Detritus (bottom) samples were taken monthly with an Eckman dredge at three randomly selected water sampling locations. After appropriate pretreatment, these were assayed in the same manner as the water samples.

In addition to water and detritus sampling, oysters were used to monitor changes in arsenic level. Because these mollusks are filter feeders, the arsenic content of their bodies was correlated with that of their environment.

Oyster racks were established in Choctawhatchee Bay at the mouths of Basin, Trout, Grassy, Mullet, and Rucker Creeks. A control rack was also located in the bay at the mouth of Eagle Creek, which does not drain the grid area (Figure VI-1). Each rack contained approximately 2,000 oysters in the 1 to 3 inch diameter range, and these were sampled periodically. The small size of the oysters was intended to discourage removal from the racks. Samples obtained from the racks were frozen and taken to the laboratory for analysis. There the sample was acidified, hydrolyzed, and neutralized before undergoing standard atomic absorption analysis for arsenic (Reference VI-2).

Water samples were collected for picloram analysis from a small bayhead of Basin Creek just north of sampler station A-11. The bulk of herbicide White was disseminated on Grid 3, with the remainder being sprayed on Grid 4 (see Section I, *Figure* I-5 and *Table* I-7). Thus, most of picloram was probably concentrated around the northern portion of the one square mile area. Other water samples for picloram analysis were collected in the bayhead of Long Creek, which is located approximately 3 miles northwest of the one square mile grid and which has a water source not associated with TA C-52A.

c. Results

Twenty-one species of fishes were collected, with three species occurring within the boundaries of the one square mile grid and 20 species from the surrounding streams (Table VI-2). Habitat and spatial isolation seemed to be the major limiting factors on the grid.

Reference:

VI-2. Hamme, N. A., A. L. Young, J. H. Hunter: A Rapid Analysis of Soil and Water by Atomic Absorption. AFATL-TR-70-106, Air Force Armament Laboratory, Eglin Air Force Base, Florida, 1970. Unclassified.

TABLE IV-2. FISH SPECIES FOUND IN PONDS AND DRAINAGE AREAS OF THE ONE SQUARE MILE GRID AND IN BASIN, MULLET, AND TROUT CREEKS

SPECIES AND COMMON NAME	AREAS WHERE COLLECTED	
	ON GRID	OFF GRID
1. Ambloplites rupestris - southern rock bass	-	+B
2. Anguilla rostrata - American eel	-	+BT
3. Aphredoderus sayanus - pirate perch	-	+BT
4. Elassoma okefenokee - Okefenokee pigmy sunfish	-	+T
5. Erimyzon sucetta - lake chubsucker	+*	-
6. Esox americanus - red-fin pickerel	-	+B
7. Esox niger - chain pickerel	-	+B
8. Etheostoma edwini - brown darter	-	+BT*
9. Fundulus notti - starhead topminnow	-	+T
10. Gambusia affinis - mosquito fish	-	+BMT*
11. Ichthyomyzon gagei - southern brook lamprey	-	+BM
12. Ictalurus natalis - yellow bullhead	+	-
13. Lepomis punctatus - spotted sunfish	+	+BMT
14. Micropterus punctulatus - spotted bass	-	+T
15. Minytrema melanops - spotted sucker	-	+B
16. Notropis hypselopterus - sailfin shiner	-	+BMT*
17. Notropis texanus - weed shiner	-	+B
18. Noturus funebris - black madtom	-	+T
19. Noturus gyrinus - tadpole madtom	-	+T
20. Noturus leptacanthus - speckled madtom	-	+BMT*
21. Percina nigrofasciata - blackbanded darter	-	+BMT*

*Denoted large population in area.
B = found in Basin Creek
M = found in Mullet Creek
T = found in Trout Creek

The lake chubsucker was abundant in one of the ponds on the grid but was not found in the three streams within a 2 mile radius of the center of the grid, however, the species occurs several miles downstream in more sluggish waters. The employment of a diversity index (i.e., a statistical comparison of the fish populations before and after the spray missions, representing a time period of 8 months) showed a population change in one fish species at one of the six stations studied. This change, however, was probably due to an unidentified variable (e.g., variation in collecting techniques) rather than to arsenic residue. The arsenic analyses for 588 water samples and 68 silt samples were negligible (less than 1 ppm and not significantly different from control streams). A comparison of arsenic contents of 73 oyster samples taken from sampling stations established in Choctawhatchee Bay showed no significant differences from control samples taken elsewhere in the bay at the 95% probability level (1.32 ppm arsenic versus 1.45 ppm).

The results of water samples collected from Basin, Trout, and Long Creeks, and analyzed for picloram content are shown in Table VI-3. Picloram residues were still being detected in the small bayhead north of sampler station A-11 as late as December 1971. The last mission with herbicide White was in May 1970.

TABLE IV-3. RESULTS OF CHEMICAL ANALYSIS OF WATER SAMPLES FOR PICLORAM, 1971 DATA

SAMPLE LOCATION	DATE COLLECTED	PICLORAM[a], ppb
Basin Creek, North of Sampler Station A-11 in NE Corner of one square mile grid	11 Jun 1971	11
Trout Creek, South of Sampler Station 0-11 in SE Corner of one square mile grid	11 Jun 1971	2.4
Basin Creek, same as June 1971 location	3 Dec 1971	11, 9.4
Trout Creek, same as June 1971 location	3 Dec 1971	1.4
Control; Long Creek, approximately 3 miles from one square mile grid	11 Jun 1971	< 0.1

[a]Analysis performed by the Dow Chemical Company; Method ACR 68-14

2. CURRENT STUDIES OF AQUATIC ORGANISMS

The objectives of the current studies were (1) to reaccomplish the 1969 - 1970 aquatic studies and to compare population and diversity data, (2) to accomplish an in-depth survey of the aquatic organisms in the test grid ponds, and (3) to obtain samples of aquatic vertebrates from streams and grid ponds for arsenic and TCDD residue analyses.

a. Methods and Materials

Sampling of the ponds on TA C-52A was accomplished using dip nets and, where aquatic vegetation permitted, a 4 by 15 foot seine and a variable mess gill net. Sampling of the ponds was performed twice with identical collection methods employed both times. Tadpoles (Rana pipens subsp. sphenocephala Hyla gratiosa) and lake chubsuckers (Erimyzon sucetta) were frozen for arsenic and TCDD residue analyses. Those aquatic organisms caught only for species diversity and relative quantity were preserved in 10% formalin. Tadpoles were also collected at a control pond (north of TA C-52A) and were frozen for TCDD residue analysis.

Sampling of the streams draining TA C-52A (Mullet, Trout, and Basin Creeks) and the control stream (little Basin Creek) was accomplished also using the 4 by 15 foot seine. Approximately 100 yards of each stream was worked for 2 hours. The identical sampling technique was employed, and each stream was sampled three times. (This technique was that described in Reference VI-1). Species collected only for diversity and relative quantity were preserved in 10% formalin. Crayfish (Ordonectes sp.), speckled madtoms (Noturus leptacanthus), brown and blackbanded darters (Etheostoma edwini) and (Percina nigrofasciata) and any larger fish, e.g., redfin pickerel (Esox americanus) and spotted sunfish (Lepomis punctatus), were frozen for subsequent analysis of TCDD and arsenic residue. The selection for residue analyses of the crayfish and smaller fish species was based on the fact that they are bottom feeders or primary/secondary consumers and thus likely to ingest organic matter containing TCDD and arsenic. The larger fish were selected for residue analysis because they had been in the stream for a longer time and were predators, filling niches at the top of the aquatic food web - hence, a greater likelihood of residue accumulation taking place if bio-magnification was occurring. In addition to these species, oysters were collected for arsenic analysis from the mouth of Mullet and Trout Creeks where they drain into Choctawhatchee Bay. The samples collected for TCDD analysis were sent to the Interpretive Analytical Services Laboratory, Dow Chemical U.S.A., while the samples collected for arsenic analysis were sent to the Pesticide Degradation Laboratory, United States Department of Agriculture. The analysis of arsenic was by atomic absorption of arsine generated with N_2BH_4.

All of the streams that were sampled for fish were also sampled for aquatic invertebrates. Benthic samples were taken near the stream margins and in mid-stream at each station using a modified Surber Sampler with number 15 mesh. The margins were covered with a thin layer of organic debris and entangled with the root systems of neighboring plants, while the center of the stream bed was composed almost entirely of sand. The sampler was sunk about 6 inches into the stream bottom with the net on the downstream portion; then, the sand and debris enclosed by the sampler were placed in the net - going down about 6 inches into the stream bottom. The netting was taken to a deep spot on the stream and washed so that sand and debris would pass out through the netting. The remaining contents in the mesh were transferred into an enamel pan. The debris was examined, and all invertebrate organisms were removed and placed in plastic bottles containing water from the stream. The bottles were labled and taken to the laboratory. There, the organisms were placed in boiling water for 5 minutes, transferred to containers with 70% ethanol solution, classified, and counted.

Ten-foot strip samples of the aquatic areas of the grid were taken by using an insect net to make a 10 foot linear scoop along the bottom of the pond. The debris collected was then sorted for invertebrate organisms. Figure IV-2 shows the location of the major bodies of water at the time of survey, June 1973. It should be noted that the first 6 months of 1973 were abnormally high in rainfall, and thus, the 1973 survey showed more water on the grid than observed in 1969 to 1971. The three aquatic invertebrate samples were taken from the ponds located near sampler stations F-7, F-13, and G-13.

Biological specimens were forwarded to Dow Chemical U.S.A., Midland, Michigan for determination of TCDD levels. Analysis was accomplished using a modification of the technique of Baughman and Meselson (Reference VI-3).

b. Results and Discussion

Table VI-4 compares those fish species caught in the streams (draining TA C-52A) in 1969 (Reference VI-1) and those caught in 1973. The methods of collection and the sampling stations were the same for both studies.

In order to compare the fish populations caught in 1969 with those caught in 1973, three assumptions were made:

(1) That fish caught per sampling is proportional to the total fish population at that site, so long as the methods employed are sufficiently similar.

(2) That the sampling methods remained sufficiently similar to justify assumption one during all seining operations both in 1969 and 1973.

(3) That the frequency distribution of fish caught per sampling is approximately normal.

The data for fish populations per sampling for 1969 and 1973 can be shown as:

Sampling Period	Number of Observations	Mean Number of Fish Per Sampling	Standard Deviation
Before Spraying Blue (Mar 1969)	36	84	29.6
After Spraying Blue (Oct 1969)	16	84	49.2
1973 Sampling	13	141	60.2

As can be seen from these data, the fish caught per sampling before and immediately after the dissemination of Blue in 1969 remained constant. Moreover, a significant increase in fish caught per sampling occured in 1973 as compared to 1969. If the control stations (Little Basin and Fox Creeks) are compared for population changes during this time period, the following data are obtained.

Control Stations	1969 Means Per Sampling	1973 Means Per Sampling
Little Basin Creek	81	94
Fox Creek	83	84

Reference:

VI-3. Report Number IAS-405, Dow Chemical U.S.A., Midland, Michigan

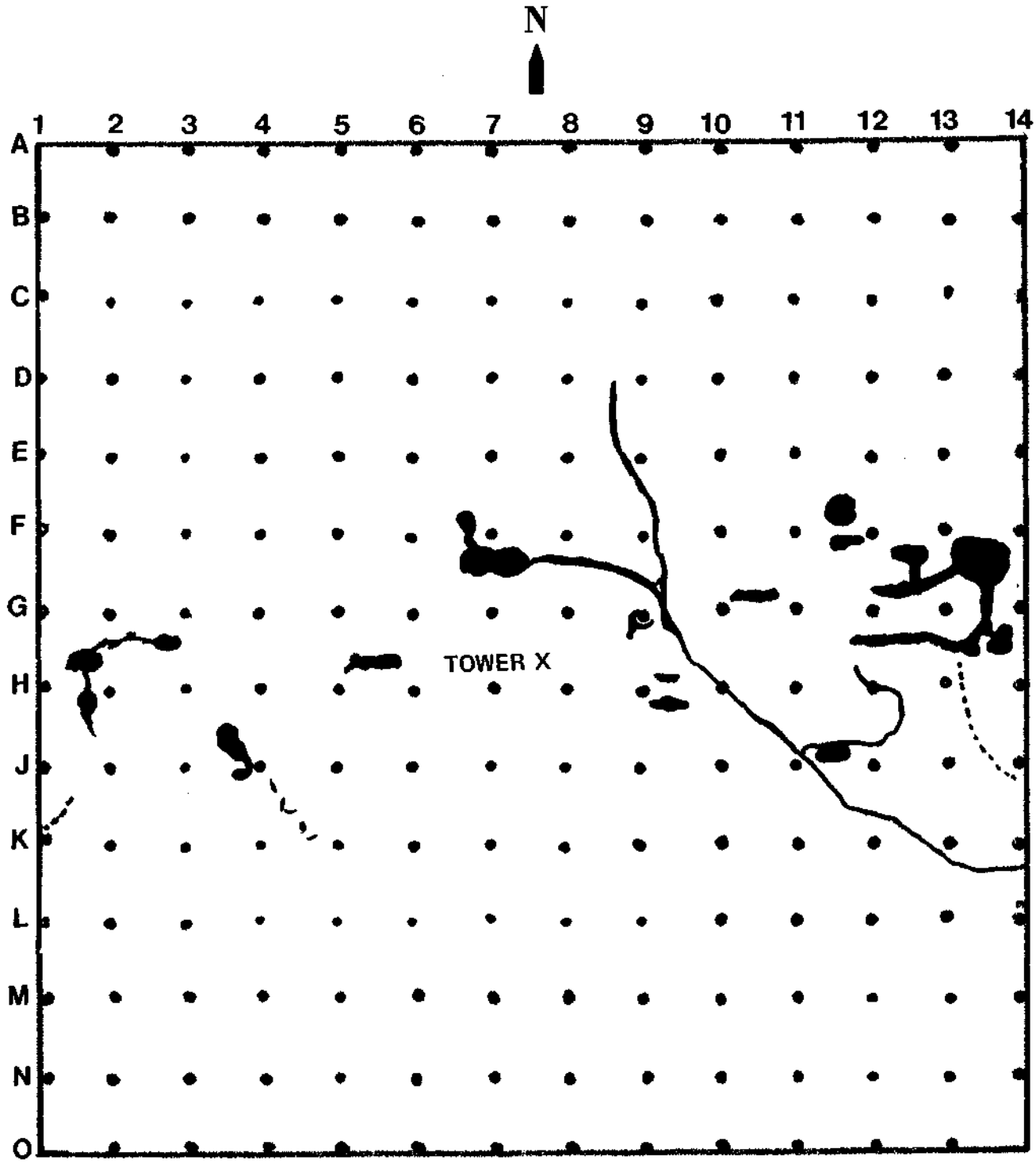

Figure VI-2. Location of Water and Major Drainage Ditches on the One Square Mile Grid of TA C-52A, 1973 Data

TABLE VI-4. FISH SPECIES COLLECTED IN 1969 AND 1973 FROM THREE STREAMS DRAINING TA C-52A AND A CONTROL STREAM

SPECIES	COMMON NAME	TROUT CREEK		MULLET CREEK		BASIN CREEK		LITTLE BASIN[a]	
		1969	1973	1969	1973	1969	1973	1969	1973
Notropis hypselopterus	sailfin shiner	+[b]	+	+	+	+	+	+	+
Gambusia affinis	mosquito fish	+	+	+	+	+	+	+	+
Percina nigrofasciata	blackbanded darter	+	+	+	+	+	+	+	+
Etheostoma edwini	brown darter	+	+	+	+	+	+	+	+
Lepomis punctatus	spotted sunfish	+	+	+	+	+	+	+	+
Noturus leptacanthus	speckled madtom	+	+	+	+	+	+	+	+
Ichthyomyzon gagei	southern brook lamprey	–[c]	+	+	+	+	+	+	+
Notropis texanus	weed shiner	–	–	–	–	+	+	–	–
Esox niger	chain pickerel	–	–	–	–	+	–	–	–
Aphredoderus sayanus	pirate perch	+	+	+	+	+	+	+	+
Esox americanus	redfin pickerel	–	+	–	–	+	–	+	+
Anquilla rostrata	American eel	+	–	–	–	+	–	+	+
Minytrema melanops	spotted sucker	–	–	–	–	+	+	+	–

[a] Control Stream
[b] Species Present (+)
[c] Species Absent (–)

TABLE VI-4. CONCLUDED

SPECIES	COMMON NAME	TROUT CREEK		MULLET CREEK		BASIN CREEK		LITTLE BASIN[a]	
		1969	1973	1969	1973	1969	1973	1969	1973
Ambloplites rupestris	southern rock bass	–	+	–	–	–	–	–	–
Mugil cephalus	common mullet	–	–	–	+	–	–	–	–
Ictalurus natalis	yellow bullhead	–	+	–	–	–	–	–	–
Micropterus punctulatus	spotted bass	+	–	–	–	–	–	+	+

[a]Control Stream

There is no significant change in the fish populations at the control sites. There are insufficient data on other variables (e.g., nutrient fluctuations), on other environmental factors, or on food chain growth data to warrant pinpointing the direct cause of the fish population increase other than that it appears to be associated with the general recovery phenomenon of vegetation, animal, and insect populations as noted in other sections of this report.

The species diversity was determined by the same method employed in the 1969 study (Reference VI-1). The mean diversity for 1969 (before and after the spraying of Blue) and 1973 for the control sites are:

Control Sites	Number of Samplings	Mean Diversity	Standard Deviation	Variance
Before Blue, 1969	36	0.9779	0.3049	0.0930
After Blue, 1969	16	1.3286	0.4903	0.2404
1973 Sampling	13	1.5934	0.1952	0.0381

The 1973 sample size of 13 may be too different from that of 1969 to compare diversity indices. The dependence of the diversity index (d) on the number of samples taken (N) may exist in such a way as to bias d when large or small values of N are used. If the diversity index is plotted as a function of N (using actual data) then the difference in d values before and after spraying Blue (and hence, the 1973 data) is too greatly dependent on N to use without either correcting for the sample size difference or re-sampling (thus using nearly the same sample sizes) A correction technique was employed. A description of this method is included and is in fact an analysis of the diversity of species using Monte Carlo normalized diversity indices.

In attempting to make comparisons between d values it appeared desirable to factor out the influences of N by making all N's the same. A small simulation was undertaken in which the observed frequency of species was assumed to be the expected value. A sample size of 80 was chosen as the common sample size because it is near the mean of the actual sample taken. Then, using the observed distribution to establish the probability of the occurrence of each species, 80 "fish" were drawn from the population. This closely simulated the process in which fish are captured until exactly 80 were caught in each sample and then the specimens classified and the data tabulated. One source of error for the simulation is the fact that the number of species, S, cannot exceed the S value for the observed case; i.e., if a species did not appear in the original sample, then the probability of its appearance in the "redrawn" sample is zero. This error, however, should be insignificant in cases where the original sample size was 30 or larger.

In a further effort to make comparisons of diversity more meaningful, the expected values of sample size, N_e, and expected number of species, S_e, were calculated assuming that the variety of fish life had not decreased; i.e., the d value now is no worse than the d value for the time period before the herbicide was applied. A comparison of N_e and S_e were made with the respective observed values N_o and S_o.

The equations used are:

$$N_e = e^{\frac{S_o - 1}{d}}$$

and

$$S_e = d \log_e (N + 1)$$

The d values were calculated for the redrawn samples. The only tendency, if any, was for the diversity index to increase after spraying. Linear correlation coefficients between d and average sample size compared to distances of the sampling stations from the center of the spray area were very small and insignificant. The S_e and N_e values for 1969 both before and after spraying were compared to those for 1973 using the two control sites to establish the expected diversity index. In both cases S_o was 10 to 20 percent higher than S_e, and N_e was grossly larger than N_o. These observations both tend to imply that the collecting sites considered to be within the spray zone are richer in fish life than the control sites outside the spray zone.

By using the correction technique on the mean diversity for the control sites only, a chronologically higher diversity in fish is evidenced from before spraying Blue in 1969, through the after spray period, to the 1973 sampling.

Control Sites	Before Blue 1969 Diversity	After Blue 1969 Diversity	1973 Diversity
Little Basin	1.324	1.806	1.770
Fox Creek	1.138	1.212	1.580

This same trend was noted for the streams draining the test area. However since the control sites are assumed to be either too far away from the grid area to be affected by the herbicides or are experiencing the recovery phenomenon noted for TA C-52A, no significant changes are evident in the diversity of fish life from 1969 to 1973.

In order to compare the species proportions for the 1969 data to the 1973 data, the following assumptions were made:

(1) That the average of the percentages of a given species found in the sample is a reasonable estimate of the actual percentage of that species in the fish population in the stream.

(2) That the percentages of rare species found in the samples are not valid for comparisons because sample sizes are not large enough for a sufficiently high confidence in the percentages.

Using these assumptions, only the two most common species were compared from the 1969 and 1973 data for significant changes. The rare species, therefore, were treated as part of the general diversity analysis.

Table VI-5 compares the 1973 mean percentages for the sailfin shiner (Notropis hypselopterus) and the mosquito fish (Gambusia affinis) to the 1969 data of before and after spraying of Blue and the combined mean for 1969. The significance of the differences was tested using the t test. The sailfin shiner had a significant decrease in its proportion of the fish population in 1973 as compared to the before spray (March 1969) fish populations, but the difference was not significant when 1973 data were compared to after spray data (October 1969). For the overall comparison, however, of 1973 data to 1969 data (combined) no significant difference existed. If data for the sailfin shiner are compared only for the control sites a significant decrease occurs in the percent of the population between the March 1969 data and the 1973 data. However, such a significant decrease does not occur when 1973 data is compared to October 1969 data.

Control Station	March 1969 Means	1973 Means	October 1969 Means
Little Basin	0.869	0.623	0.788
Fox Creek	0.928	0.714	0.758

As a result of these data, it is apparent that some factor (unrelated to the herbicide or recovery phenomenon) was at work between the spring of 1969 and the fall of 1969. As a result, it is more prudent to assume no significant proportional changes existed between spring 1969 and 1973 for the most abundant species.

The three aquatic areas on the grid were observed to be areas of 80% to 100% vegetative cover with the vegetation chiefly composed of grassy plants. The pond at station F-7 is located on Rutledge Sand, and the ponds at F-12 and G-13 have Chipley Sand underlying them. The F-7 pond had a pH reading of 5.51 and was heavily congested with algae and aquatic grasses. The two other ponds had a pH reading of 6.39 and were much less overgrown with aquatic grasses and algae. Both of the ponds were known to be intermittent; partially drying up once in the last 5 years. An alligator was sighted in F-7 pond and two 6-inch lake chubsuckers (Erimyzon sucetta) were taken from it. In the east grid ponds (F-12 and G-13), sightings were made of turtles, but no fish were taken from these ponds. The results of bottom sampling these ponds for specimens of invertebrate are shown in Table VI-6. The dominant order is Odonata. Without exception, the members of this order are predacious; therefore, their supply in these ponds must be relatively extensive.

The Serber sampling of the streams is shown in Table VI-7. Fox Creek yielded very few aquatic organisms, either invertebrate or vertebrate. The yields of invertebrates were so few, in fact, that Fox Creek was considered too different from Basin, Trout, or Mullet Creeks to effectively serve as a control stream. Perhaps the low yield in organisms in Fox Creek is related to its depth (mean depth of 18 inches with pools reaching to five feet) and/or swiftness. The majority of the organisms found in the other three streams were caddis fly larvae and snails. The caddis fly larvae are omnivores, while the snails are herbivores. Presumably, there is an extensive food web associated with these invertebrates that was not sampled by the Serber sampler.

TABLE VI-5. POPULATION CHANGES IN THE TWO MOST COMMON FISH SPECIES (ALL SITES ARE GROUPED TOGETHER) 1969 AND 1973 DATA

SPECIES	DATE	NUMBER OF OBSERVATIONS	MEAN	STANDARD DEVIATION	PROBABILITY OF CHOOSING SAMPLES WITH MEANS HAVING THIS OR GREATER DIFFERENCE
Notropis hypselopterus					
	Mar 1969	8	0.79	0.117	0.01[a]
	Oct 1969	8	0.58	0.172	0.94
	Combined 1969	16	0.69	0.179	0.91
	Jun 1973	5	0.51	0.161	- -
Gambusia affinis					
	Mar 1969	8	0.102	0.101	0.92
	Oct 1969	8	0.188	0.132	0.99
	Combined 1969	16	0.145	0.122	0.96
	Jun 1973	5	0.182	0.194	- -

[a]95% level of significance

TABLE VI-6. NUMBER OF AQUATIC INVERTEBRATE SPECIMENS COLLECTED FROM BOTTOM SAMPLING THREE PONDS ON TEST AREA C-52A[a]

ORDER	COMMON NAME	LOCATION OF PONDS[b]		
		F-7	F-13	G-13
Coleoptera	scavenger beetle	0	1	0
Hemiptera	backswimmers	3	10	6
	giant water bugs	0	1	0
Odonata	dragonflies/	10	8	1
	damsel flies	2	17	9
Trichoptera	caddis flies	1	0	0
	Total Specimens	16	37	16

[a]Each sample represents three collections with a 1-square foot Serber Sampler

[b]Ponds designated by the closest permanent sampler station

TABLE VI-7. NUMBER OF AQUATIC INVERTEBRATE SPECIMENS COLLECTED FROM BOTTOM SAMPLING THE STREAMS DRAINING TEST AREA C-52A[a]

ORDER	COMMON NAME	CREEK			
		MULLET	TROUT	BASIN	FOX[b]
Annelida	aquatic earthworms	10	11	10	1
Coleoptera	beetles	5	19	8	1
Decopoda	crayfish	1	1	0	0
Gastropoda	snails	48	96	15	0
Odonata	dragonflies	0	0	2	2
Plecypoda	freshwater clams	0	0	9	0
Trichoptera	caddis flies	51	75	158	49
	Total Specimens	115	202	202	53

[a]Ten-foot strip sample from bottom of pond using a 15-inch insect net
[b]Control station

The results of residue analysis for arsenic in aquatic organisms are shown in Table VI-8. The level of arsenic in the oysters was considerably lower than those values reported for oysters in 1970 - 1971 (average of 0.28 ppm arsenic versus 1.32 ppm, respectively). The lower levels of arsenic in 1973 may be due to the employment of different analytical procedures or to the increased stream flow noted this year, and hence, to a greater purging of the arsenic by the large volumes of freshwater entering Choctawhatchee Bay. No control tadpoles were analyzed for arsenic content, but presumably the level of arsenic was probably higher than it would be in control samples. This would be evident from data of the arsenic levels in 1973 grid soils as shown in Table II-7 (Section II).

The results of residue analysis of aquatic organisms for TCDD are shown in Table VI-9. The analysis were performed by the Interpretive Analytical Services, Dow Chemical U.S.A., Midland, Michigan. The duplicate samples were analyzed independently by high resolution mass spectrometry (see Section II for methods and materials). Duplicate samples of all biological specimens were also submitted to the Pesticide Degradation Laboratory, Agricultural Environmental Quality Institute, Beltsville, Maryland, for an independent check on results. However, none of the methods employed by the Degradation Laboratory could lower the limit of detection below 0.1 - 0.2 ppb TCDD.

3. CONCLUSIONS

From examining the data, certain observations support the idea that a recovery phenomenon is occurring in the streams draining TA C-52A. These observations are difficult to document because of insufficient data. For example, in 1969 the southern brook lamprey (Ichthyomyzon gagei) was never collected in Trout Creek, yet in 1973 it was taken in relatively large numbers. It now appears that the lamprey is breeding in Trout Bayhead south of sampler station 0-11. Moreover, all of the specimens of lamprey collected this year in Trout Creek are immature

TABLE VI-8. CONCENTRATION OF ARSENIC IN BIOLOGICAL SPECIMENS COLLECTED ON OR ADJACENT TO TA C-52A

BIOLOGICAL SPECIMEN[a] (Common Name)	LOCATION COLLECTED (June 1973)	CONCENTRATION OF ARSENIC (μg As/gram Fresh Tissue)
Oyster	Mouth of Trout Creek	0.44
Oyster	Mouth of Mullet Creek	0.12
Blue Crab	Mouth of Trout Creek	0.32
Blue Crab	Mouth of Mullet Creek	0.32
Crayfish	Little Basin Creek[b]	0.29
Crayfish	Trout Creek	0.30
Black-banded Darters	Little Basin Creek[b]	0.75
Black-banded Darters	Trout Creek	0.15[c]
Speckled Madtom	Little Basin Creek[b]	0.30
Speckled Madtom	Trout Creek	0.38
Redfin Pickerel	Trout Creek	0.23
Tadpoles	Grid Pond (F-7)	1.47

[a]Samples for analysis were either aliquots of homogenates or the entire homogenate depending on sample size.

[b]Control Samples

[c]Part of the tissue was lost in digestion of sample.

TABLE VI-9. CONCENTRATION OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) IN BIOLOGICAL SPECIMENS COLLECTED ON OR ADJACENT TO TEST AREA C-52A

BIOLOGICAL SPECIMEN[a] (Common Name)	LOCATION COLLECTED	CONCENTRATION OF TCDD (parts per trillion)
Cub Sucker	Mouth of Trout Creek	< 10
Crayfish	Trout Creek	< 10
Crayfish	Control	< 10
Oyster	Mouth of Trout Creek	< 10
Rock Bass	Trout Creek	< 10
Spotted Sunfish	Trout Creek	< 10
Spotted Sunfish	Control[b]	< 10
Tadpole	Grid Pond (F-7)	< 10
Tadpole	Control	< 10

[a]All samples were run in duplicate and analyzed independently by high resolution mass spectrometry.

[b]Control locations are noted in text.

indicating that the population was recently established (within the past two years). However, statistical comparisons of 1969 and 1973 data confirm a chronologically higher diversity in fish populations for even the control streams. Thus, the presence of the lamprey may or may not reflect a change in the habitat due to recovery from herbicide exposure.

The data on picloram in waters draining from the test grid would support the need for production studies of these streams. However, a review of toxicological data for picloram (Reference VI-4) suggests that concentrations of 1000 ppb do not seem to effect aquatic organisms. Moreover, the lack of baseline data and adequate control streams would probably make such studies futile or of doubtful value.

It is apparent from the results of samples analyzed for TCDD that representative organisms living in streams draining Test Area C-52A or in the ponds on the test area were free from TCDD contamination at a lower detection limit of less than 10 ppt. These data are not unexpected knowing the low solubility of TCDD in water and its apparent lack of movement in the soil profile (Reference IV-1).

Reference:
VI-4. Pimentel, David: Ecological Effects of Pesticides on Non-Target Species. Executive Office of the President, Office of Science and Technology, June 1971.

SECTION VII

STUDIES ON THE MICROFLORA OF TEST AREA C-52A

The soil persistence of herbicides is influenced by many environmental and biological factors. Perhaps one of the most important of these is that of the presence or absence of microorganisms. In an area such as Test Area C-52A, Eglin AFB Reservation, where the soils were subjected to repetitive applications of four different herbicides (2,4-D, 2,4,5-T picloram, and cacodylic acid) over a period of 8 years (1962 - 1970), the organisms had to either adapt to the presence of the chemicals or be adversely affected (i.e., reduction in population). For this reason, studies were initiated to examine population levels of various microflora found occurring on the one square mile grid. The initial studies[4,5] were conducted from 1967 to 1969. These studies provided data on the soil algal populations and are included as a synopsis in this report. In June 1970, a survey was conducted[6] of the soil bacterial, fungal, and Actinomycete populations found at specific sites on the grid and in control areas. The data from this study (identified in this report as the 1970 study) have been the basis for comparisons in the current study. In addition, the current studies also include a preliminary examination of aquatic algae found in the ponds in the center of the one square mile grid.

1. SYNOPSIS OF PREVIOUS RESEARCH , 1967 - 1970

In 1967, three areas were soil sampled for algal flora. Area I was Grid 1 located immediately south of the present one square mile grid. This area received a total accumulative concentration of 1,894 pounds of 2,4-D and 2,4,5-T from June 1962 through July 1964. Area II was Grid 2 located in the southwest portion of the present grid. This area received a total accumulative concentration of 1168 pounds of 2,4-D and 2,4,5-T from May 1964 through September 1966. Area III was a control area and was located 3 miles northwest of the present grid. (See Figure I-5).

Samples were taken from two levels in the soil. The first level included the surface litter and the first centimeter of soil. The second level samples included an amalgam of the soil between one and 15 cm. Two methods of culture were used. In the first, sterile filter paper was placed in sterile Petri dishes, after which approximately 10 gm of the sample soil were added. The cultures were moistened with sterile Bristol's solution and placed under fluorescent lights with an intensity of 300 ft-candles. The second method of culture preparation was identical to the first, except an additional piece of sterile filter paper was placed directly on the soil and moistened with the nutrient solution.

The number of algae was found to be low but no significant differences could be noted between the sprayed area and plots that had not received herbicides or only minimal amounts due to drift (Table VII-1). Only green and bluegreen algae were considered for identification. A total of 38 organisms were identified (Table VII-2). At least one species of Chlamydomonas, Chlorococcum, Chlorella, Micrococcus, Nostoc, Oscillatoria and Schizothrix was in every sample. In the majority of cases, Chlorococcum, Nostoc, and Schizothrix were represented by two or more species. Most of the other algae were located sporadically through the sampling period, and few were not universally distributed in all samples. A species of Sponiococcum was the only alga found repeatedly in a single location. The most frequently located alga was Schizothrix calcicola.

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[4] Arvik, J. H.: Soil Algae of the Eglin AFB Defoliant Test Range and the Response of Selected Species to Military Herbicides. Air Force Armament Laboratory Unpublished Data. 1969. Unclassified.

[5] Arvik, J. H. and J. H. Hunter. Soil Algae of a Herbicide Test Area, Eglin AFB, Florida, and the Response of Selected Species to Military Herbicides. Air Force Armament Laboratory Unpublished data. 1971. Unclassified.

[6] Hunter, J. H. Soil Microorganism Study of TA-C52A, Eglin AFB, Florida. Air Force Armament Laboratory Unpublished Survey. 1970. Unclassified.

TABLE VII-1. NUMBER OF SOIL ALGAE FOR GRAM OF SOIL FROM GRIDS I AND II, TEST AREA C-52A, AND THE CONTROL AREA, 1967 DATA

SAMPLING AREA	SOIL pH	SURFACE (0 - 1 cm)	CORE (1 - 15 cm)
Grid I (Area I)	5.4	2,360[a]	820[a]
Grid II (Area II)	5.2	2,243	567
Control	5.3	2,468	570

[a]Data are averages of three samples and three replications taken 30 days apart from September 1967 through November 1967.

TABLE VII-2. SOIL ALGAE FOUND ON OR NEAR TEST AREA C-52A, EGLIN AFB RESERVATION

CHLOROPHYTA

- Characium ambiguum Herm
- Characium sp.
- Chlamydomonas pyrenoidosa Deason and Bold
- Chlamydomonas typica Deason and Bold
- Chlorella vulgaris Beyer
- Chlorella sp.
- Chlorococcum ellipsoideum Deason and Bold
- Chlorococcum diplobionticum Hern
- Closteridium sp.
- Cylindrocystic brebissonii Meneg.
- Euglena sp.
- Homidium subtillissimum Mattox and Bold
- Hormidium flaccidum Mattox and Bold
- Protococcus viridis C. A. Agardh.
- Spongiococcus bacillaris Naeg.
- Ulothrix tenerrima Kuetz.
- Zygogonium ericetorum Kuetz.

CYANOPHYTA

- Anacystis marina Drouet and Daily
- Arthrospira brevis (Kuetz.) Drouet
- Calothrix parictina (Naeg.) Thuret.
- Coccochloris aeruginesa Drouet and Daily
- Coccochloris peniocystis Drouet and Daily
- Fischerella ambigua (Naeg.) Gom.
- Microcoleum lyngbyaceus (Kuetz.) Crouan
- Microcoleus vaginatus (Vauch.) Gom.
- Nodularia sp.
- Nostoc commune Vauch
- Nostoc ellipsosporum (Desmaz.) Raben.
- Nostoc muscorum Ag.
- Oscillatoria lutea Ag.
- Oscillatoria submembranaceae Ard. and Straff
- Porphyrosiphon Natarisii (Menegh.) Gom.
- Rivularia sp.
- Schizothrix arenaria (Berk.) Gom.
- Schizothrix calcicola (Ag.) Gom.
- Schizothrix friezii (Ag.) Gom.

2. CURRENT STUDIES ON MICROFLORA

In the June 1970 study, soil samples were selected for microbial analysis from sites which had been separated into four general areas on the basis of a prior bioassay experiment to determine phenoxy herbicide residues. The four areas were as follows: (a) relatively moist soil with high residue, (b) relatively moist soil with low residue, (c) relatively dry soil with high residue, and (d) relatively dry soil with low residue. The lowest fungal counts were obtained from Area c: 4×10^3 to 7×10^3 propagules per gram of soil. The highest fungal counts were obtained from Area b: 4×10^4 to 1×10^5 propagules per gram of soil. Actinomycetes and bacteria were considerably higher on wet sites than on dry sites, but the amount of herbicide residue did not seem to affect the count.

The objective of the present study was to analyze the microflora in the C-52A Test Grid (population levels of bacteria, fungi, and Actinomycetes, and identification of predominant genera) and then compare the results with a microfloral analysis of an adjacent non-treated area (control) and with a similar C-52A Test Grid analysis carried out in the 1970 study.

3. LITERATURE REVIEW

The general role of microorganisms in the biodegradation of herbicides has been thoroughly investigated and well documented. As part of the study, a literature review was conducted of the items listed in Table VII-3. Most research has centered around the biodegradation of various commercial herbicides by fungi and bacteria, both alone and in combination (Table VII-3, items 2 to 9, 11 to 14, 16, 17, 20, 21, and 27 to 31). In many such efforts, various soils have been used to effect the breakdown of herbicides with little attention paid to the microbial genera involved in the process. Total numbers, rather than types of organisms, have been stressed (Table VII-3, items 12 to 14, 16, 17, 20, 21, 27, and 30 to 32).

Pfister has investigated the role of soil organisms in biodegradation of pesticides, especially the breakdown of halogenated hydrocarbons (Table VII-3, item 27). In that study, soil fungi, Actinomycetes, and bacteria are implicated in the breakdown of various pesticide compounds. Kearney has extensively studied the role of microorganisms in the soil degradation of halogenated hydrocarbons and has likewise concluded that they do function in the breakdown of these products. Kaufman, Kearney, Sheets, and Beall have been involved in elucidating the role of total bacterial count and individual enzymes in the breakdown process (Table VII-3, items 20 to 23).

Bollag has researched the biochemical transformation of pesticides by soil fungi, and considers the fungi to be an important group of organisms in this transformation process (Table VII-3, item 7). He implicates specific organisms, particularly Geotrichum candidum, in connection with polymerization of an aniline compound to an azo-derivative.

Tyagny-Ryadno, in determining the effect of herbicides on the microflora and agrochemical properties of the soil, concludes that some herbicides actually increased bacterial populations (2,4-D was a notable exception). He states also that some herbicides promote the growth of fungi, but seem to inhibit Actinomycetes. In his experiments, Simazine caused the greatest increase in populations of Clostridium pasteurianum (Table VII-3, item 32).

In investigating the action of 2,4-D on Azotobacter spp. in sugarcane soils, Colmer concludes that the population levels of these organisms are not affected if herbicide application rates are within those normally recommended for sugarcane (Table VII-3, item 1). Johnson and Colmer have included in their work the effect of 2,4-D and 2,4,5-T on metabolic activities of various bacteria including specific Azotobacter organisms, Bacillus cereus and Pseudomonas fluorescens (Table VII-3, items 11, 18, and 19). Johnson and Colmer have determined that concentrations of 2,4-D less than 5,000 ppm have little effect on Pseudomonas fluorescens.

TABLE VII-3. LITERATURE REVIEWED IN 1973 STUDY

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2. Alexander, M. and M. I. H. Aleem. 1961. Effect of Chemical Structure of Microbial Decomposition of Aromatic Herbicies. Ag. and Food Chem. 9:44-47.

3. Audus, L. J. 1950. Biological Detoxification of 2,4-dichlorophenoxyacetic Acid in Soils: Isolation of an Effective Organism. Nature 166:356.

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5. Bell, G. R. 1960. Some Morphological and Biochemical Characteristics of a Soil Bacterium Which Decomposes 2,4-D. Can. J. of Microbio. 3:821-840.

6. Bell, G. R. 1960. Studies on a Soil Achromobacter which Degrades 2,4-dichlorophenoxyacetic Acid. Can. J. of Microbiol. 6:325-337.

7. Bollag, J. M. 1972. Biochemical Transformation of Pesticides by Soil Fungi. CRC Critical Reviews in Microbiology. 2(1):35-37.

8. Bollag, J. M., G. G. Briggs, J. E. Dawson, and M. Alexander. 1968. Enzymatic Degradation of Chlorocatechols. J. Agr. Food Chem. 16(5):829-833.

9. Bollag, J. M., C. S. Helling, and M. Alexander. 1968. Enzymatic Hydroxylation of Chlorinated Phenols. J. Agr. Food Chem. 16(5):826-828.

10. Cairney, W. J. 1973. Summary of Results: Populations of Soil Microorganisms from Desert Soil Treated with 2,4-D/2,4,5-T Herbicide. Presented to Ad-hoc subcommittee of the U. S. Scientific Advisory Board. 22 - 23 February 1973.

11. Colmer, A. R. 1953. The Action of 2,4-D upon the Azotobacter of Some Sugarcane Soils. Applied Microbiology 1:184-187.

12. Fletcher, W. W. 1966. Herbicides and the Bio-activity of the Soil. Invitation lecture given at Wageningen at meeting organized by the Dutch Working Party for Weed Control. 22 March 1966.

13. Fletcher, W. W., and J. E. Smith. Herbicides and Soil Microbes. New Scientist 24:527-528.

14. Hirsch, P. and M. Alexander. 1960. Microbial Decomposition of Halogenated Propionic and Acetic Acids. Can. J. of Microbiol. 6:241-249.

15. Hunter, J. H. and A. L. Young. 1972. Vegetative Succession Studies on a Defoliant-Equipment Test Area, Eglin AFB Reservation, Florida. AFATL-TR-72-31. Air Force Armament Laboratory, Eglin Air Force Base, Florida. February 1972. Unclassified.

16. Jensen, H. L. 1957. Decomposition of Chloro-substituted Aliphatic Acids by Soil Bacteria. Can. J. of Microbiol. 3:151-164.

TABLE VII-3. Concluded.

17. Jensen, H. L. 1957. Decomposition of Chloro-organic Acids by Fungi. Nature 180:1416.

18. Johnson, E. J. and A. R. Colmer. 1955. I. The Effect of 2,4-dichlorophenoxyacetic Acid on some Phases of the Nitrogen Metabolism of Bacillus cereus. Applied Microbiology 3:123-126.

19. Johnson, E. J. and A. R. Colmer. 1955. II. The Effect of 2,4-dichlorophenoxyacetic Acid on some Phases of the Nitrogen Metabolism of Pseudomonas fluorescens and the Microorganisms of a Soil Suspension. Applied Microbiology 3:126-128.

20. Kaufman, D. D., P. C. Kearney, and T. J. Sheets. 1963. Simazine: Degradation by Soil Microorganisms. Science 142:405.

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27. Pfister, R. M. 1972. Interactions of Halogenated Pesticides and Microorganisms: A Review. CRC Critical Reviews in Microbiology 2(1):1-34.

28. Rogoff, M. H. and J. J. Reid. 1956. Bacterial Decomposition of 2,4-dichlorophenoxyacetic Acid. J. Bacteriol. 71:303-307.

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30. Steenson, T. A. and N. Walker. 1956. Observations on the Decomposition of Chlorophenoxyactic Acids by Soil Bacteria. Plant and Soil. 8:17-32.

31. Thiegs, B. J. Fall 1962. Microbial Decomposition of Herbicides. Down to Earth. 18(z):7-10.

32. Tyagny-Ryadno, M. G. 1967. Effect of Herbicides on the Microflora and Agrochemical Properties of the Soil. Trudy Kamenetzpodolsk. sel',-khoz. Inst. 9:43-48.

Two recent studies, however, have indicated that the 2,4-D/2,4,5-T herbicide combination has a short term effect on levels of soil microorganisms, especially bacteria (Table VII-3, items 1 and 10). Analyses of desert soil to which herbicide had been applied three months earlier have revealed that bacteria levels are still considerably reduced from levels in control soil samples. Fungal levels were also affected but not to the same degree as the bacteria.

4. MATERIALS AND METHOD

Samples were taken from the C-52A Test Grid on 13 June 1973. Eight sampling sites were selected to correspond with those sampled in the 1970 study. Three samples were taken from each of the eight 400 by 400 foot grid areas according to the pattern in Figure VII-1. Samples were taken from depths of 0 to 6 inches and from 6 to 12 inches at each site. Four control samples were taken from the same depths in an area 1/4 mile distant from the C-52A site, but similar to it in soil and vegetative cover. The control area was upwind from prevailing wind patterns, upstream from natural test grid water drainage, and never subjected to concentrated herbicide application.

In selecting areas for sampling within the 400 by 400 foot grid squares, an attempt was made to sample sites with varying vegetative cover. A system was devised to approximate cover which employed a rank ordering of the sites from 0 to 5; 0 indicated a 0 to 5% vegetative cover, 1 a vegetative cover of 5 to 20%, 2 a cover of 20 to 40%, 3 a cover of 40 to 60%, 4 a cover of 60 to 80%, and 5 a cover of 80 - 100%.

In obtaining the samples, a shovel was used to bare a slightly more than one foot deep vertical cross section of soil. The side of the cross section was marked at the 6 and 12 inch points. Soil was skimmed from the side of the hole, first from the 0 to 6 inch depth, then from the 6 to 12 inch depth. Samples were placed in plastic bags and labelled. The soil was kept at $4^{o}C$ for no more than 2 days before plating on media for microorganism analysis.

Three media were used to enumerate microorganisms. Potato dextrose agar medium plus Tergitol NPX (100 ppm) and chlorotetracycline (40 ppm) was used for maximum development of soil fungi. Nutrient agar plus 150 ppm Actidione was used for development of bacteria. Sodium caseinate medium (DIFCO Actinomycete Isolation Agar) plus 50 ppm Actidione was used for determination of Actinomycetes.

Thirty grams of each sample to be analyzed were blended with 300 ml of sterile distilled water for one minute. Dilution series were made using subsequent sterile distilled water blanks to achieve dilutions of 10^{-3}, 10^{-4}, and 10^{-5}. Dilutions were dispensed in three media in sterile petri plates with three replicates per dilution for each sample. All plated samples were incubated at $25^{o}C$.

Potato dextrose agar plates were examined for fungi after 3 days. Nutrient agar plates were examined for bacteria after 4 days, and the sodium caseinate agar plates were examined for Actinomycetes after 6 days. Counts were made from each plate and predominant organisms were isolated in pure culture for subsequent identification.

In addition to enumeration of microorganisms, 10 samples (0 to 6 inch depth) were analyzed for water content. Samples were selected on the basis of Hunter's previous estimations of relatively high or relatively low water content of a given area of the C-52A Grid and on the basis of relative vegetative cover (0 to 5). Samples tested were as follows: (1) five from relatively high moisture areas, (2) two from areas with a vegetative cover of 1, (3) one from an area with a vegetative cover of 3, (4) two from areas with a vegetative cover of 5, (5) five samples from relatively low moisture areas, (6) three from areas with zero vegetative cover, and (7) two from areas with a vegetative cover of 1.

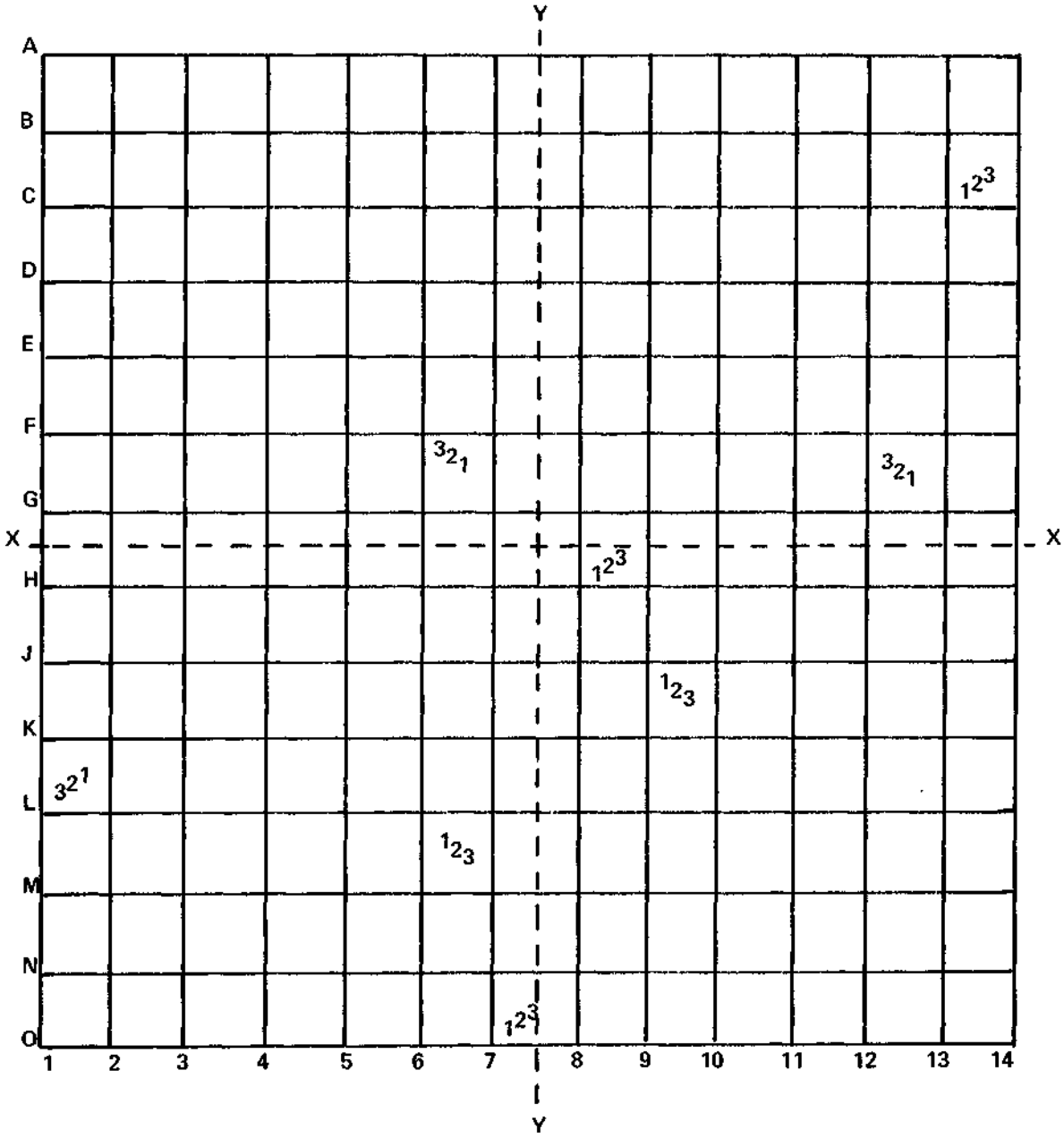

Figure VII-1. Schematic of the Test Area C-52A Grid Showing Soil Sampling Pattern (also see Figure I-5)

5. RESULTS AND DISCUSSION

The average number of organisms per gram of soil are shown in Tables VII-4 and VII-5. Table VII-4 indicates the average number of bacteria, fungi, and Actinomycetes for each grid location sampled. Table VII-6 indicates the average number of organisms per gram of soil in terms of relative vegetative cover. Table VII-5 is a summary of the data from the 1970 study arranged for comparison with Table VII-4. Table VII-7 is a summary of the data from the 1970 study arranged for comparison with Table VII-6. Tables VII-8 and VII-9 show water content and percent organic matter, respectively, for each of the samples analyzed.

There were no large differences in the numbers of Actinomycetes, bacteria, or fungi between the sampling sites on the grid for the 0 to 6 inch depth. Comparing these data with the 1970 population levels shows an increase in the average number of Actinomycetes in the J-9 and K-2 locations and an overall increase in the number of bacteria in all sampling areas. In the K-2 area, particularly, the number of bacteria per gram of soil shows an order of magnitude increase over the 1970 level. This increase might be partially explained by the marked increase in overall vegetative cover around J-9 and K-2 since 1970.

Differences in microorganism levels in 1970 correlated to an extent with vegetative cover, the lower populations existing where cover was minimal (see Table VII-7). The 1973 data (Table VII-6) shows a significant increase in microorganisms in poorly covered areas. The 1973 data indicates no strong correlation between vegetative cover and microorganism populations. Control areas had population levels similar to those found for the grid.

Predominant bacteria isolated from the test grid were Bacillus sp. and Pseudomonas sp.. Predominant fungi were Penicillium spp., Aspergillus spp., and Fusarium spp.. In addition, Nigrospora sp., Helminthosporium sp., Pullularia sp., and Curvularia sp. were recovered. The predominant Actinomycetes were Streptomyces sp. and Nocardia sp.

Although number of organisms from the 6 to 12 inch depth were not tabulated for this report, the numbers of fungi were approximately 40 to 50% reduced from the corresponding 0 to 6 inch – depth averages. The average numbers of Actinomycetes and bacteria were about the same as those from the corresponding 0 to 6 inch depth.

Water content varied very little in the 10 samples tested; the range being 0.35% to 1.22%. The average for all samples was 0.54%. There was no correlation between microorganism population levels and the slight differences in water content.

Organic matter variation was also minimal[7]. Percent organic matter variations did not correlate with differences in microorganism populations.

6. CURRENT STUDIES ON SURVEY OF AQUATIC ALGAE

The role of phytoplankton in the productivity of both soil and aquatic ecosystems is well documented. Algae have been identified (Reference VII-1) as being important in the initial

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[7] This confirms data from personal communication between A. L. Young with the Department of Life and Behavioral Sciences, United States Air Force Academy, Colorado, 1973.

Reference:
VII-1. Shields, L. M., and L. W. Burrell, 1964. Algae in Relation to Soil Fertility, Bot. Rev. 30:90-128.

TABLE VII-4. AVERAGE NUMBER OF ORGANISMS PER GRAM OF SOIL FOR EACH GRID LOCATION SAMPLED (0 - 6 INCHES DEPTH) 1973

GRID LOCATION	ACTINOMYCETES	BACTERIA	FUNGI
C-13	394,000	870,000	58,750
G-13	366,670	722,500	30,000
G-7	255,000	630,000	24,375
H-8	290,000	980,000	60,000
J-9	360,000	575,000	39,909
K-2	285,000	428,000	55,000
L-6	353,330	ND	20,710
0-7	363,000	468,000	32,166

TABLE VII-5. AVERAGE NUMBER OF ORGANISMS PER GRAM OF SOIL FOR EACH GRID LOCATION SAMPLED (0 - 6 INCHES DEPTH), 1970

GRID LOCATION	ACTINOMYCETES	BACTERIA	FUNGI
C-13	440,000	ND	23,400
G-13	612,000	ND	78,750
G-7	460,000	173,000	74,500
H-8	536,000	224,000	27,500
J-9	21,465	ND	17,968
K-2	54,333	29,000	11,366
L-6	276,000	ND	10,315
0-7	NOT SAMPLED IN 1970		

TABLE VII-6. AVERAGE NUMBER OF ORGANISMS PER GRAM OF SOIL (0 - 6 INCH DEPTH), VEGETATIVE COVER, 1973

VEGETATIVE COVER	ACTINOMYCETES	BACTERIA	FUNGI
5	310,000	1,015,000	50,000
4	300,000	1,070,000	110,000
3	1,860,000	722,500	30,000
2	283,330	890,000	32,166
1	357,000	416,000	30,800
0	326,360	529,000	25,800
CONTROL 5	235,000	ND	48,370
CONTROL 4	370,000	810,000	55,000
CONTROL 3	303,000	740,000	51,000

TABLE VII-7. AVERAGE NUMBER OF ORGANISMS PER GRAM OF SOIL (0 - 6 INCH DEPTH), VEGETATIVE COVER, 1973

VEGETATIVE COVER	ACTINOMYCETES	BACTERIA	FUNGI
5	460,000	173,000	74,500
5	612,000	ND	78,750
3	536,000	224,000	27,500
2	440,000	ND	23,400
1	54,333	29,000	11,366
0	21,465	ND	17,968
0	276,000	ND	10,315

TABLE VII-8. WATER CONTENT OF TEN C-52A SOIL SAMPLES (0 TO 6 INCH DEPTH)

SAMPLE	VEGETATIVE COVER	GRID LOCATION	PERCENT WATER
1	5	G-13	1.22
2	1	C-13	0.45
3	5	C-13	0.63
4	3	G-13	0.52
5	1	C-13	0.48
6	0	K-2	0.35
7	0	L-6	0.40
8	1	J-9	0.44
9	0	0-7	0.41
10	1	J-9	0.47

TABLE VII-9. ORGANIC MATTER OF SIX C-52A SOIL SAMPLES (0 TO 6 INCH DEPTH)

SAMPLE	VEGETATIVE COVER	GRID LOCATION	PERCENT ORGANIC MATTER
1	0	K-2	0.75
2	0	J-9	0.81
3	1	J-9	1.19
4	4	G-13	[a]2.58
5	5	C-13	[a]4.29
6	3	G-13	1.88

[a]Samples taken from directly beneath a clump of panicum grass and contained root material.

ecological succession of barren areas. Other investigations (Reference VII-2) have indicated the effects pesticides have on algae (Reference VII-3).

Grab samples were obtained from the pond located near the one square mile grid. Two types of samples were collected: Sample One was a collection of the suspended and precipitated algal material in the pond and Sample Two was a collection of the dense algal mat which occurred just beneath the surface of the water. The one liter samples were returned to the laboratory for algal genera identification.

Seven genera of algae were identified from the samples collected (Table VII-9). All genera were present in both samples: Sample Two being predominantly Zygnema and Sample One being predominantly Zygnema and Triploceras. The seven genera represent two divisions, Chlorophyta, the green algae, and Chrysophyta, the yellow-green or yellow-brown algae.

Data collected during the course of this study included some physical data. Of interest specifically with respect to the aquatic algae is the pH which was found to be 5.51, or slightly acid. Genera represented in the samples collected are those expected to be found under conditions of this type[8].

The previous study, conducted from September to November 1967 was more extensive than the current study and encompassed the periodicity of algal species with time. In the previous study, genera representative of two divisions were found. Representative of Chlorophyta (green algae) and Cyanophyta (blue-green algae) were identified. The present study, based on a single collection time, would be expected to identify less diversity of orders and families. No genera were found to be common to the two studies. The previous study, however, addressed only Chlorophyta (green) and Cyanophyta algae (blue-green). These data do not however necessarily indicate changes in algal populations as the previous study dealt exclusively with soil populations.

7. CONCLUSIONS

In tests performed 3 years after the last application of 2,4-D/2,4,5-T herbicide, the Test Area C-52A grid, Eglin AFB Reservation, exhibits a population level of soil microorganisms identical to that in an adjacent control area of similar soil and vegetative characteristics not exposed to massive quantities of herbicide. There are increases in Actinomycete and bacteria populations in some test site areas over levels recorded in 1970. This is possibly due to a general increase in vegetative cover for those sampling sites and for the entire test grid. No significant permanent effects could be attributed to the presence of herbicides.

Data on aquatic algae populations from ponds previously exposed to repetitive applications of herbicides indicate that the genera present are those expected in warm, acid (pH 5.5), seepage, or standing waters.

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[8]Personal Communication with R. Lynn, Utah State University, Department of Botany, Logan, Utah, 1973.

References:

VII-2. Schluter, M. 1966. Investigations of the Algacidal Characteristics of Fungicides and Herbicides. Int. Rev. Gestamten Hydrobiol. 51:521-541.

VII-3. Wolf, F. T. 1962. Growth Inhibition of Chlorella Induced by 3-amino, 1,2,4, Triazole, and its Reversal by Purines. Nature, 193:901-902.

TABLE VII-10. AQUATIC ALGAE* FROM PONDS OF TEST AREA C-52A

DIVISION	CLASS	ORDER	FAMILY	GENERA
Chlorophyta	Chlorophyceae	Zygnematales	Desmidiaceae	Closterium Cosmarium Triploceras
Chlorophyta	Chlorophyceae	Zygnematales	Zygnemataceae	Zygnema
Chlorophyta	Chlorophyceae	Tetrasporales	Palmellaceae	Asterococcus
Chrysophyta	Bacillariophycaeae	Pennales Suborder: Fragilarineae	Fragilariaceae	Asterionella
Chrysophyta	Bacillariophyceae	Naviculineae	Naviculaceae	Navicula

*Smith, G. M. 1950. The Fresh-Water Algae of the United States. McGraw-Hill. 2nd. Ed. 709 pp.

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