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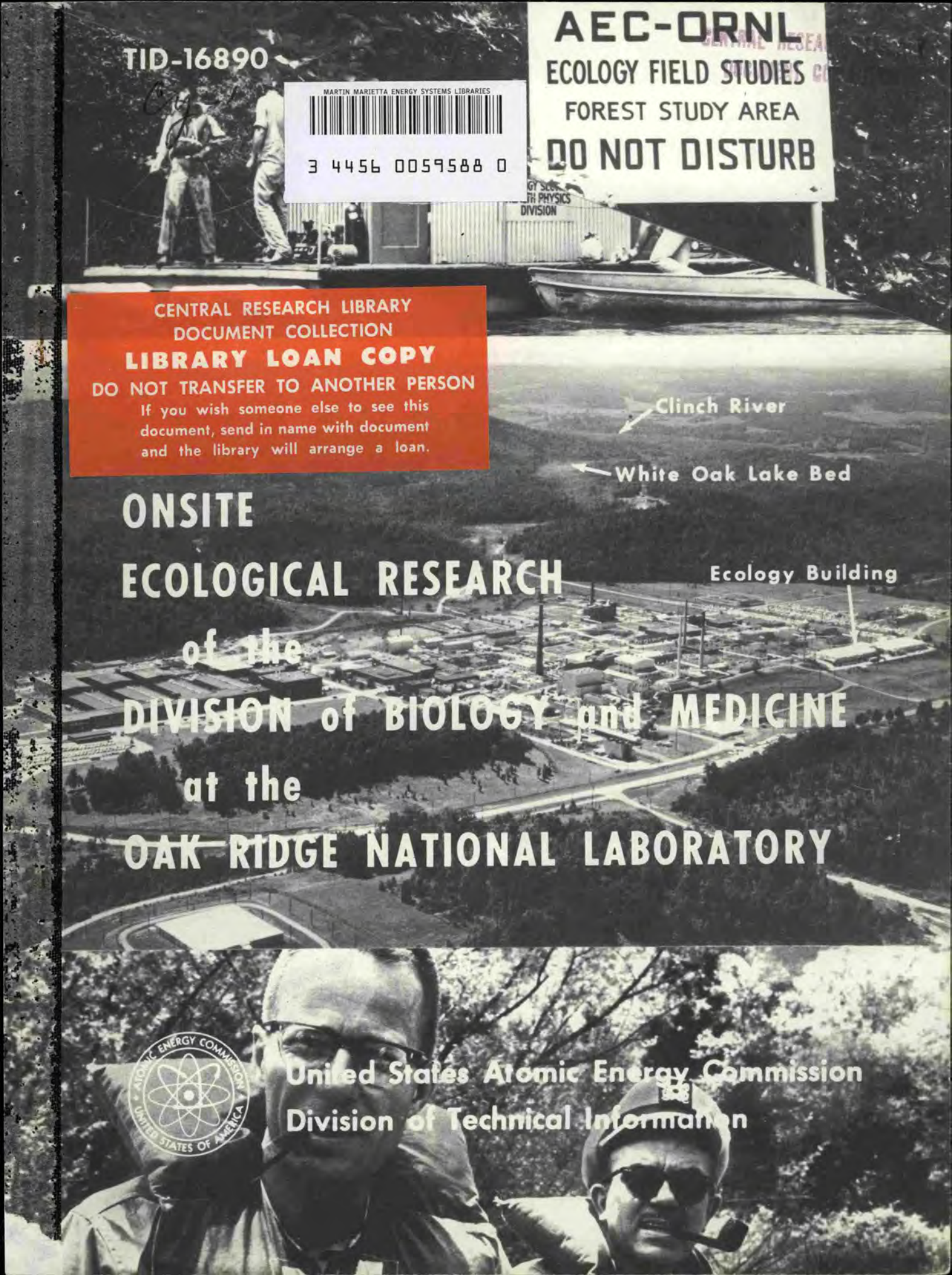


# ONSITE ECOLOGICAL RESEARCH of the DIVISION of BIOLOGY and MEDICINE at the OAK RIDGE NATIONAL LABORATORY

United States Atomic Energy Commission
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# ONSITE ECOLOGICAL RESEARCH OF THE DIVISION OF BIOLOGY AND MEDICINE AT THE OAK RIDGE NATIONAL LABORATORY

Compiled and Edited by

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United States Atomic Energy Commission

October 1962

UNITED STATES ATOMIC ENERGY COMMISSION

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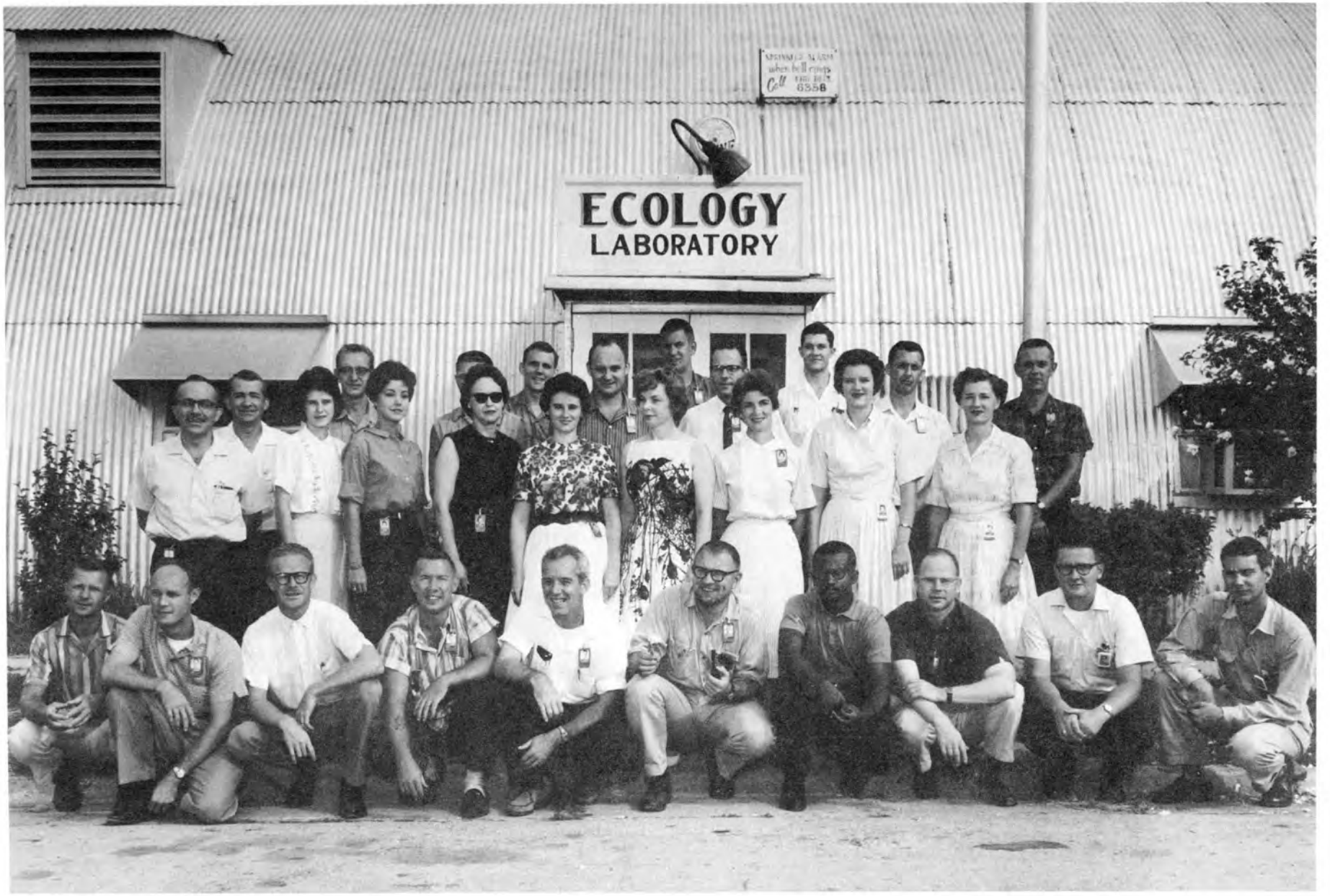
ECOLOGY
LABORATORY

## FOREWORD

This booklet on the radiation ecology research program at Oak Ridge National Laboratory is one in a series of such publications in preparation to describe research programs supported by the Division of Biology and Medicine, U. S. Atomic Energy Commission. Its purpose is to acquaint interested scientists with the biomedical program of the Commission, its objectives and justification. Further, experimental procedures are described with the hope of stimulating and improving research in radioecology.

The terrestrial and fresh-water ecology program is the responsibility of the Environmental Sciences Branch, Division of Biology and Medicine, and is currently under the supervision of John N. Wolfe, Chief of the Branch and an Ecologist, and Vincent Schultz, Ecologist, who with S. I. Auerbach, Chief, Radiation Ecology Section, Oak Ridge National Laboratory prepared and edited this brochure.

The Environmental Sciences Branch program is conducted through U. S. Atomic Energy Commission installation contractors and contracts with universities and colleges. The former is known as the on-site program and the latter as the off-site program. This brochure describes only the on-site program in terrestrial and fresh-water ecology at Oak Ridge National Laboratory which is operated for the Commission by the Union Carbide Corporation.

The project descriptions in this brochure were prepared by the staff of the Radiation Ecology Section, ORNL at our request, edited in the Branch, and approved for publication by the ORNL staff. The brochure

contains descriptions of the majority of research projects conducted since the initiation of the program at ORNL. The intent here was to demonstrate the continuity and overall cohesiveness of the program underway, even though portions of the project descriptions herein presented may be obsolete at this time.

Publications and reports listed are those which have been published or accepted for publication by July 1, 1962. Manuscripts in preparation are not listed.

The usefulness of the series will depend upon the reaction of our readers. Suggestions for improvement would be most welcome.

Charles L. Dunham, M.D.
Director
Division of Biology and Medicine

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Summer 1962

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Not Present-J. S. Olson, H. D. Waller, W. T. Lammers (Davidson College), and Tom Grizzard (Tennessee Wesleyan College).

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# INTRODUCTION

The challenging environmental problems created by nuclear technology and the opportunities for ecological research to contribute to the resolution of these problems are combined uniquely at the Oak Ridge National Laboratory. Here scientific and engineering technologies produce many degrees of environmental modification in forests, fields, and bodies of water.

Within the confines of the Oak Ridge Reservation are a variety of biotic habitats which have received an input of radioactive materials. These habitats are both terrestrial and aquatic. Fields and forests in various stages of succession, streams, swamps, and ponds are included in these habitats. These contaminated areas comprise a relatively small fraction of the 30,000 acres which make up the Oak Ridge AEC Reservation. A system of long parallel ridges and valleys is found throughout the Reservation. Prior to government occupancy in 1942, the bottom lands in the valleys were extensively farmed while the ridge tops were left wooded, although the prime timber was removed selectively. For the past twenty years much of this land has been allowed to undergo succession back to its native forested state. In addition, numerous plantations of different coniferous species have been established in parts of the valleys. This mosaic of forest stands and their associated biota is superposed on soils of contrasting chemical content which have been derived from the several geologic formations which underlie the Reservation. These combinations of biological and physical factors provide a large-scale field research facility, analogous to the reactors, cyclotrons, and chemical and

biological laboratories of the National Laboratory.

Ecology is becoming increasingly an interdisciplinary science. To this end the National Laboratory can contribute a unique combination of skilled personnel and facilities for applying chemical, physical, mathematical and biological methods to ecological research. The resources include expert advice and large-scale service for chemical analyses, including spectrographic and activation analyses; a variety of data processing equipment and analog computers, and specialists on the biology of organisms and effects of ionizing radiation. Health Physics support and rigid control are available over a reservation of sufficient size that large scale tracer experiments can be conducted with safety.

Ecology at Oak Ridge National Laboratory is part of the Health Physics Division. Historically this division has been concerned with the protection of man from radiation in the broadest sense of the term. This concern has manifested itself in a broad interdisciplinary program in the environmental sciences, covering both basic science and engineering aspects, in relation to the behavior of radionuclides released to the environments.

Chiefly responsible for the initiation of a radiation ecology program at ORNL were Karl Z. Morgan, Director, and Edward G. Struxness, Assistant Director, Health Physics Division. Both have had a long and abiding interest in environmental problems related to radiation protection and the foresight to recognize that many aspects of the radiation problem were ecological in nature. In 1953, after taking charge of the Health Physics Division Waste Disposal Research and Engineering Section,

Struxness, with Morgan's approval and backing, solicited the assistance of Orlando Park, Professor of Ecology, Northwestern University to aid in establishing an ecology program at the Laboratory. In view of the unfamiliarity with ecology that existed both in the Laboratory and in the Commission, Park and Struxness were singularly successful in obtaining an authorization to establish a program the following year (1954).

The undersigned was appointed and joined the Laboratory in the latter half of 1954 to take charge of the program. Program development, acquisition of facilities, and staff procurement were carried along simultaneously with exploratory researches in a number of areas of interest.

The following year two events occurred which proved to be of major importance to the future development of radiation ecology. An ecologist was appointed to the staff of the Division of Biology and Medicine, AEC, Washington, and a radioactive waste impoundment (White Oak Lake) was drained at Oak Ridge. The former led to the official recognition of ecology as part of the Commission's program and resulted ultimately in the formation of the Environmental Sciences Branch. The latter event produced the first relatively large terrestrial area contaminated by radioactive waste water and thus provided the means and motivation for development of a field program directly related to nuclear technology.

During the ensuing years, additional scientists joined the staff, adding their knowledge to the development of the program. By 1959 the program had achieved its present form. The radiation ecology program has two research themes which for purposes of effective organization and administration are pursued under the four projects described in this brochure.

One theme of radioecology research at the Oak Ridge National Laboratory emphasizes the processes of movement and behavior of radionuclides and other chemical elements in natural ecosystems such as forests, fields, and rivers. Laboratory and field studies make use of radioactive tracers and waste radionuclides to determine: (1) uptake, accumulation, and movement of isotopes in terrestrial and aquatic food chains; (2) rates of translocation in plants; (3) consumption of food and turnover of isotopes by terrestrial and aquatic insects and other invertebrates, fish, and mammals; (4) reentry of nutrients and isotopes into the soil through breakdown of organic litter by fungi, bacteria, and soil animals.

The second theme of radioecological research deals with the effects of ionizing radiation as a factor influencing organisms, populations, and ecosystems. Pioneering laboratory work several years ago revealed effects of ionizing radiation on predator-prey relations and differential fecundity in populations of mites and insects. So far no definite proof of significant damage by radiation from nuclear operations has been found under field conditions at Oak Ridge, but the possibilities of subtle effects (or of more pronounced effects of future intentional irradiation) cannot be ruled out until careful research is continued over several years. The present program encompasses field research on the effects of chronic ionizing radiation derived from radioactive waste disposal operations on natural populations both at the cytogenetic and organism level. Parallel field and laboratory studies, employing an unshielded reactor, will consider the interaction of ionizing radiation and such factors as temperature, light, and humidity on the responses of plants to varying levels of radiation.

In addition to its research function, the Radiation Ecology Section has provided many university and college faculty members and students with opportunities for learning the application of new techniques to the solution of problems while carrying on publishable research. The radiation ecology program has grown rapidly since its inception in 1954, but interest in this new field has grown even faster. Not all well-qualified applicants can be supported but we hope to make the ecological research facilities of the Oak Ridge National Laboratory as widely available as possible in the future, and to aid university programs in this new and challenging area of research.

S. I. Auerbach, Chief
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Oak Ridge, Tennessee

## PROJECT I: LABORATORY STUDIES OF THE EFFECT OF RADIOACTIVITY ON NATURAL POPULATIONS

Over-all Objectives:

To measure the effects of ionizing radiation on ecological parameters in natural populations.

Justification:

Any evaluation of the effects of acute and chronic ionizing radiation on ecosystems and their components will require critical laboratory studies to differentiate between effects induced by radioactivity and those caused by other environmental factors.

Subproject 1.001 -- Instantaneous and delayed effects of gamma radiation on mixed arthropod populations.

Date initiated: September 1954 Date completed: September 1955

Research personnel: S. I. Auerbach, H. F. Howden, and M. D. Engelmann

Objectives: To obtain preliminary data on the immediate and delayed effects of acute gamma radiation on mixed arthropod populations.

Justification: At the time of the study, little information was available in the literature on the effects of radiation on arthropods, especially natural populations.

Procedures: Weighed samples of tree-hole mold containing an average of 1,500 arthropods each were exposed to various doses of radiation from a $Co^{60}$ source. Exposures ranged from 3,000 to 125,000 r. At intervals of 30, 60, 90 days, samples were removed from culture, arthropod populations extracted by means of Berlese

funnels, and subsequently counted. Dose response curves were prepared for the abundant species. These curves were evaluated insofar as possible in terms of the biologies of the species involved.

Reports:

Auerbach, S. I. 1958. The soil ecosystem and radioactive waste disposal to the ground. Ecol. 39(3): 522-529.

Auerbach, S. I. 1956. Delayed effects of gamma radiation on tree hole arthropods. In Health Physics Division Semiannual Progress Report for the period ending January 31, 1956, ORNL-2049.

Auerbach, S. I. 1956. Delayed effects of gamma radiation on tree hole arthropods. In Health Physics Division Semiannual Progress Report for the period ending July 31, 1956, ORNL-2151.

Subproject 1.002 -- Investigations on sterility and deformities of *Onthophagus* (Coleoptera, Scarabaeidae) induced by gamma radiation.

Date initiated: June 1955 Date completed: September 1955

Research personnel: H. F. Howden and S. I. Auerbach

Objectives: To determine effects of gamma radiation on aspects of the autecology of ground burrowing beetles and to provide baseline information for further experiments using chronic exposures to radiation.

Justification: The probability of release of radioactive wastes into soil indicated a need for information on the effects of radiation on soil organisms.

Procedures: Adult and larval beetles were subjected to 2,000, 3,000, 4,000, and 5,000 r. of $Co^{60}$ radiation. Animals were raised in the laboratory and subsequent effects on fertility and life span noted. Sexual differences in response to radiation also were evaluated.

Reports:

Auerbach, S. I. 1956. Effects of gamma radiation on Onthophagus texanus (Schaeffer). In Health Physics Division Semiannual Progress Report for the period ending January 31, 1956, ORNL-2049.

Howden, H. F. 1957. Investigations on sterility and deformities of Onthophagus (Coleoptera, Scarabaeidae) induced by gamma radiation. Ann. Ent. Soc. Amer. 50(1): 1-9.

Subproject 1.003 -- Effects of gamma radiation on a natural microbial population.

Date initiated: June 1955 Date completed: September 1955

Research personnel: R. J. Davis, V. L. Sheldon, and S. I. Auerbach

Objectives: To determine the lethal effects of known dosages of gamma radiation upon segments of a natural microbial population and to compare dose mortalities of natural microbial populations with those reported for pure cultures of well-known strains.

Justification: There is a need to know the effects of ionizing radiation upon natural populations in their native substrate.

Procedures: Duplicate samples of tree-hole mold were subjected to

various doses of gamma radiation from a $Co^{60}$ source. Controls were plated out from suspensions made from the same material prior to irradiation. Microbial populations in experimentals and controls were evaluated, using the scheme of Lockhead and Chase. Dose response curves were plotted on semilog paper.

Reports:

Davis, R. J., V. L. Sheldon, and S. I. Auerbach. 1956. Lethal effects of gamma radiation upon segments of a natural microbial population. Jour. Bacteriol. 72(4): 505-510.

Auerbach, S. I. 1956. Ecology. In Health Physics Division Semi-annual Progress Report for period ending July 31, 1956, ORNL-2151.

Subproject 1.004 -- Differential effects of gamma radiation on predator and prey species of mites.

Date initiated: October 1955 Date completed: May 1956

Research personnel: S. I. Auerbach, C. J. Rohde, and A. L. Broseghini

Objectives: To obtain basic autecological data on the life cycle of mites and to evaluate the effect of gamma radiation on various life history stages of these mites.

Justification: This investigation was part of a series of studies concerning the effect of ionizing radiation on soil organisms.

Procedures: Mite eggs were irradiated with gamma radiation from a $Co^{60}$ source. Exposures ranged from 250 to 3,750 r. Adults of both sexes were subjected to doses ranging from 4,000 to 12,000 r. Experiments crossing irradiated males with nonirradiated

females and vice versa were performed to test for doses required for sterility.

Reports:

Auerbach, S. I. 1956. Radiation studies on a single species. In Health Physics Division Semiannual Report for period ending July 31, 1956, ORNL-2151.

Rohde, C. J., Jr. 1956. A modification of the plaster-charcoal technique for the rearing of mites and other small arthropods. Ecol. 37(4): 843-844.

Rohde, C. J., Jr. 1959. Studies on the biologies of two mite species, predator and prey, including some effects of gamma radiation on selected developmental stages. Ecol. 40(4): 572-579.

Subproject 1.005 -- Effects of gamma radiation on Collembola population growth.

Date initiated: August 1956 Date completed: February 1957

Research personnel: S. I. Auerbach, D. A. Crossley, Jr., and M. D. Engelmann

Objectives: To determine effect of gamma radiations on population growth rate.

Justification: No such information available in literature at that time.

Procedures: Sixty-one reproducing population units of ten individuals each were used in this experiment. Increase in population size was measured by bidaily counts of individuals at food points and by counts of total numbers at end of experiment. Three

doses of gamma radiation (3,000, 5,000, and 7,000 r.) were given in single exposures to these units. Sixteen replicates were used for each dose level; thirteen were controls. An analyses of variance was applied to the data.

Reports:

Auerbach, S. I. 1957. Laboratory studies on arthropods. In Health Physics Division Annual Progress Report for the period ending July 31, 1957, ORNL-2384.

Auerbach, S. I., D. A. Crossley, Jr., and M. D. Engelmann. 1957. Effects of gamma radiation on Collembola population growth. Science 126(3274): 614.

Subproject 1.006 -- Effects of acute and fractionated doses of gamma radiation on *Trogoderma* (Coleoptera, Dermestidae).

Date initiated: September 1956 Date completed: April 1957

Research personnel: H. F. Howden and S.I. Auerbach

Objectives: To evaluate the effect of gamma radiation on Coleopterous insects of high productive capacity and to compare the effects of single and fractioned doses on larval development time and metamorphosis.

Justification: This was the second study in a projected series of investigations on the comparative effects of ionizing radiation on various species of Coleoptera.

Procedures: Duplicate jars, each containing 50 larvae, were exposed to doses of gamma radiation ranging from 1,000 to 10,000 r. One series received these exposures in single doses. The second

series received one-fifth of their dose per day for five consecutive days. Controls and experimentals subsequently were checked weekly for emergence of adults which were transferred to fresh jars. These were checked also at weekly intervals for appearance of $F_1$ larvae. Weekly checks were made over a 2-month period, after which checking was less frequent. Final counts of the $F_1$ and $F_2$ generations were made 6 months after irradiation.

Reports:

Auerbach, S. I. 1957. Radiation effects on Trogoderma sternale. In Health Physics Division Annual Progress Report for period ending July 31, 1957, ORNL-2384.

Howden, H. F. and S. I. Auerbach. 1958. Some effects of gamma radiation on Trogoderma sternale Jayne. Ann. Ent. Soc. Amer. 51(1): 48-51.

Subproject 1.007 - Effects of moisture and temperature stress on irradiated shortleaf and scrub pine seedlings.

Date initiated: August 1962 Date to be completed: August 1964

Research personnel: J. P. Witherspoon, Jr.

Objectives: To determine the effects of sublethal gamma and fast neutron irradiation on physiological tolerance ranges of locally important conifer species, to establish levels of sublethal radiation doses which alter the normal plant-environment interaction, and to establish criteria for more detailed assessment of radiation damage to natural vegetation surrounding the Health Physics Research Reactor.

Justification: In determining the total ecological effect of radiation on plant populations it is necessary to consider not only changes in morphology, growth rates, and life span, but also changes in the physiological tolerances of the organisms. Radiation-induced deviations from normal tolerance ranges may alter the plants' ability to cope with an environment free of excess radiation. Changes in response to moisture and temperature stress may effect survival rates by lowering the recovery capacity of irradiated plants.

Procedures: Two year old potted seedlings of Pinus echinata (shortleaf pine) and P. virginiana (scrub pine) will be subjected to acute doses of gamma radiation at the $Co^{60}$ seed irradiation facility of the UT-AEC Agricultural Research Laboratory. Later this year and next year additional seedlings will receive fast neutron and gamma radiation from the Health Physics Research Reactor at ORNL. Metaphosphate glass rod dosimeters will be employed to determine dose to plant parts.

These plants, subjected to sublethal dose levels, will be placed in Percival Growth Chambers where appropriate environmental conditions can be maintained. Responses (measured by leaf discoloration and wilting) and survival time of control and irradiated plants will be determined for a number of environmental stress conditions including flooding, drought, and temperature extremes.

Minimum radiation levels producing significant changes in physiological tolerance ranges will be repeated with additional

pine in an attempt to locate threshold values.

Reports:

None

Subproject 1.008 - Effects of radiation from the Health Physics Research Reactor on adjacent forest stands.

Date initiated: July 1962 Date completed: continuing

Research personnel: J. P. Witherspoon, Jr.

Objectives: To survey and map the natural vegetation around the Health Physics Research Reactor emphasizing woody species and perennial herbs, to ascertain radiation-induced changes in plant populations. These may be changes in density, growth habit, phenological behavior, or productivity, and to study structural changes in plant community composition which may be altered as a result of differential species radiosensitivity.

Justification: The bare, unmoderated Health Physics Research Reactor is surrounded by forests of the pine-hardwood type at distances varying from 30 to 300 feet. When the reactor goes critical (December 1962) gamma and neutron radiation up to several hundred rad per hour will reach these forested areas. Therefore, another opportunity exists to study the effects of relatively high radiation doses at the plant community level. This study will differ from those at the Lockheed Facility (Dawsonville, Georgia) and at Brookhaven National Laboratory in that different plant species are involved, and in that the reactor will produce relatively pure fast neutrons during short bursts.

Procedures: A grid reference system consisting of 10 x 10 m grid has been laid out around the reactor. Woody species in these grids have been mapped and records taken of tree size and general vigor. When the reactor goes critical, a study of horizontal and vertical dose gradients will be made to determine best locations for integrating dosimeters to be used to determine dose when the reactor is fully operational.

Next spring phenological observations (seasonal occurrence of buds, leaves, flowers, and fruit) will be recorded for species along dose gradients and species in a nonirradiated control area. Growth habits, particularly length of apical growth in conifers, will be similarly compared.

Radiation damage to individuals will be assessed both at the species and community level. Some interpretations of physiological damage to members of the community will be facilitated by concomitant experiments (subproject 1.007) on the effects of environmental stresses on irradiated plants.

## PROJECT II: PROCESSES AND COMPONENTS OF TERRESTRIAL ECOSYSTEMS

Over-all Objectives:

To understand the processes involved in the circulation of radioactive isotopes and other materials through such ecosystems.

To evaluate the energetics of ecosystems, including food energy which guides the circulation of materials and thermal energy which modifies the rates of circulation.

To characterize the natural populations of land plants and animals and the habitats where these processes take place and to interpret their significance for ecosystems representative of the eastern American forest region, as compared with those of other environments.

Justification:

The contrasting ecosystems near Oak Ridge provide opportunities for large-scale tracer experiments and related studies on the circulation of elements and isotopes. Research on nutrient elements not only helps to interpret data on radioactive contamination, but should contribute to a fundamental understanding of chemical circulation of ecosystems. Data and predictions on the movement of radionuclides already contaminating the environment (even outside areas affected by processed radioactive waste) are needed by health physicists guiding the safety policy of nuclear installations and a future nuclear technology.

The rates of circulation of nutrient elements and radioisotopes depend on the amount of organic matter produced (plant productivity) and on certain macro- and microclimatic conditions, and thus ultimately on the energy transformations of the system. These matters are pertinent to

predicting the implications of radioactive contamination, as well as interest for the quantitative theory of ecology.

Identification and quantitative records of organisms occupying the Oak Ridge Reservation are needed as background for more specialized research. They provide a basis for objectively detecting future changes of this area and for comparing it with other regions.

Subproject 2.001 -- Nature of the oribatid mite found in pine litter in the Oak Ridge area.

Date initiated: June 1956 Date completed: September 1956

Research personnel: K. K. Bohnsack and D. A. Crossley, Jr.

Objectives: To investigate the abundances of the various oribatid mite species in pine litter (*Pinus echinata* and *P. virginiana*) and to examine local variation in numbers for these species, both between and within similar stands of trees.

Justification: Oribatid mites are numerous in many soil and litter situations. It would appear that they play a role in the decomposition of leaf litter, but details of bionomics and ecology are known for only a few of the species. Data on the structure and variation of the litter oribatid fauna are prerequisites to studies of the importance of the litter fauna in the soil ecosystem. This study was designed to provide a baseline for future work on the influence of these arthropods on the release of radiation fission products from the litter layer.

Procedures: A stratified sampling program was used. Three areas (different stands) of pines each contained three stations.

Three samples per week were withdrawn from each station for 8 consecutive weeks (a total of 216 samples). Arthropods were extracted from the samples and counted. An analysis of variance of the data was made.

Reports:

Crossley, D. A., Jr. 1958. Microarthropods of pine-needle litter. In Health Physics Division Annual Progress Report for the period ending July 31, 1958, ORNL-2590.

Crossley, D. A., Jr. and K. K. Bohnsack. 1960. Long-term ecological study in the Oak Ridge area. III. The oribatid mite fauna in pine litter. Ecol. 41(4): 785-790.

Subproject 2.002 -- A sampling device for soil microarthropods.

Date initiated: June 1956 Date completed: December 1956

Research personnel: S. I. Auerbach and D. A. Crossley, Jr.

Objectives: To develop and test a sampling tool and extraction device for soil microarthropods.

Justification: This equipment is necessary for routine sampling of soil microarthropods in other subprojects.

Procedures: Paired samples were taken in a variety of soil and litter situations. One sample of each pair was treated in the new extraction device, the other in the standard Tullgren funnel. In addition, 40, 60, and 100-watt bulbs in the Tullgren funnel were used on a portion of a series of samples; the remainder of the series was treated in the new extraction equipment.

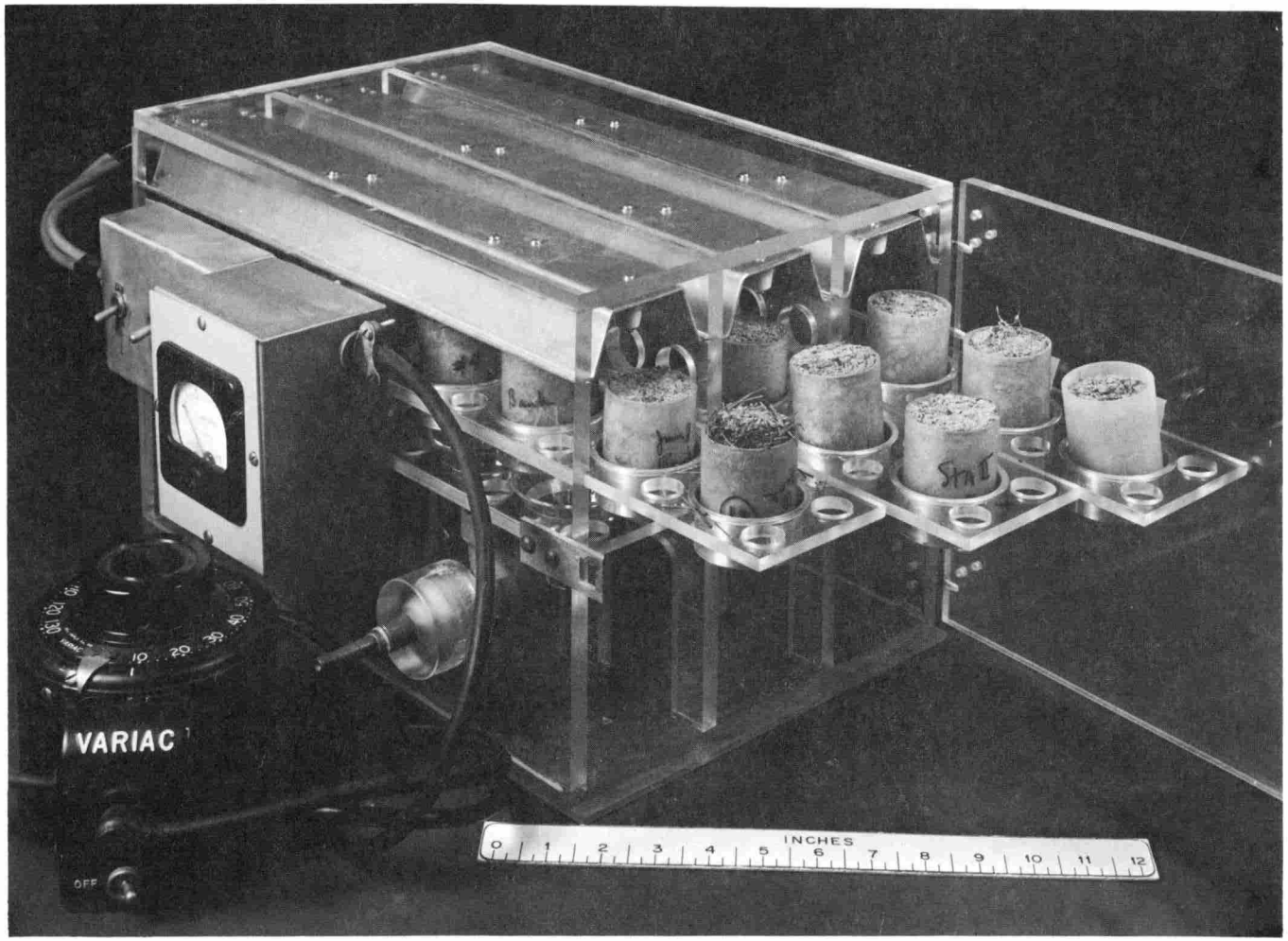


Fig. 1. High-gradient microarthropod extraction device modeled after Macfadyen design (Subproject 2.002).

Reports:

Auerbach, S. I. and D. A. Crossley, Jr. 1960. A sampling device for soil microarthropods. Acarologia t. II fasc. 3: 279-287.

Crossley, D. A., Jr. 1957. Use of Berlese extraction apparatus. In Health Physics Division Annual Progress Report for period ending July 31, 1957, ORNL-2384.

Subproject 2.003 -- Exponential equations relating production, decay, and accumulation of dead organic matter in ecosystems.

Date initiated: September 1958 Date completed: August 1959

Research personnel: J. S. Olson

Objectives: To provide simple quantitative hypotheses concerning the rates of transfer of chemical elements and isotopes between compartments of an ecosystem. Such studies relate empirical data from other subprojects and from widely scattered literature.

Justification: Ecology needs a more theoretical framework to relate the data on individual steps in the general circulation of elements and isotopes, and on the whole process of circulation. First approximations are suggested by the first-order differential equations which have previously been applied by health physicists to the movement of radionuclides between organs of the body. Obvious limitations on simple models, when compared to the complexities of real ecosystems, can gradually be overcome, at the expense of mathematical complications and the use of digital and analog computers.

Procedures: The balance of income and loss of carbon and other material was first considered for a single compartment--the dead organic matter unincorporated into mineral soil, e.g., the forest floor. Jenny's parameter k' for annual rate of breakdown was estimated from the ratio (annual income of litter)/(total mass of forest floor). For cases where equilibrium was not yet attained, the solution of first order differential equations leads to asymptotic exponential equations, in terms of the exponent $k = -\ln(1-k') = .693/$(half time for litter breakdown). Theoretical values for the accumulation of forest floor for contrasting values of productivity and decay parameters were calculated and compared with estimates based on data from several climatic regions of the world.

Reports:

Olson, J. S. 1959. Development of theoretical ecological models. In Health Physics Division Annual Progress Report for period ending July 31, 1959, ORNL-2806.

Olson, J. S. 1959. Exponential equations relative productivity, decay and accumulation of forest litter. Proc. 9th Inter. Bot. Cong. 2: 287.

Olson, J. S. 1959. Energy storage and the balance of producers and decomposers in ecological systems. Submitted to Ecology.

Subproject 2.004 -- Use of analog computers for predicting the transfer of matter and energy in ecosystems.

Date initiated: September 1959 Date completed: August 1960

Research personnel: R. B. Neel and J. S. Olson

Objectives: To extend simple exponential equations used in subproject 2.003 for the movement of elements or isotopes through several compartments of an ecological system. To evaluate the use of analog computers to simulate ecological model systems.

Justification: Analog computers not only aid the solution of complicated systems of differential equations which emerge from mathematical models, but can also be used so that the transmission of electrical potential through the computer circuit simulates the transfer of matter or energy through the ecological model. Methods of adapting these circuits to ecosystems not only facilitate the prediction of contamination hazards that could be important in health physics, but also provide a thought-providing tool for ecological theory.

Procedures: Familiarity with the elements of computer theory and practice were gained by simulating simple models on a Donner computer whose capacity is limited to 10 operational amplifiers and a smaller number of "compartments" in the ecological model. Extension of this experience to the large ORNL Analog Computer facility provided for a larger number of compartments, and more complicated forms of input, with various kinds of seasonal cycles, and arbitrary introduction of radioactive contaminants by means of a diode function generator. Examples involved the major element carbon, and the neutron-activated contaminant, $C^{14}$.

Reports:

Neel, R. B. and J. S. Olson. 1962. Use of analog computers for simulating the movement of isotopes in ecological systems. ORNL-3172.

Subproject 2.005 -- Chemical composition of forests.

Date initiated: October 1958 Date completed: continuing

Research personnel: J. S. Olson, J. A. Wolfe, R. M. Anderson, T. C. Rains, L. C. Bate, and S. R. Koirtyohann

Objectives: To estimate masses of total organic matter and specific major and minor elements.

Justification: Information on the natural distribution and circulation of chemical elements is needed in order to understand movements of radioisotopes and nutrients. Analyses of representative samples of plants and soils will be needed for calculations of specific activities of radioisotopes. Differences in the chemical requirements of species are probably also important in limiting their distribution and growth.

Procedures: Shortleaf pine (*Pinus echinata*) forests were chosen for first collections of foliage, branches, and trunk samples. Cooperative studies are covering other important species. Fresh and dry weights and pertinent form measurements are taken for estimating the biomass of trees and stands. Some samples have been used for chemical analyses, including flame photometry for Ca, Sr, Mg, and K, and arc spectrography and activation analysis for many other elements. IBM 1401 and 7090 computers are used for statistical analysis.

Reports:

Olson, J. S. 1959. Analysis of forest growth. In Health Physics Division Annual Progress Report for period ending July 31, 1959, ORNL-2806.

Olson, J. S. 1961. Studies of the Oak Ridge reservation. In Health Physics Division Annual Progress Report for period ending July 31, 1961, ORNL-3189.

Subproject 2.006 -- Origin of radioactive contamination of the environment.

Date initiated: February 1958 Date completed: April 1960

Research personnel: J. S. Olson and R. M. Anderson

Objectives: To estimate the extent and kinds of contamination outside the waste disposal areas where most previous ecological field work had been done. In particular, discrimination between regional fallout and local stack dispersal is desirable.

Justification: Contamination from fallout affects much broader areas than that from processed wastes, and involves several interesting isotopes other than the long-lived $Sr^{90}$ and $Cs^{137}$ which have received most attention. Ruthenium-106 and especially $I^{131}$ were found to be dispersed for several miles around the Laboratory. Cesium-137, $Zr^{95}$-$Nb^{95}$, $Ce^{144}$-$Pr^{144}$, and $Co^{60}$ exceeded regional fallout levels over more limited areas.

Procedures: Careful sampling of vegetion (mostly foliage, some twigs, wood, and sawdust) is followed by gamma spectrometry, at the earliest possible time in the case of short-lived isotopes like $I^{131}$. Comparisons with meteorological predictions were

made with an IBM 610 program. Some suites of samples were designed to show differences among many species growing at a few places (October, November, 1958; May, June, July, 1959). Others standardized on a favorable species sampled along a systematic network of stations (flowering dogwood, mulberry, September, October, 1959). Trips for vacation and meetings were used for collecting samples from distant areas (July, August, November, December, 1959). Localized contamination within the ORNL area was studied by special samples (foliage and trunk of a tree contaminated by $Co^{60}$ and $Cs^{137}$; grass, litter and soil contaminated with $Ru^{106}$, $Ru^{103}$, $Zr^{95}$-$Nb^{95}$, and $Pu^{239}$.)

Reports:

Olson, J. S. 1959. Foliage contamination by fallout. In Health Physics Division Annual Progress Report for period ending July 31, 1959, ORNL-2806.

Olson, J. S. 1961. Ecological sampling and meteorological calculations of fallout on forests near Oak Ridge. ORNL-3181.

Subproject 2.007 -- Natural dispersal of contaminated plant materials.

Date initiated: September 1958 Date completed: March 1959

Research personnel: J. S. Olson

Objectives: To evaluate briefly the possibility of spreading very localized contamination by wind movement of tree leaves.

Justification: Health Physics experience in the isotopes area indicates that blowing and tracking of "hot tree leaves" (e.g., leaves reported to have dose rates of 1 r./hr. from $Co^{60}$) have

occasionally been responsible for spread of certain kinds of contamination (see subproject 2.006). Similar surrounding areas deserve attention for long-term problems of waste disposal and accident hazards. The dispersal of leaves, seeds, spores, and insects by wind has fundamental implications for distribution of organisms and nutrients.

Procedures: The regular decrease in weight of leaves per unit area was measured by collections from plots along 16 radii from an isolated sycamore tree near ORNL, which had relatively slight contamination by fallout and by a late-September inoculation of $Sr^{89}$. The direction of maximum dispersal (NE and WSW) was found to agree with expectations based on wind frequency, especially when winds are weighted by velocity and by the proximity to time of maximum leaf fall (late October). Mathematical models making allowance for the terminal velocity of leaves, seeds and other materials deserve further attention.

Reports:

Olson, J. S. 1959. Patterns of leaf dispersal by wind. In Health Physics Division Annual Progress Report for period ending July 31, 1959, ORNL-2806.

Subproject 2.008 -- Translocation of $Sr^{85}$, $Ca^{45}$, and $K^{42}$ in trees.

Date initiated: April 1959 Date completed: November 1960

Research workers: J.S. Olson, J. P. Witherspoon, and J. A. Wolfe

Objectives: To estimate rates of translocation of radionuclides and normal nutrient elements after being taken inside plants.

To indicate whether Sr follows Ca exactly, or whether there is significant discrimination against this element. They also provide tagged leaves for experiments on movement of nuclides from litter.

Justification: The value of radioactive tracers for showing direction and rates of movement of normal chemical nutrients can be extended into field conditions at ORNL because of the control of surrounding areas and the availability of less widely used isotopes in quantity. Planning of future experiments requires knowing how quickly different isotopes move through plants. Unequal movement of Sr and Ca would help explain ecological discrimination among alkaline earth elements in $Sr^{90}/Ca$ ratios.

Procedures: In early May, $Ca^{45}$, $Sr^{85}$ (and in one case $K^{42}$) in 0.1 N KCL were pumped into holes which had been drilled in dogwood trees growing in soils of each of several geologic formations. Pathways and rates of movement of tracers in trunk and branches were determined by portable survey meters. Replicated and composite leaf samples were collected systematically several times during the growing season. Potassium-42 was estimated by gamma counts before and after decay (t 1/2 = 12 hr.). Excellent discrimination between $Sr^{85}$ was achieved by using NaI scintillation detectors (integral well counter and single channel analyzer) for the pure gamma radiation of $Sr^{85}$ and detecting the weak beta radiation of $Ca^{45}$ in a liquid scintillation counter.

Reports:

Olson, J. S. 1959. Translocation of tracer radionuclides in trees. In Health Physics Division Annual Progress Report for period ending July 31, 1959, ORNL-2806.

Subproject 2.009 -- Translocation of $Cs^{134}$ by white oak (*Quercus alba*).

Date initiated: Spring 1960 Date completed: Autumn 1961

Research personnel: J. P. Witherspoon, J. S. Olson, and S. I. Auerbach

Objectives: To estimate the movement and storage of radiocesium and natural alkali metal ions (Cs, K) within white oak trees and in forests dominated by oak.

Justification: Movement of $Cs^{137}$ inside plants and out of plant litter should follow quite a different pattern from that of Sr, which is covered in other subprojects. Experiments to determine these patterns are needed by those who are evaluating hazards and should be of fundamental interest for forest nutrition.

Procedures: General procedures will be similar to those of subproject 2.008, involving inoculation of isotopes into trees, tracing of their movement inside trees, and using the tagged litter in further experiments on breakdown of litter.

Reports:

Witherspoon, J. P., S. I. Auerbach and J. S. Olson. Cycling of $Cs^{134}$ in white oak trees on sites of contrasting soil type and moisture. ORNL-3328.

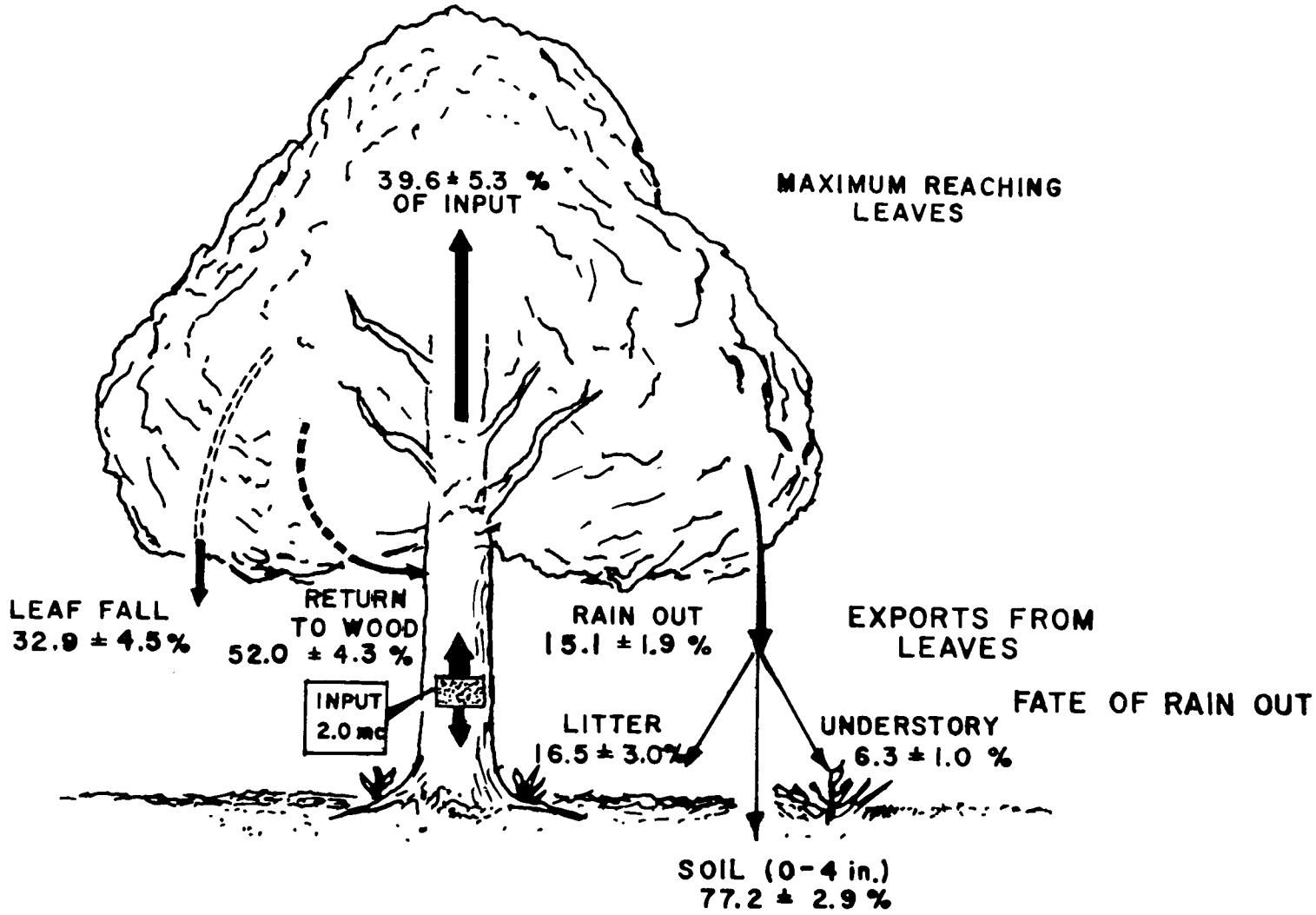


Fig. 2. Cycle of $Cs^{134}$ in white oak (Quercus alba). These data represent the average values determined from 12 trees at the end of the growing season (Subproject 2.009).

Subproject 2.010 -- Rate of breakdown of litter of several deciduous tree species in contrasting climates of Oak Ridge and the Great Smoky Mountains. (cooperative project with the University of Tennessee)

Date initiated: November 1958 Date to be completed: January 1964

Research personnel: J. S. Olson and R. E. Shanks

Objectives: To estimate the rate of breakdown of organic matter and nutrient elements from litter to mineral soil. To estimate the dependence of this rate on cover (evergreen vs. deciduous), macroclimate and slope microclimate, and on the species of litter.

Justification: Most foliage contamination is carried with dead organic matter to the ground surface, where further movement into the soil is hastened by physical and chemical activities of decomposer organisms rather than herbivores and carnivores feeding on live tissue. General experience and calculations from models (see subproject 2.003) indicated great differences between forests in their rate of litter breakdown. The litter-bag method was developed to overcome the confounding of differences in the kind of litter with differences between forests. Such experiments aid planning of further work (e.g., subproject 2.011) with litter tagged by radionuclides as in subproject 2.008.

Procedures: Bags of nylon net served to (1) standardize the maximum size of organisms acting on the litter, (2) standardize the size of fragment that was arbitrarily considered "broken down", and

(3) permit periodic collection for purposes of weighing and extraction of microfauna by Berlese funnels.

Experiment 1, started in December 1958, was a 2 x 3 factorial: cover x climate (Oak Ridge vs. two elevations in the Smokies). Five species were compared in randomized split plots at all stations. Extra groups of five kinds of leaves were provided at the two Oak Ridge Stations included additional sources of leaves of two species, plus two extra species.

Experiment 2, started in December 1958, in a 2 x 3 x 2 factorial, cover x macroclimate x slope (north vs. south); level stations are added at Oak Ridge.

Experiment 1 involved sampling twice the first year and once each year through 1962. Sampling in experiment 2 will be the end of each of the four seasons in 1960 and 1961. Chemical analyses are being made by flame photometry to show differences in mobility of major cations.

Reports:

Olson, J. S. 1959. Rates of litter breakdown in contrasting forests. In Health Physics Division Annual Progress Report for period ending July 31, 1959, ORNL-2806.

Shanks, R. E. and J. S. Olson. 1961. First-year breakdown of leaf litter in southern Appalachian forests. Science 134(3473).

Subproject 2.011 -- Arthropods associated with litter breakdown in decimeter litter bags.

Date initiated: December 1959 Date completed: December 1960

Research personnel: D. A. Crossley, Jr. and M. P. Hoglund

Objectives: To discover which arthropods of the soil fauna become associated with freshly-fallen leaf litter, to test the effect of the different soil faunas on leaf breakdown, to compare the decomposition rates of three leaf species (pine, oak, and dogwood), and to measure the rate of release of certain incorporated fission products ($Sr^{85}$, $Ru^{106}$, $Co^{60}$) from leaf litter.

Justification: This experimental approach is designed to answer questions concerning the role of microarthropods in the breakdown of fallen leaf litter. Frequent sampling is necessary so that microclimatic effects upon weekly variation in numbers may be included. Decomposition rates for litter are an important phase of the cycling of materials in the forest system. The objectives posed above are basic questions which must be answered before more sophisticated experiments can be designed.

Procedures: The leaf materials used were inoculated with radionuclides ($Sr^{85}$, $Co^{60}$, $Ru^{106}$) in previous experiments. The litter bag size (1 decimeter square) was chosen to give a sample of arthropods of optimal size. Forty-eight litter bags containing three leaf species (16 each of oak, dogwood, and pine) were distributed between two stations (oak and pine woods), where the bags were arranged in 4 x 6 plots. In a systematic sampling

regime, a set of six bags representing each leaf species from each station is brought into the laboratory each week. After removal of arthropods the bags are returned to the field.

Reports:

Crossley, D. A., Jr. and M. P. Hoglund. 1962. A litter-bag method for the study of microarthropods inhabiting leaf litter. Ecol. 43. (In press).

Olson, J. S. and D. A. Crossley, Jr. 1962. Tracer studies of the breakdown of forest litter. Radioecology. Proc. of First Nat. Symp. on Radioecology. Reinhold Publ. Corp., N. Y., N. Y. (In press).

Subproject 2.012 -- Role of earthworms in breakdown of contaminated oak and ash leaves.

Date initiated: September 1959 Date completed: June 1962

Research personnel: S. I. Auerbach and R. M. Anderson

Objectives: To obtain preliminary data on the role of earthworms in the breakdown of certain species of tree litter and to determine the ability of earthworms to accumulate $Co^{60}$ and $Ru^{106}$.

Justification: Previous studies at the laboratory showed that earthworms have negligible accumulation of radiostrontium. Recent work by Russians (Peredelskii) reports earthworms as being effective accumulators of $Co^{60}$. Earthworms are a major soil turning organism. They may be important for moving fallout contaminants into the soil. Likewise, by aiding in the breakdown of litter they may hasten entry of radionuclides into the soil.

Procedures: This experiment is a 2 x 2 factorial (2 species of leaves vs. 2 species of radionuclides) with duplicate pots for each factor. Earthworms were removed, allowed to evacuate their guts, analyzed spectrometrically, and evaluated as a percentage of total original leaf burden. Rate of loss from leaves into soil as a function of watering was also obtained.

Reports:

Auerbach, S. I. 1955. Uptake of $Sr^{89}$ by Eisenia foetida. In Health Physics Division Semiannual Progress Report for period ending July 31, 1955, ORNL-1942.

Auerbach, S. I. 1956. Uptake of $Sr^{89}$ by Allolobophora caliginosa. In Health Physics Division Semiannual Progress Report for period ending January 31, 1956, ORNL-2049.

Subproject 2.013 -- Forest map and sample of the Oak Ridge Reservation.

Date initiated: April 1960 Date completed: August 1962

Research personnel: J. S. Olson and R. Doub

Objectives: To show the distribution of areas suitable for ecological experiments and sampling, to estimate volumes, rate of growth and successional change. To relate the composition and properties of the forest ecosystem, as a function of substrate, topography, hydrology, biotic factors and time. To provide the basis for planning land management on the AEC Reservation.

Justification: Systematic records of composition and topographic situation of the forest and other vegetation on the several geologic belts of the Reservation are needed to aid for planning

research. Long-term records of natural ecological succession, or its modification by human activities, are especially valuable assets for ecological research on the Reservation. Future use of Reservation lands depends on an inventory of forest conditions.

Procedures: Arrangements through AEC with the Tennessee Air National Guard provided excellent 1:6,000 black and white photos in March and October, 1959, and 1:12,000 color photos in early November, 1959, when autumn foliage contrast was at a maximum. TVA foresters mapped current conditions and 1935 conditions directly on photo prints and later onto maps. Forest survey procedures and IBM tabulation provided stand data of special interest for characterizing major ecological communities.

Reports:

Forest map of the Oak Ridge Reservation (8 sheets 1:6,000).

Subproject 2.014 -- Quantitative aspects of the microflora of the leaf litter of five tree species on the forest floor of two main forest types (hardwood and coniferous) at three exposures (north and south slope and level) and three altitudes (850, 3,400, and 5,200 ft.) in the four seasons.

Date initiated: June 1960 Date completed: December 1961

Research personnel: M. Witkamp

Objectives: To determine the influence of species, forest type, season, exposure, and altitude on the development of microorganisms on constituents of forest litter.

Justification: Knowledge of the influence of the above mentioned ecological factors on microbial development on decomposing natural substrates is necessary for the understanding and prediction of this development and subsequently of the rate of turnover of matter, including radionuclides, in various biotypes and seasons.

Procedures: Leaf samples (approximately 5 g.) are periodically collected from litter bags (2 x 2 dm.) left on the forest floor of the various biotopes. Collected leaves are cut into 2.5 mm wide strips. One gram is stirred magnetically in 1 liter of sterile water for 15 minutes. From this suspension a dilution series is prepared. Fungal and bacterial counts are obtained on rose-bengal - streptomycin and on nutrient agar plates respectively after incubation for 5 days at 25°C. Counts are calculated on a 1 g. dry weight basis.

Reports:

Witkamp, M. 1963. Microbial populations of leaf litter in relation to environmental conditions and decomposition. Submitted to Ecology.

Subproject 2.015 -- Quantitative aspects of the microflora in the surface soil (0-25 mm. deep) of two main forest types (hardwood and coniferous) at three exposures (north and south slope and level) and three altitudes (850, 3,400, and 5,200 ft.) in the four seasons.

Date initiated: June 1960 Date completed: September 1961

Research personnel: M. Witkamp

Objectives: To determine differences in microbial development in the soil of various biotopes and the influence of forest type, exposure, altitude, and season on these differences.

Justification: Knowledge of microbial development in soil of various biotopes and the effect of various ecological factors governing this development is necessary for the understanding and prediction of microbial activity and subsequently of the rate of microbial turnover of matter, including radionuclides, in various biotopes and seasons.

Procedures: Soil samples (5 - 10 ml.) are periodically collected from soil directly under the organic layer (L+F+H). After thorough mixing 1 g. is stirred magnetically for 15 min. in 1 liter of sterile water. From the suspension a dilution series is prepared. Fungal and bacterial counts are obtained on rose-bengal-streptomycin and on nutrient agar plates respectively after incubation for 5 days at 25°C. Counts are calculated on a 1 g. dry weight basis.

Reports:

Witkamp, M. 1963. Microbial populations of leaf litter in relation to environmental conditions and decomposition. Submitted to Ecology.

Subproject 2.016 -- Quantitative aspects of the development of fungus mycelium in the surface soil of two main forest types (hardwood and coniferous) at three exposures (north and south slope and level) and three altitudes (850, 3,400, and 5,200 ft.) in the four seasons.

Date initiated: June 1960 Date completed: December 1961

Research personnel: M. Witkamp

Objectives: To determine differences in fungal development in soil of various biotopes and the influence of forest type, exposure, altitude, and season on these differences.

Justification: Knowledge of fungal development in the soil of various biotopes and the effect of various ecological factors governing this development in soil of various biotopes is necessary for the understanding and prediction of the rate of turnover of matter, including radionuclides, in various biotopes and seasons. Previous work indicated that mycelium growth is a better criterium for fungal development in soil than fungal plate counts.

Procedures: Glass slides (37 mm. wide; 50 mm. long) are inserted vertically in the forest floor and removed after 7 days. After straining, hyphal development on the slides is determined microscopically by counting the number of intersections of fungal hyphae and crosswires in the eyepiece. Development is calculated in cm./cm.$^2$/week.

Reports:

Witkamp, M. 1963. Microbial populations of leaf litter in relation to environmental conditions and decomposition. Submitted to Ecology.

Subproject 2.017 -- Respiratory activity of biota in litter and soil of two main forest types (hardwood and coniferous) at three exposures (north and south slope and level) in the four seasons.

Date initiated: June 1960 Date completed: December 1961

Research personnel: M. Witkamp

Objectives: To determine differences in the respiratory activity of soil biota in various biotopes and the influence of forest type, exposure, and season on these differences. To correlate the results of the respiration measurements with quantitative aspects of the soil biota (microflora and meso-fauna) and with the loss of weight of litter constituents contained in litter bags.

Justification: Knowledge of the respiratory activity of the biota in litter and soil in the various biotopes and of the effect of various ecological factors governing this activity is necessary for the understanding and prediction of the dissimilative activity and subsequent turnover of matter, including radionuclides, by soil biota in various biotopes and seasons. Measurement of respiration of biota of the forest floor is necessary to establish a correlation between development of the biota, their physiological activity and the breakdown of organic matter and subsequent remineralization of elements, including radionuclides, in various biotopes and seasons.

Procedures: Plastic jars (dia. 15 cm.; 5 cm. high) are placed, open side down, on the forest floor over a petri dish (dia. 5.5 cm.)

containing 5 ml. of 0.2 N KOH. After 2 hrs. the amount of KOH neutralized by $CO_2$ diffusing from the forest floor is determined titrimetrically. Respiratory activity of dissimilative soil biota is calculated in ml. $CO_2/dm^2./hr$.

Reports:

None

Subproject 2.018 -- Accumulation of various radionuclides by microorganisms in soil.

Date initiated: June 1960 Date completed: June 1961

Research personnel: M. Witkamp and J. Shoopman

Objectives: To determine the accumulation of various radionuclides by fungi, bacteria, and actinomycetes in soil.

Justification: Radioactive nuclides may be removed from adsorption points of organic matter and inorganic soil constituents and from soil moisture by microorganisms. Knowledge of the rate of incorporation of various radionuclides by soil organisms may further understanding and prediction of pathways and rate of cycling of these radionuclides and of related elements essential for plant growth.

Procedures: Ethylene oxide sterilized soil will be amended with radionuclides and inoculated with single or mixed culture of microorganisms and incubated for four weeks. During this period the soil cultures will be sampled periodically and the organic and inorganic fractions will be separated. The biomass of microorganisms developing and the simultaneous consumption of

soil organic matter will be calculated from respiration measurements. Changes in count rates in the organic and inorganic fractions after leaching will indicate the microbial accumulation of radionuclides during incubation. A noninoculated series will be used as a blank.

Reports:

None

Subproject 2.019 -- Competition among individual trees within a planted 10-year-old loblolly pine (*Pinus taeda*) community.

Date initiated: July 1960 Date completed: September 1962

Research personnel: R. J. Hutnik, J. S. Olson, and S. I. Auerbach

Objectives: To trace the uptake of radioactive nuclides by individuals within a forest community. To determine the area influenced by a dominant member of a plant community, and to determine the extent of root transfer among loblolly pines and minor species.

Justification: The extent to which a nuclide is spread by the vegetation will depend initially upon the distribution within the contaminated area of the roots of the individual plants and upon their ability to absorb the nuclide in competition with the roots of other vegetation. A basic knowledge of competition and specially of the part played by a dominant individual should result in a better understanding of the uptake, distribution, and movement of radioactive nuclides within a plant community.

Fig. 3. Monitoring loblolly pines (Pinus taeda) for radioactive $Rb^{86}$ applied to stumps of nearby trees as a test of root transfer of nutrients between members of a forest community (Subproject 2.019).

Procedures: Ten millicuries of $Rb^{86}$ 100 ml. of a 1 per cent KCl solution have been applied on the top of the cut stump of each of 10 trees spaced at least 30 feet apart. Significant increases in radioactivity of the surrounding trees is taken as evidence of occasional root grafting or other intimate root connections, not only between loblolly pines but with sumac and red maple. A second exploratory study used $Rb^{86}$ plus $Ca^{45}$, and $Zn^{65}$, applied in surface soils. Each plot is circular, 1/40-acre in size with a dominant tree at the center. Uptake from soil in late summer was limited to a few trees, but these were not always the ones closest to the source. This shows how root patterns and competition are complex, even in relatively simple stands. The methods tested suggest possibilities and needs for further study.

Reports:

None

Subproject 2.020 -- Initial release of three radionuclides from redbud leaves in five habitats.

Date initiated: October 1960 Date completed: October 1961

Research personnel: R. Petty and J. S. Olson

Objectives: To observe release rates of $Cs^{134}$, $Sr^{90}$, $Ca^{45}$, and $Co^{60}$ from tagged redbud leaves in 5 basic habitats, with emphasis on the magnitude of earliest release (within days of exposure) and movement into an underlying soil/humus substrate.

Justification: Previous tracer experiments (subproject 2.011) with

$Sr^{85}$ and $Co^{60}$ in leaves suggested that the rate of release of these isotopes was similar to the rate of litter decay during most of the first year following leaf fall. Other experiments (subproject 2.009) showed that $Cs^{134}$ begins moving out of leaves rapidly and becomes readily available for recycling into previously uncontaminated vegetation. The present experiment provides a confirmation of such differences in rates of movement from a singly species of leaf, and compares two kinds of counting technique (loss from leaves; gain to underlying soil) in measuring the extent of these losses.

Procedures: Redbud leaves tagged with $Cs^{134}$, $Co^{60}$, and $Sr^{90}$-$Y^{90}$ were placed in bags of woven fiberglass curtain material appropriately numbered and weighed. Four bags each of $Cs^{134}$, $Co^{60}$, and $Sr^{90}$ tagged leaves were placed in each of five contrasting habitats. These were: a mesophytic sinkhole, an upland oak stand, a shortleaf pine stand, lowland hardwoods and an old field situation. Approximately 1 inch of organic or surface mineral soil was obtained from each of these habitats, a decimeter square, and placed in plastic boxes having drilled holes in sides and bottom. Fiberglass screen wiring was placed in the bottom of the boxes to guard against undue soil loss, yet permit movement of microorganisms. The litter bags were then placed on top of these decimeter soil samples and the sample unit replaced in the soil surface. These units were set out on October 17. On the night of October 18, the area received 1.07 inches of rainfall. On October 20 one sample each of the 3 isotopes from

each of the habitats were brought in to the laboratory and counted. These were returned on October 27. No rain occurred during the time elapsed between collections. Counting of bags, soils and standards over a 3 x 2 in. NaI scintillation crystal after 6 days showed 0.5 to 4 per cent of original $Sr^{90}$ and $Co^{60}$ counts moved to soil, compared with 6 to 13 per cent for $Cs^{134}$. Further sampling has continued through one calendar year.

Reports:

None

Subproject 2.021 -- Sequence of release of radionuclides from mulberry, white oak, pine, and redbud leaves in three forests in the outcrop belt of Conasauga shale.

Date initiated: November 1960 Date completed: December 1961

Research personnel: J. S. Olson, H. D. Waller, and W. C. Cate

Objectives: To follow release of several radionuclides ($Cs^{134}$, $Co^{60}$, $Sr^{90}$) as a function of time without disturbance of litter prior to collection. To compare rates for leaves previously shown to represent maximum contrast in litter breakdown rates. To make these comparisons in upland pine and oak forests and a typical lowland hardwood forest.

Justification: Subproject 2.020 confirmed that $Cs^{134}$ is released from litter much faster than the other isotopes studied earlier (subproject 2.011), and suggested that release of the same isotope of cesium be followed by a technique which is not subject to the possible criticism of handling prior to litter collection.

Subproject 2.010, showed that mulberry (Morus rubra) epitomized species having extremely rapidly decomposable litter supporting very high populations of bacteria (subproject 2.014). Redbud (Cercis canadensis) was included as another rapidly decomposing species, for comparison with litterbox results for subproject 2.020 being carried on simultaneously. White Oak (Quercus alba) is a widespread and important species of intermediate decay rate, supporting fungal as well as bacterial populations (subproject 2.014). Loblolly pine (Pinus taeda) was added as a widespread, important conifer having needles of slower decay rate than any of the deciduous leaves. The three forests included the white oak and pine stations used previously (subprojects 2.010, 2.011) plus a forest of red maple (Acer rubrum), tulip poplar (Liriodendron tulipifera), red gum (Liquidambar styraciflua) with occasional mulberry (Morus rubra), typical of many lowland sites in the Conasauga shale belt and elsewhere.

Procedures: Trees were tagged by the use of Mauget feeders. Leaves were collected in early autumn, stored briefly under refrigeration and folded into bags of 2 mm. mesh nylon net (used in subproject 2.010) or finer-meshed fiberglass cloth (holes slightly greater than 1 mm. mesh). Bags were placed in fenced plots, grouped so that all those of the same radionuclide are together. Sampling is scheduled on a 8 week cycle, with fiberglass bags having $Cs^{134}$ collected after 2 weeks, $Sr^{90}$ after 4 weeks, and $Co^{60}$ after 6 weeks; nylon net bags with several radionuclides are to be collected on the 8th week of the cycle (along with

extra untagged leaves of beech, to round out the species comparisons with subproject 2.010). The result is that every species will be collected on a biweekly basis throughout 7 cycles (56 weeks) or slightly over 1 year, and a few extra bags, mostly 2 x 2 dm. size, will be left for a few later collections for extension of a few more points on the decay curve.

One collection in each cycle will be included for estimation of loss rates for each isotope-leaf-bag type combination. Unlike subproject 2.011, these points will not be repeated measurements on the same bag, and statistical analysis may proceed either by analysis of variance of time-species-radionuclide (or bag-type) combinations, or by regression estimates of time loss functions, with subsequent analysis of variance on the resulting parameter estimates. IBM punchcard data storage and 7090 computer processing will be used in the analysis (subproject 2.025).

Reports:

None

Subproject 2.022 -- Entry and retention of radionuclides from several leaf species in surface soil.

Date initiated: November 1960 Date to be completed: November 1962

Research personnel: J. S. Olson, H. D. Waller, and W. C. Cate

Objectives: To provide for repeated measurement of radionuclide uptake of the elements used in subprojects 2.020 and 2.021 and the litter species used in subprojects 2.021 and 2.011. To estimate rates of gain and loss of radionuclides from surface soil

which explain the timing of increase, equilibration, and eventual decrease of soil radioactivity.

Justification: The rapid intial release of $Cs^{134}$, compared with $Sr^{90}$ and $Co^{60}$, in subproject 2.020 indicated the desirability of additional comparisons of litterbox soil uptake for other leaf species at the same time that experiments are proceeding with other litter bags that are unconfined on the forest floor (subprojects 2.020, 2.021, and 2.023). It is important to know whether isotopes released from litter are retained almost completely in surface layers of mineral soil. If there is an equilibration as losses by further leaching out of the soil occur and eventually balance the rate of gain of the isotope by litter decay, its promptness should be known in order to help predict the mobility of isotopes through lower layers of the soil.

Procedures: Sets of 4 white oak leaves tagged with $Cs^{134}$, along with litterboxes (see subproject 2.020) with surface soils from each of the three forest sites, will be collected and counted at biweekly intervals for more detailed records of rapid Cs movement than the 8-week counting schedule of subproject 2.021. Additional collections counted once at the end of each 8-week cycle will be made for $Sr^{90}$ with rapidly decomposing mulberry leaves and slowly decomposing pine needles. Pine needles will also be compared for $Co^{60}$ to complete the list of isotopes of subproject 2.021. Cobalt-60 and $Ru^{106}$ uptake will also be estimated for southern red oak and ash

leaves and $Sr^{85}$-$Ca^{45}$ dogwood leaves for comparison with subproject 2.011 and a few 1 square decimeter bags of unconfined leaves of these species previously placed on the forest floor without litterboxes. Subproject 2.020 indicated that integral scintillation counting of soil and litter with a 0 Mev pulse height baseline was sufficient to get thousands of counts per minute even from $Sr^{90}$-$Y^{90}$ (probably including strong betas from the latter in addition to bremsstrahlung). However, experience with such counting should indicate whether differences in self-absorption with varying moisture content of soils precludes more widespread use of this method for unprocessed field samples.

Reports:

None

Subproject 2.024 -- Litterfall and fallout for evergreen and deciduous forests.

Date initiated: May 1960 Date to be completed: December 1962

Research personnel: J. S. Olson, W. C. Cate, H. D. Waller, Leo Thompson, and J. C. Ritchie

Objectives: To estimate litterfall as a measure of an important fraction of forest production(energy fixation by photosynthesis), and as the major income of organic matter to the forest floor litter and humus layers that are being studied in several subprojects (2.010, 2.011, and 2.017, 2.020 through 2.022). To use this material as indicators of radioactive fallout rates for the growing season preceding leaf fall or for total cumulation on the ground.

Justification: Estimates of litter production complement the other experiments measuring litter decay as steps toward quantitative interpretation of the forest floor organic matter, in terms of models for gain and loss of material and energy (subprojects 2.003, 2.004, and 2.026). The material so collected accumulates radioactive nuclides, from rainfall and the atmosphere. It is desirable to get records of levels attained by a relatively simple suite of long-lived radionuclides after shorter-lived radioactivity has decayed during the moratorium on atmospheric testing. If atmospheric testing resumes, the promptness of accumulation of different specific isotopes on different litter fractions will be pertinent to interpreting movement of these materials through forest systems.

Procedures: The basic routine of litter collection will use 6 to 10 bushel baskets, perched on wooden stakes so that there is opportunity for air circulation beneath the baskets to dry them without contact with moisture in the ground. Collections will be made at approximately monthly intervals, with occasional extra collections during October or November for sharper definition during periods of peak deciduous litter fall. Samples are tc be oven dried as promptly as feasible. Counts of gamma radioactivity are made in bulk containers around scintillation crystals connected with a multichannel analyzer.

Reports:

None

Subproject 2.025 -- Digital data programming for ecological samples.

Date initiated: June 1960 Date completed: February 1962

Research personnel: J. S. Olson and D. J. Nelson

Objectives: To provide forms for recording of data which can be used for IBM punchcard input for storage and rudimentary manipulation. To utilize existing computer programs for statistical and mathematical processing of data. To prepare programs particularly suited for our more voluminous data, such as the output of the automatic sample changer (single channel or multichannel analysis), or the combined weight and radioactivity data of our litter studies (especially subprojects 2.020 through 2.023, 2.031 and 2.032.)

Justification: As the ecology program develops gradually from small experiments to larger ones, the time required for hand manipulation exceeds the time needed for arranging for machine processing. Some problems like the estimation of individual radionuclides from mixed gamma spectra are particularly tedius and uncertain by graphical and hand arithmetic methods, and experience is needed with various ecological samples of high and low activity to judge the extent to which automatic processing can replace these methods for these kinds of samples. There is a tendency for data in larger experiments or surveys to be useful in more than one particular kind of analysis, and there is an advantage in having basic forms sufficiently flexible and interchangeable so that different analyses of the same data are

feasible. In particular, punchcard storage of the unit data has advantages for subdividing the data into groups for separate, more homogeneous analyses, and for recombining data accumulated over different time periods into larger summary analyses.

Procedures: Existing general purpose programs determine the input form for certain data. The nonlinear or multiple regression "poly-and-plane" least squares regression program and general-purpose (AA ANVA) analysis of variance forms use SHARE programming data forms with numbers (including decimal) in IBM columns 12-72 (commas but no space between numbers). "Simplified Program Translator" (SFT) forms for small, infrequently used arithmetical problems use four fixed fields per card, each consisting of 2 digits for address (e.g., col. 1,2) and 14 numbers (e.g., col. 3-16). The order in which data are taken conveniently in the field (e.g., in our TVA-ORNL forest survey) determines the initial format of some punchcards, but machine reproduction of the cards rearranges data in the form needed for analysis (in this case, an existing IBM 704 program of TVA for forest mensuration).

Identification of the investigator and his program, the date (year and day within year), and standardized sample indexing systems will be kept common for many ecology samples. Certain columns for counting time, counts, channel number (if needed) will be standardized for multichannel analysis of

Isotope Data Analysis (IDA) and single channel counting (as with the litter Weight and Isotope Loss data (WIL). Three groups of columns will be kept for weight data (fresh, air dry, oven dry, gross, net). The Isotope Data Analysis (IDA) provides for Friden punchtape output to IBM cards for individual channel times and counts; from these cards and a single sample description card per sample to cards suitable for input on magnetic tape for the IBM 7090. Matrix inversion and matrix multiplication programs provide for estimation of picocuries per gram of radioactivity of sample, with printed and punchcard output. Evaluation of statistical errors will proceed along with evaluation of other errors inherent in sample handling and instrumentation. The Weight and Isotope Loss program (WIL) will be used particularly for the larger litterbag and litterbox experiments, but can readily be adapted to a wide variety of experiments involving single channel counts of radioactivity (for which an efficiency factor can be included in input) and substraction of weights.

Reports:

None

Subproject 2.026 -- Analog computer development of ECOS--"ecological computer, organizer and simulator."

Date initiated: January 1961 Date to be completed: September 1962

Research personnel: J. S. Olson, O. W. Burke, and E. R. Mann

Objectives: To use analog computers for integration of differential

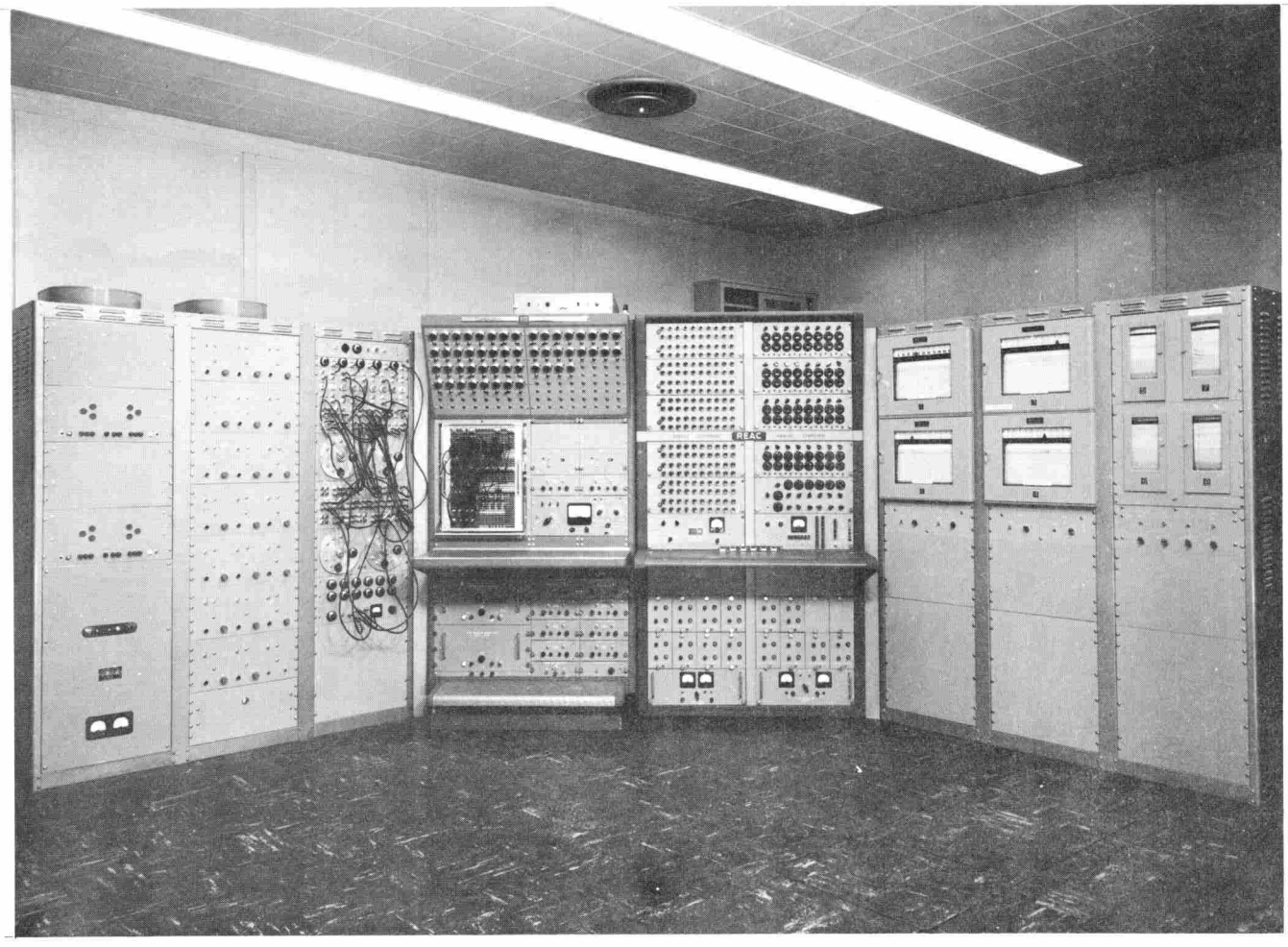


Fig. 4. ECOS-A, an analog "Ecological Computer, Organizer, and Simulator" based on the Reactor Controls Analog Computer Facility (RCAF), Oak Ridge National Laboratory shown above (Subproject 2.026).

equations in which there may be several positive and negative rates of change affecting any one variable--particularly if the rates of increase or loss depend on the quantities being integrated (nonlinear differential equations). To develop models suggested by subprojects 2.003 and 2.004 which will help to organize miscellaneous field data into a form suitable for eventual computer treatment, or to focus attention on ways in which data collection may need to be modified or extended to achieve this purpose. To simulate an ecosystem model by the electronic circuits of the computer itself provides an encouragement to consider the behavior of an ecosystem as a whole, and its response to changes in given parameters of the model, without requiring continual revision of the mathematical equations and solutions which formalize this situation.

Justification: The need for quantitative predictions of the changes expected from radioactive contamination which has not yet occurred (and which we hope may never occur) requires the use of mathematical models. The larger number of integrators and the servomultipliers of the ORNL Analog Computer facility are useful for complex ecological situations. The range of values and combinations for certain parameters which are appropriate within real situations need exploration so that the use of computers for special problems which come along later will not be discouraged by the need for many additional machine runs on facets of the problem which are then of peripheral interest.

Procedures: The ORNL Analog Computer Facility provides the basic equipment for the ecological computer here called "ECOS." It includes three consoles which are controlled by removable panels on which the wiring is done, so that a previously setup circuit can be plugged in ready for operation after potentiometer settings and other minor adjustments are made. Normal operations involve use of at least two consoles of the computer for a week at a time, but it may be feasible to handle special problems on a single console during short periods, even when other problems are simultaneously using other parts of the computer. Desk-top computers are likely to be instructive for many small problems. One console is used primarily for simulating changes in the mass or size (or carbon or energy content) per square meter of the major compartments of the system, and a second one primarily for changes in radioactivity (expressed in terms of units per gram of material in the case of plants and animals, and in units per square meter of ground area in the case of soil and litter activities). On one console certain auxiliary circuits are used for creating periodic oscillations (seasonal or possibly daily cycles) and certain limiting conditions needed for approximating realistic circumstances (e.g., a limit imposed by competition for space).

Reports:

Olson, J.S. 1962. Analog computer models for movement of nuclides through ecosystems. Radioecology. Proc. of First Nat. Symp. on Radioecology. Reinhold Publ. Corp., N. Y., N. Y. (In press).

Olson, J. S. 1961. Compartment models and analog computer simulation. In Health Physics Division Annual Progress Report for period ending July 31, 1961, ORNL-3189.

Olson, J. S. 1962. Analog computer model for biomass and cesium-137 in an herbaceous system. In Health Physics Division Annual Progress Report for period ending July 31, 1962, ORNL-3347.

Subproject 2.027 -- Effect of natural ecological factors on movement of $Cs^{134}$ in soils derived from Conasauga shale.

Date initiated: May 1961 Date to be completed: September 1963

Research personnel: Hubert Waller, W. C. Cate, D. A. Crossley, Jr., and J. S. Olson

Objectives: To determine the relative effects of soil type, forest type, and the presence or absence of forest litter, insects and ground cover plants on the movement of $Cs^{134}$ within the soil profile and ground cover plants.

Justification: In general, the movement of $Cs^{134}$ radionuclide within a soil system may be correlated closely with the mineralogy, tending to be retarded due to cesium fixation by high illite content. However, much of the work on fixation has been done by using soil columns in a laboratory where many of the natural physical and biological properties of a soil may be disrupted or destroyed. Recent work (subproject 2.009) has indicated the movement and availability of this radionuclide to be more than might have been expected from the laboratory experiments. This suggests that mobility of $Cs^{134}$ may not be explained entirely

by soil chemistry or soil mineralogy, but that the physical properties of soil and natural ecological factors may alter the effect of soil chemistry and mineralogy. Four areas of the Conasauga shale formation have been chosen for this experiment. This formation has an unusually high illitic clay content and has been selected for waste disposal operations by the Oak Ridge National Laboratory. Because of the mineralogy within this geological formation, $Cs^{134}$ should be rendered immobile and relatively unavailable for recycling into the ecological system, unless the effectiveness of clay mineralogy on fixation of cesium is somewhat altered by the other naturally occurring factors.

Procedures: The basic experiment consists of four separate areas which were selected to give the natural effect of soil types and forest type on the movement of this radionuclide. Contrasting coniferous vs. hardwood forest types on wet vs. dry soil types are represented within these areas. The presence or absence of litter layer, insects and undercover plants are imposed variables that are repeated on each of the above four areas. Imposed variables of presence or absence of litter, insects and undercover plants are thus represented by a factorial experiment arranged as randomized block design, replicated in the four areas. These eight treatments can be considered as "within plot" treatments of a split-plot design and estimates of their effects would be tested for significance by comparison

with the interaction terms for within-area treatments. The four areas being compared could be considered as providing "between plot" comparisons for which differences are to be estimated. The four areas chosen for this experiment are located within the outcrop area of Conasauga shale formation. A residual soil that is relatively shallow to bed rock (2-3 ft.) and low in available moisture supplying capacity is contrasted with an alluvial soil that is deeper to bed rock and has higher moisture supplying capacity. Eight plots, one square meter, are to be sprayed with $Cs^{134}$ at each of the four experimental areas, after treatments are given to the plots within each experimental area so that the relative effect of litter, ground cover plants, and insects may be measured independently. Removal of ground cover plants will be accomplished by cutting the stems at ground level and sprouts will be clipped later. Insecticides will be used to eradicate arthropods. A mixture of 74 per cent chlorodane and 17.6 per cent dieldrin insect spray will be diluted and sprayed over plots using a 2 gallon pressure spray tank. The insecticide will be applied one to two days before inoculating with the radionuclide. Two hundred sixty-one microcuries of $Cs^{134}$ will be applied in a 50 ml. water solution per one square meter plot using a pressure tank with jet spray attachment. Woody ground cover plants of the oak species, *Quercus alba* and *Quercus falcata*, will be used as an indicator for $Cs^{134}$ availability. To minimize disturbance

of limited plant cover, leaf discs will be sampled by cork borer during the growing season.

Sampling -- plant leaf materials will be sampled within one or two days after inoculation at one plot and resampled again at all sites three days after inoculation. A complete sampling of soils (in 1/4 or 1/2 inch intervals) and plants at all sites will be made five weeks after inoculation. Soils will be sampled two or three times per year at depths judged by movement of the radionuclide as time progresses. Vegetation will be sampled in the second growing season by taking leaf discs in Spring and Summer, 1962, and by complete harvesting in September. Final sampling of selected plots will be made on undisturbed areas. From this sampling, we will attempt to detect and interpret trends and irregularities of movement related to roots, animal burrows, and physical properties of the soil. All samples will be counted for gamma activity of the .59 and .79 Mev photopeaks of $Cs^{134}$ by differential counting (.50 to .90 Mev).

Reports:

None

Subproject 2.028 -- Biomass of larger arthropods in forest litter.

Date initiated: June 1961 Date completed: September 1961

Research personnel: D. A. Crossley, Jr. and J. G. Stoffolano

Objectives: To estimate the biomass (weight per unit area) of larger arthropods (exclusive of mites and collembola) in leaf litter in oak and pine forests.

Justification: This subproject is ancillary to investigations on the influence which arthropods may have on rates of mineral cycling, and hence rates of movement of radionuclides in forests. The biomass of arthropods in leaf litter is one of several pieces of information which will be necessary for estimating the amounts of radionuclides which may be expected to pass through the soil arthropod compartment of a forest ecosystem.

Procedures: These large samples (0.1 square meter) will be taken weekly in pine and oak stands for 8 consecutive weeks. Of the three replicates, one will be used for identification of arthropods and the other two for estimates of arthropod biomass. Samples will be extracted on Tullgren funnels and arthropods will be collected in 70 per cent alcohol. Oven-dry weights will be obtained. Statistical analysis will involve confidence limits for estimates of biomass, comparisons of biomasses in the two forest types, and changes with time during the 8-week period.

Reports:

None

Subproject 2.029 -- Gross effects of soil arthropods on leaf litter breakdown and release of radionuclides.

Date initiated: November 1961 Date to be completed: November 1962

Research personnel: D. A. Crossley, Jr., M. Witkamp, C. Corley, and G. J. Dodson

Objectives: To measure the rates of leaf litter breakdown in plots from which soil arthropods have been excluded by the use of insecticides, and to compare these rates with those from control

plots which contain a normal arthropod fauna. To measure the rates of release of $Cs^{134}$ from leaf litter in the arthropod-free plots, and make comparisons with controls.

Justification: Arthropods may hasten the breakdown of fallen leaf litter by feeding upon it, by pulverizing it, and by mixing it with the underlying soil. Recent work in Russia (Kurcheva 1960) suggests that treatment of experimental plots with insecticides may provide a means for quantifying the effect of arthropods on leaf litter breakdown. The importance of soil animals in the forest ecosystem is still an unresolved problem but experiments such as these should provide estimates of at least the magnitude of their influence upon mineral cycling. In addition, this experiment is preliminary to others concerning the effects of irradiation on the soil community, in which doses of radiation rather than insecticides will be used.

Procedures: Preliminary tests with dieldrin, chlordane, lindane, paradichlorobenzene (PBD), and naphthalene showed that the latter two insecticides reduced soil arthropod populations by two orders of magnitude and the others by one order of magnitude. Naphthalene is more residual than is PBD and should have less effect on the microflora. Twelve 1 square meter experimental plots in an oak stand (4 levels of naphthalene x 3 replicates) will be used. Initial insecticide applications will be 100, 35, 10, and 0 grams per square meter. Five litter bags containing oak leaves tagged with $Cs^{134}$ will be placed in each plot. Each week 1 litter bag from each plot (12 bags) will be collected,

weighed, counted for contained radioactivity, arthropods extracted, and the bags then returned to the plots. Biweekly measurements of respiration and microfloral counts will be made to determine possible influences of naphthalene on bacteria and fungi. For statistical analysis, each bag (60 in all) will be considered an observation and least squares regressions will be used to express weight loss and radionuclide loss from each bag. IBM programs will be utilized to obtain the 60 regression coefficients for weight loss and for radionuclide loss. Comparisons of the slopes by analysis of variance will be used to express differences between levels of insecticides. Measurements of organisms are secondary in this experiment, and are made only to discover the magnitude of response to the insecticide. Values will be expressed in percentages of control and square-root transformations of numbers will be used where appropriate.

Reports:

None

Subproject 2.030 -- Radionuclide tracer estimates of feeding rates for a forest floor millipede (Diplopoda) (*Dixidesmus erasus* Loomis)).

Date initiated: November 1961 Date to be completed: May 1963

Research personnel: D. A. Crossley, Jr., and C. L. Corley

Objectives: To estimate the feeding rates of forest floor millipedes, using radioactive tracer techniques. To evaluate the importance of these arthropods in the turnover of radionuclides in forest ecosystems.

Justification: Millipedes (Dixidesmus erasus (Loomis)) are dominant arthropods in the forest floors of mixed mesophytic forests (predominantly Liriodendron) which occur in limestone sinks in the Oak Ridge area. Numbers of millipedes may exceed 100 per square meter. These animals undoubtedly influence the rapid rate of breakdown of leaf litter in the limestone sink forest stands, and an evaluation of their importance in breakdown of litter and release of minerals will contribute substantially to our knowledge of the position of arthropods in forest floor ecosystems. The isotope-equilibration method for estimating feeding rates has so far been used only in contaminated areas, and this subproject offers an opportunity to develop such techniques for use in uncontaminated areas.

Procedures: For measurements of radionuclide accumulation under field conditions, small enclosures (one-quarter of a square meter) will be erected in field areas. Leaf litter will be removed and replaced by contaminated leaves containing $Cs^{134}$, $Sr^{85}$, $Co^{60}$, $Fe^{59}$, or $Zn^{65}$. Twenty-five millipedes will be placed in each enclosure. Periodically the millipedes will be collected, counted for radioactivity, and returned to the enclosure. Changes in radionuclide concentration in food (due to leaching and/or breakdown of litter) will be estimated by periodic measurements on litter bags placed nearby. Elimination rates using animals dosed in the laboratory also will be measured for these isotopes under field conditions, by the use of similar enclosures stocked with uncontaminated leaf litter.

These measurements, in combination with estimates of the equilibrium concentration in the millipedes, and the changes in food concentration of radionuclides (if any), should permit a calculation on the rate of feeding of the millipedes. Where the concentration of isotope in food remains constant, the model used will be:

$$r = kQ_e$$

where $\underline{r}$ is the rate of feeding, $\underline{k}$ is the elimination constant, and $Q_e$ is the equilibrium concentration in the millipedes. Where the concentration of isotope in food decreases, an exponential rate of loss will be assumed and the model used will be:

$$Q_t = \frac{r}{k_b - k_1} \quad (e^{-k_1 t} - e^{-k_b t})$$

where $Q_t$ is the concentration in the millipedes at time $\underline{t}$, $\underline{k_b}$ is the elimination constant, and $\underline{k_1}$ is the leaching constant for isotopes from leaf litter. Laboratory experiments will duplicate the field procedure, since interpretation of the field results will be greatly aided by experimentation under constant conditions and with more frequent measurements than can be obtained under field conditions. In addition, laboratory experiments will be added to answer specific questions posed by field conditions, such as the influence of molting upon elimination rates, accumulation of radionuclides from soil or from water, and the influence of temperature upon feeding and elimination rates. Since millipedes are active over a broad range of temperatures, laboratory experiments may be conducted with smaller

experimental units using fewer millipedes measured at more frequent intervals, at ambient or controlled laboratory temperatures higher than those of the microenvironment in field conditions. In addition, weekly measurements will be made of millipede biomass, density, and growth rate, as well as phenomena concerned with reproduction and dispersal. Local distribution will be examined in one sink-hole, and local migrations will be estimated from the movement of millipedes tagged with radionuclides. These population measurements, combined with estimates of feeding rates, should permit an evaluation of the importance of these animals in leaf litter breakdown and mineral cycling in these forests.

Reports:

None

Subproject 2.031 -- Release of $Fe^{59}$ and other radionuclides from tulip poplar and white oak leaves.

Date initiated: December 1961 Date to be completed: December 1963

Research personnel: J. S. Olson, H. D. Waller, and W. C. Cate

Objectives: To compare release rates of $Fe^{59}$ from tagged leaf litter with rates for several other radionuclides, to determine whether they are about the same as the rate for litter decay (as previous experiments indicate for $Sr^{85}$ and $Co^{60}$) or more rapidly (as for $Cs^{134}$). To compare these rates with the rates of litter fragmentation and decay for rapidly decomposing leaves of the important mesophytic species tulip poplar (*Liriodendron tulipifera*),

as well as the previously studied white oak (Quercus alba). To estimate the relative importance of contrasting general geologic and soil mineralogy conditions (Melton Valley-Conasauga shale soils vs. Melton Hill sinkhole soils derived from Knox dolomite) and of local forest cover and topographic-soil moisture contrasts within each of these areas. To estimate differences in retention of $Fe^{59}$, $Sr^{86}$-$Ca^{45}$ mixture, and $Cs^{134}$ in surface soil layers of contrasting mineralogy and humus conditions of the four sites.

Justification: Radionuclides that were previously incorporated within natural plant materials, should provide tracers for the natural cycles of important classes of materials. The $Fe^{59}$ represents a trace element of interest for enzyme nutrition and of possible secondary interest as a radioactive contaminant. The $Sr^{85}$ is the only gamma emitter conveniently indicating the movement of $Sr^{90}$, a contaminant of major interest, and can be compared with movement of $Ca^{45}$, a major nutrient element. The $Cs^{134}$ represents a class of rapidly moving soluble elements that would also include the major nutrient, K. The addition of tulip poplar leaves and tulip poplar forest cover (in addition to oak leaves and oak cover), and extension of studies into the Knox dolomite soils near the Health Physics Research Reactor site in addition to the Melton Valley stations included in most previous experiments, constitute a distinct step toward the broadening of applicability of our studies of isotope mobility over a wider range of forest conditions.

Procedures: Leaves tagged with the above mentioned isotopes (and

additional Liriodendron leaves tagged with $Co^{60}$ for an extra comparison) were collected in early October, 1961, shortly before they normally would fall. Some will be folded into 1 square decimeter bags before drying and becoming brittle, while others will be tied to twisted nylon cord, sealed by brief exposure to an alcohol lamp flame. Weight loss and radionuclide loss for leaves on the cord may be expected to exceed losses for bagged leaves because of the gross fragmentation of leaves by animal activity. The extent of this discrepancy in weight and isotope loss between the two methods and close observation of leaves will indicate the importance of this or other modes of fragmentation on a comparative basis, between the sites. At the two main forests (tulip tree-sinkhole; white oak station), four of the bags of seven species-isotope combinations (28 bags in each of 2 stands) will be placed on previously counted slabs of soil contained in plastic boxes with holes and fiberglass screening over the bottom (subprojects 2.020 and 2.022). These bags and their underlying boxes will be counted at monthly intervals to provide estimates of uptake and loss rates for surface layers of soil (which can be compared with redistribution of isotopes in plots with unconfined litter, in subproject 2.032). The litter bags from these boxes will be air-dried and weighed only quarterly and counted by comparison with "control" boxes with soil and bags of untagged leaves.

Other bags are scheduled for collection without previous disturbance over a 2 year period (1, 2, 3, 6, 12, 18, and 24 months for $Sr^{85}$-$Ca^{45}$, $Cs^{134}$, and $Co^{60}$). Because of the 45 day half-life for $Fe^{59}$, its leaves are scheduled only for 1, 2, 3, 6, 9, and 12 month collections. In the minor stations (oak on Knox dolomite; maple-tulip lowland in Melton Valley), comparative collections are scheduled only for 6 and 12 months for $Fe^{59}$, and 6, 12, 18, and 24 months for the other radionuclides. The 6 and 12 month collections will thus provide a basis for an analysis of variance of a factorial split-plot design involving all combinations of 4 stands (two leaf types, 3 radionuclides (plus $Co^{60}$ for tulip only). Additional times of collection will be used for closer estimation of loss as a function of time. This is of particular interest for the two main stations where unconfined litter is being used in other experiments. The strings of litter are scheduled for duplicate collections for each leaf-radionuclide combination (6 strings in all for oak, 8 strings for tulip poplar) at each of the 7 collections at the main stations, to estimate the mean losses and variance associated with this method.

Reports:

None

Subproject 2.032 -- Recycling of radionuclides released from oak and tulip poplar litter.

Date initiated: December 1961 Date to be completed: December 1963

Research personnel: J. S. Olson, H. D. Waller, and W. C. Cate

Objectives: To estimate the promptness and extent of recycling of several radionuclides (including $Sr^{85}$, $Ca^{45}$, $Co^{60}$, $Fe^{59}$, and $Zn^{65}$ in addition to $Cs^{134}$) from tagged litter to undercover vegetation and small trees. To diminish border effects, by scaling up litter experiments (which have previously used units of $10^{-2}$ or $10^{-1}$ square meters) to a small "pilot operation" using plots of 1 square meter or larger. To compare $Cs^{134}$, $Sr^{85}$ + $Ca^{45}$, along with $Co^{60}$, $Fe^{59}$, and $Zn^{65}$ in tulip poplar (Liriondendron tulipifera), as well as white oak (Quercus alba).

Justification: Liriodendron was added to the list of tagged species because of its importance as a major dominant in mesophytic forests, and as a representative of species with rapidly decomposing leaves. In our forests which are dominated by Liriodendron, litter decay has been notably more rapid than elsewhere, and the unconfined, uncaged leaves should supplement the millipede experiment with caged litter (subproject 2.030) as a means of indicating how this rapid breakdown proceeds without the interference of litter bags. The reason for inclusion of isotopes intimately incorporated in litter is to simulate the pathways which the corresponding elements would follow in normal nutrient cycling. The only previous experiment operating on a scale of 1 square meter involved the liquid application of a single isotope ($Cs^{134}$; subproject 2.027). Its results show prompt uptake of small fractions of $Cs^{134}$; the hypothesis of the present experiment is the likelihood of less prompt but ultimately more extensive movement of Sr and Ca.

Procedures: The basic plot units will be 1 or 2 meters squared totalling four square meters depending on quantities of litter available. Sampling results will be considered separately for a buffer strip having half the total plot area, to minimize border effects. Associated litterbags and litter on string (subproject 2.031) will be used for indicating details of breakdown during the first year. Herbs will be mapped and sampled at appropriate seasons (probably in June for spring species, and September for summer species). Seedlings will be mapped and leaves sampled before dropping. One-half of each plot will be harvested, layer by layer, in late summer of the first- and second years to determine vertical redistribution through the soil profile and into root systems. Calcium-134, $Co^{60}$, $Sr^{85}$, $Zn^{65}$, and $Fe^{59}$ will be counted by gamma spectrometry while $Ca^{45}$ will be determined by digestion and liquid scintillation counting, probably with oxalate precipitation if needed for purification and concentration. The experiment will be carried out in both the white oak station on Sequoia soil derived from Conasauga shale, and in tulip poplar forest in the sinkhole station, on soil derived from Knox dolomite.

Reports:

None

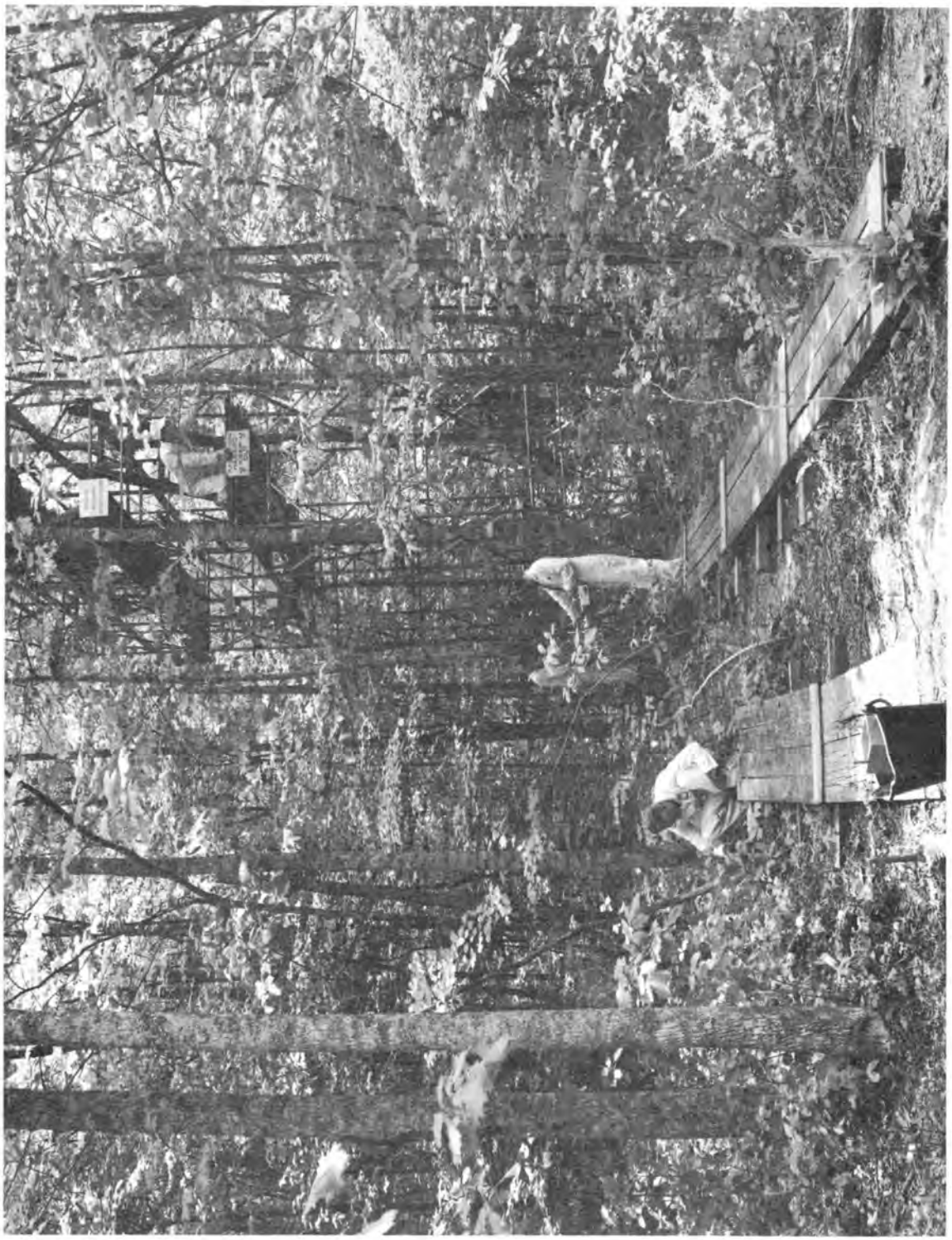

Fig. 5. Sinkhole forest dominated by tulip poplar (Liriodendron tulipifera) which has been tagged with 0.5 curie of $Cs^{137}$, in a long term experiment on the behavior of this radionuclide in the different components of a forest. Four story scaffold tower in right rear is used for sampling leaves and insects in the canopy layer (Subproject 2.033).

Subproject 2.033 -- Circulation of cesium and other cations in vegetation of a tagged tulip poplar forest.

Date initiated: May 1962 Date to be completed: June 1967 or later

Research personnel: J. S. Olson, W. C. Cate, and H. D. Waller

Objectives: To use the movement of long-lived $Cs^{137}$ (and to a limited extent, short-lived $K^{42}$) to estimate rate parameters for transfer of alkali metal elements from boles to foliage of a whole stand of tulip poplar (*Liriodendron tulipifera*) and into understory trees and lesser vegetation over a period of several years.

Justification: Tracer experiments under field conditions on whole ecosystems are needed for developing and evaluating theoretical ecological models for the circulation of chemical elements. The present subproject extends earlier tagging of smaller trees (subprojects 2.008, 2.009), decomposing litter (2.020-2.022; 2.030-2.032), and soil (2.027). Previous work on white oak (*Quercus alba*) showed that cesium and presumably potassium circulate rapidly through short-term cycles involving rain leaching from foliage, annual cycles involving foliage maturation, abscission and decay, and longer-term cycles involving tree mortality and bole and root decay. Use of a long-lived tracer in an usually productive and floristically and nutritionally rich forest of tulip poplar should provide a suitable site for several concurrent and subsequent studies of the development, maintenance, and ecological interactions of a deciduous forest.

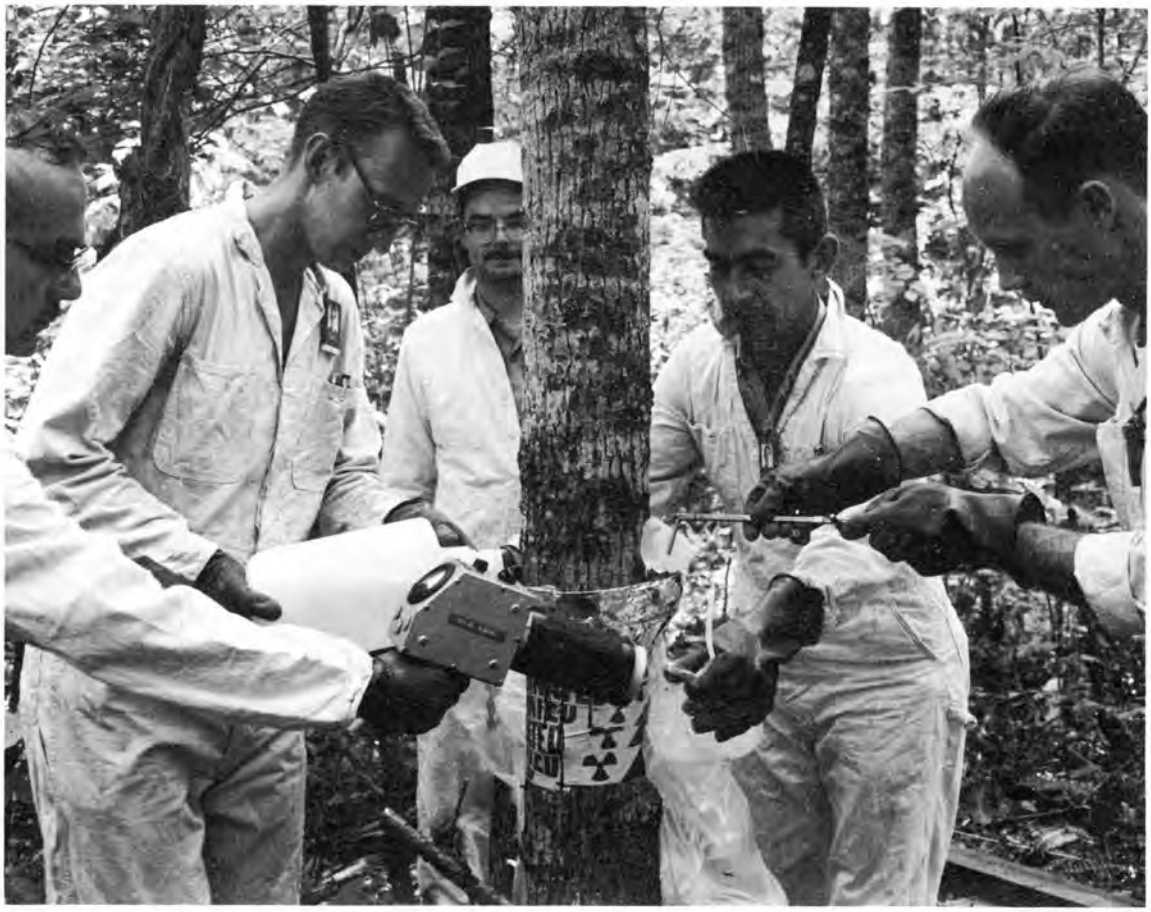

Fig. 6. Inoculating a tulip poplar (Liriodendron tulipifera) with $Cs^{137}$. Man on right is pouring radionuclide solution into a previously prepared trough from which isotope moves into the trunk through slits chiseled through bark. Technician on left is adding water to facilitate transfer of radionuclide from the trough. Man in left foreground is monitoring and timing operation (Subproject 2.033).

Procedures: A nearly flat-bottomed sinkhole area, to be separated from the public by chain-link fence and guarded gates, was selected for this initial long-lived contamination experiment. The area has no external surface drainage, and has a deep fill of colluvial (Emory) soils which should adsorb cesium before it reaches groundwater. A 20 x 25 meter plot was mapped and surface vegetation was sampled in nested plots in each 5 x 5 meter square. A tower of scaffolding was constructed to provide a 1.5 x 7 meter working area at several levels up to 11 meters above the ground, which is treetop level for saplings and is within pruning distance of most of the crown of selected dominant and codominant trees.

Cesium-137 (and potassium-42 in a few cases) will be introduced into tree boles through horizontal chisel slits made under water in troughs constructed of perma-gum sealing compound and aluminum foil. Translocation from lower bole to crown will be followed by nearly simultaneous measurements with several survey meters. Tree leaves from marked branches from four poplars (3.6, 5.7, 6.1, and 12.5" DBH) will be sampled weekly until foliage radioactivity appears to have passed its maximum, and monthly thereafter. Composite samples from as many trees as possible in the stand will be made and counted near the time of maximum activity, and again in late September, and will be repeated at approximately these times each year. Undercover vegetation will be clipped from 0.1 $m^2$ areas in each 5 x 5 m

Fig. 7. Cages containing caterpillars (geometrids) tagged with $Cs^{137}$ are being positioned on untagged tulip poplar (Liriodendron tulipifera) trees, to measure rates of radionuclide elimination under field conditions (Subproject 2.033).

area at the time of midsummer crown sampling, and from a few selected stations at monthly intervals thereafter, including evergreen Christmas fern throughout winter months. Decomposing litter will be collected in plastic bags from these 0.1 $m^2$ areas, counted, subsampled for moisture and chemistry, and the remainder returned. Freshly fallen litter will be collected twice monthly from baskets lined with cloth, and an extra collection will be made from nylon netting stretched on the ground at the time of maximum leaf fall.

## PROJECT III: RESEARCH IN AREAS CONTAMINATED BY RADIOACTIVE WASTES

Over-all Objectives:

To obtain information on the behavior of specific fission products in a contaminated, relatively discrete ecosystem.

To use the contamination itself to obtain data on basic ecologic processes.

To characterize ecological phenomena as a basis for evaluating possible effects of chronic ionizing radiation.

To determine the effects of chronic, low-level radiation on organisms living in contaminated habitats.

Justification:

White Oak Lake bed and other contaminated sites in the Oak Ridge National Laboratory area are among the few such sites in the country. They present an opportunity to obtain ecological information in relation to radioactivity under field conditions that cannot be found elsewhere at the present time. Such information will aid in predicting movement of radionuclides in areas which may become contaminated in the future.

White Oak Lake bed, as a result of its radioactive contamination, has radiation fields ranging from 10 to several hundred mrad/hr. These dose rates, being at a level of presumed biological effect, offer an excellent opportunity to study the effects of chronic ionizing radiation on natural populations, especially vertebrates. Vertebrate populations are well established on the lake bed and several species are suitable for study.

Fig. 8. White Oak Creek drainage basin and White Oak Lake bed. View is toward the northeast. In left foreground is the standing pool of the lake backed up by White Oak Dam. In the upper right corner is Oak Ridge National Laboratory. White Oak Creek and the adjacent lake bed is located in the right center of the photo. Radioactive waste pits are just above the bed between the Laboratory and the Creek (Project III).

Fig. 9. Ecology study area of lower White Oak Lake bed. View is toward the northeast. Permanent 10 x 10 meter grid system covers approximately eleven acres of this area (Project III).

Subproject 3.001 - Uptake of fission products by trees growing in vicinity of radioactive waste pits.

Date initiated: April 1957 Date completed: continuing

Research personnel: S. I. Auerbach, D. A. Crossley, Jr., R. M. Anderson, C. Krauth, and J. C. Ritchie

Objectives: To determine the concentrations of $Ru^{106}$ and other radionuclides in tree components and ground litter, to ascertain if $Ru^{106}$ and other fission products were being accumulated above soil levels, and to compare the geographical distribution of radionuclides in trees and ground litter with that obtained from engineering monitor well data.

Justification: Information on the ability of trees to accumulate particular radionuclides under given soil and hydrologic conditions is needed for the design of more detailed experiments on the cycling of radionuclides in forest ecosystems. Likewise these studies will provide information on the usefulness of trees and other vegetation as biological indicators of contamination. Since the soils of the waste pit area have over 120,000 curies of long-lived $Cs^{137}$ (t 1/2 = 33 yrs.), this project provided baseline information against which future studies could be checked as a basis for biological monitoring of changes in the fixation of $Cs^{137}$ by soil.

Procedures: Leaves, bark, and twig samples from various heights up to 24 feet were collected from 15 species of trees mainly located at the margin of radionuclide distribution in soil as

Fig. 10. Collecting foliage samples from an even aged shortleaf pine (Pinus echinata) stand growing adjacent to an underground radioactive waste seepage trench (Subproject 3.001).

indicated by monitor well data. Ground litter samples were also taken underneath trees which were significantly contaminated. Samples were taken once a month in April, May, June, and July in order to obtain data on accumulation in the prevernal and vernal periods. Ruthenium-106 was determined by both radiochemical and spectrometric techniques. Other radionuclides were determined by gamma spectrometry.

Reports:

Auerbach, S. I. 1957. Uptake of fission product seepage by vegetation. In "Ecological Research," Health Physics Division Annual Progress Report for period ending July 31, 1957, ORNL-2384.

Auerbach, S. I. 1958. Waste pit studies. In Health Physics Division Annual Progress Report for period ending July 31, 1958, ORNL-2590.

Auerbach, S. I. and J. S. Olson. 1962. Biological and environmental behavior of ruthenium and rhodium. Radioecology. Proc. of First Nat. Symp. on Radioecology. Reinhold Publ. Corp., N. Y., N. Y. (In press).

Subproject 3.002 -- Uptake of waste $Sr^{90}$ and $Cs^{137}$ by soil and vegetation.

Date initiated: June 1956 Date completed: September 1956

Research personnel: E. R. Graham and S. I. Auerbach

Objectives: To obtain a chemical characterization of the White Oak Lake bed soils, to obtain preliminary data on the kinds, amounts, and distribution of radionuclides in these soils, and to determine the concentrations of $Sr^{90}$ and $Cs^{137}$ in the dominant pioneer vegetation (*Polygonum* spp).

Justification: The draining of White Oak Lake the previous fall made the area available for a terrestrial study site. Characterization, particularly of the soil, was essential for the planning of future studies. Likewise determination of concentrations of fission products in the pioneer vegetation was an essential baseline for future studies.

Procedures: Soil and plant samples were taken from various parts of the lake. Site selection was based on air dose rates determined by ionization chambers. Generally, samples were collected from areas with high, intermediate, and low dose rates. Soil chemical tests were made using a variety of published techniques (Piper, Graham, Schofield and Taylor, Bray, Woodruff, and Steinbergs). Plant samples likewise were analyzed for their chemical content by standard techniques. Strontium-90 and $Cs^{137}$ were determined radiochemically using methods outlined by Kahn and Farabee.

Reports:

Graham, E. R. 1958. Uptake of waste $Sr^{90}$ and $Cs^{137}$ by soil and vegetation. Soil Science 86(2): 91-97.

Auerbach, S. I. 1957. White Oak Lake bed studies. In Health Physics Division Annual Progress Report for period ending July 31, 1957, ORNL-2384.

Subproject 3.003 - Accumulation of $Sr^{90}$, $Cs^{137}$, and mineral elements by corn (*Zea mays*).

Date initiated: May 1957 Date completed: November 1957

Research personnel: S. I. Auerbach, D. A. Crossley, Jr., and R. M. Anderson

Objectives: To obtain baseline information on the accumulation of minerals by vegetation in this ecosystem by using a well-known crop species and to obtain more detailed information on soil-plant relationships than could be obtained by sampling the native vegetation.

Justification: Information on mineral accumulation by plants and subsequent cycling is necessary for prediction of the fate of radionuclides which may be released into various terrestrial environments. Data are needed on soil-plant relations in many field conditions.

Procedures: The corn plot (50 ft. x 50 ft.) was subdivided into 4 rows and 4 columns 12 ft. wide. The resulting 16 subplots were planted with 4 varieties of corn in a latin square design. Unequal growth of the corn varieties prevented sampling according to the design. Soil samples were taken prior to planting from each of the 144 hillocks for seed planting. Minerals were extracted by standard techniques (Piper; AOAC handbook). Inorganic cations were determined by flame photometer. Cesium-137 and $Co^{60}$ by gamma spectrometer and $Sr^{90}$ by the method of Kahn and Goldin. Plant materials were analyzed for chemical content by standard methods. Cesium-137 content was determined by gamma spectrometry. Strontium-90 was determined by the method of Farabee. Analyses of variance and other statistical techniques were used to analyze the data.

Reports:

Auerbach, S. I. and D. A. Crossley, Jr. 1959. Strontium-90 and cesium-137 uptake by vegetation under natural conditions. Proc. 2nd Internal. Conf. on Peaceful Uses of Atomic Energy, Geneva 18: 494-499.

Auerbach, S. I. 1958. White Oak Lake bed studies. In Health Physics Division Annual Progress Report for the period ending July 31, 1958, ORNL-2590.

Subproject 3.004 - The accumulation of mineral elements and radionuclides by native plants.

Date initiated: June 1956 Date completed: continuing

Research personnel: S. I. Auerbach, E. R. Graham, D. A. Crossley, Jr., G. L. Plummer, R. M. Anderson, J. H. Marks, and J. P. Witherspoon, Jr.

Objectives: To measure the accumulation of important nutrient elements and radionuclides in a variety of native plants, to compare these concentrations in plants with those in the soils, to measure interspecific and intraspecific variation of these parameters within a single ecosystem, to obtain data on the rates of turnover of mineral elements and radionuclide by the different vegetation units comprising an ecosystem, and to obtain estimates on the quantities of elements turned over by vegetation units.

Justification: Little is known about the role of mineral elements in affecting the character and distribution of native vegetation.

Also more information is needed on the cycling of minerals by vegetation. These studies aim to use the radioisotopes as tracers of the mineral nutrients in providing this basic ecologic information. The results will be applicable to the broader problem of movement of radioactive contaminants in terrestrial ecosystems.

Procedures: Various species of native plants, including grasses, forbs and trees, are sampled (several replicates) from various parts of the lake bed. Soil samples are taken at the same site. These vegetation samples are used for measurements of interspecific and intraspecific variation. Statistical procedures were used to compare samples from the upper and lower lake bed. One half square meter harvests were made in duplicate of the major forb types in order to provide data on the accumulation of minerals and radionuclides on a unit area basis. Plants and soils are processed and analyzed by standard techniques. Not enough replicates were obtained to do partial regressions on the soil values.

Reports:

Graham, E. R. 1958. Uptake of waste $Sr^{90}$ and $Cs^{137}$ by soil and vegetation. Soil Science 86(2): 91-97.

Auerbach, S. I. and D. A. Crossley, Jr. 1959. Strontium-90 and cesium-137 uptake by vegetation under natural conditions. Proc. 2nd Internal. Conf. on Peaceful Uses of Atomic Energy, Geneva 18: 494-499.

Auerbach, S. I. 1957. White Oak Lake bed studies. In Health Physics Division Annual Progress Report for period ending July 31, 1957, ORNL-2387.

Auerbach, S. I. 1958. White Oak Lake bed studies. In Health Physics Division Annual Progress Report for period ending July 31, 1958, ORNL-2590.

Auerbach, S. I. 1959. White Oak Lake bed studies. In Health Physics Division Annual Progress Report for period ending July 31, 1959, ORNL-2806.

Subproject 3.005 - Determination and evaluation of the radiation field above White Oak Lake bed.

Date initiated: September 1958 Date completed: June 1959

Research personnel: P. K. Lee and S. I. Auerbach

Objectives: To measure and characterize the radiation field above White Oak Lake bed, to map and otherwise delimit the areas of differing external radiation dose rates, and to predict the overall decrease due to radiation decay in dose rate of the radiation fields on the lower part of White Oak Lake bed.

Justification: Knowledge of the radiation fields resulting from the contamination of the lake bed is needed to implement ecological research on the effects and fates of radionuclides in a natural environment. Furthermore, the intensity of the radiation field is closely related to the distribution of the radionuclides. Therefore, the measurement of this radiation field not only defines the external dose rates to which organisms are

subjected at various parts of the lake bed, but also indicates the general distribution of the contaminants.

Procedures: Radiation intensity was determined at heights of 5, 25, 50, and 100 cm. at each of 31 stakes in the ecology 10 meter grid study area. These were made approximately 3 times a month from September 1959 to December 1959. Four readings were made at each height each time with a portable ionization-chamber type instrument (cutie pie) employing two separate chambers (gamma and beta sensitive; gamma sensitive). Type 522 Dupont Industrial film packets were used to measure the integrated doses and to check the measurements of the ionization chamber. Patterns of the radiation field were delineated by isodose lines at intervals of 10 and 15 mr./hr. Six dose rate transects across the lake bed delineated the horizontal pattern of contamination along the length of the lake bed. Soil data from other studies and dose rate information were used to solve a formula derived by K. Z. Morgan to estimate the contribution of each type of radiation to the total over the bed and to derive the expected change in average dose rate during the next 10 years.

Reports:

Lee, P. K. and S. I. Auerbach. 1960. Determination and evaluation of the radiation field above White Oak Lake bed. ORNL-2755.

Subproject 3.006 - Distribution and movement of radionuclides in White Oak Lake bed soils.

Date initiated: June 1958 Date completed: continuing

Research personnel: S. I. Auerbach, G. L. Plummer, J. A. Wolfe, and R. M. Anderson

Objectives: To determine the horizontal and vertical distribution of radionuclides in White Oak Lake bed soils and to determine rates of loss of specific radionuclides from the upper soil horizons.

Justification: Knowledge of the rates of change of radionuclide budgets in the soil horizons of terrestrial ecosystems is essential to the determination of steady state biological cycling rates. Likewise biological turnover of specific radionuclides may be related to the concentrations of these radionuclides in the soil. As more of the radionuclides are leached to lower horizons there is a possibility that the vegetation might translocate a relatively greater proportion over a period of time. Under those circumstances a continuous replenishment of the surface soils by radionuclides in hazardous quantities might occur for several decades.

Procedures: Six inch core samples were taken at each of the 200 stakes comprising the ecology grid in the lower lake bed in 1958 and 1959. These were composited and replicate samples analyzed for radionuclide content by standard techniques. Additional six inch core and other samples had been collected in various parts of the lake bed in 1956, 1957, and 1958. In 1959 six inch and deeper profile samples were taken in the same sites as the 1958 samples so as to permit paired comparisons with the latter. In addition, the 1959 points were

permanently referenced for future comparisons. All samples are being analyzed by the same laboratory to minimize variation in analysis. In addition to basic statistical treatment of these data, time trends will be studied for the different soil horizons.

Reports:

Auerbach, S. I. 1959. White Oak Lake bed studies. In Health Physics Division Annual Progress Report for period ending July 31, 1959, ORNL-2806.

Subproject 3.007 - Physiological crop ecology with respect to the uptake of $Sr^{90}$, $Cs^{137}$, $Co^{60}$, and $Ru^{106}$.

Date initiated: June 1958 Date completed: September 1960

Research personnel: S. I. Auerbach, J. P. Witherspoon, Jr., J. A. Wolfe, and R. M. Anderson

Objectives: To determine rates of uptake of mineral nutrients and radionuclides by different plant species on an individual and unit area basis and to obtain information on the relationship between microclimate and mineral uptake by plants.

Justification: Quantitative description of nutrient (energy) interchange between plants and higher trophic levels of an ecosystem requires data on the rates of accumulation of nutrients by plants in a growing season. The possible use of radionuclides as a tool for exploring these biological interrelationships with climate can be explored on a field scale in White Oak Lake bed.

Procedures: In 1958 three species of forage crops were planted in a

Fig. 11. Preparing seed beds for crop ecology experiments in study plot contaminated with mixed fission products in upper part of White Oak Lake bed (Subproject 3.007).

randomized plot design. Three 30 x 50 ft. blocks were laid out so that they spanned areas of high and low radionuclide contamination. Duplicate 4 x 4 ft. plots were planted for each species in each block after being selected at random. Each species was harvested at maturity.

In 1959 four species of forage crops (2 grasses and 2 legumes) were planted in the White Oak Lake agricultural plot. The plot was laid out in a row and column design divided into 16 subplots so that four rows containing four subplots were perpendicular to four columns containing four subplots each. Columns were 50 ft. wide and rows 15 ft. wide. The planting design resulted in alternative species in both row and column. Duplicate half-square meter harvests were taken from each subplot twice during the growing season.

All harvests were washed and processed according to standard procedures for chemical and radioactivity analyses. Flame photometry was used for chemical analyses. Strontium-90 was determined radiochemically by Kahn's procedure. Cesium-137, $Ru^{106}$, and $Co^{60}$ by gamma spectrometry.

Reports:

Auerbach, S. I. 1959. White Oak Lake bed studies. In Health Physics Division Annual Progress Report for period ending July 31, 1959, ORNL-2806.

Auerbach, S. I. 1960. White Oak Lake bed studies. In Health Physics Division Annual Progress Report for period ending July 31, 1960, ORNL-2994.

Auerbach, S. I. 1961. White Oak Lake bed studies. In Health Physics Division Annual Progress Report for period ending July 31, 1961, ORNL-3189.

Subproject 3.008 -- Plant succession on lower White Oak Lake bed.

Date initiated: June 1956 Date completed: continuing

Research personnel: S. I. Auerbach, H. F. Howden, D. A. Crossley, Jr., P. B. Dunaway, J. S. Olson, M. E. Pryor, J. P. Witherspoon, Jr., and J. A. Wolfe

Objectives: To map and follow the general successional changes in the major vegetation types and to map the succession and growth of forest tree species exclusive of willow.

Justification: Little information is available in the literature on succession related to an absolute time scale. The lake bed provides an opportunity for obtaining such data on a fixed chronological basis. Successional changes in plant distribution affect animal succession. Information on plant succession was needed for interpretation of animal studies being carried on concurrently.

Procedures: Once a season, usually in August, the distribution of vegetation types is mapped in the field. In 1957 and 1958 kodachrome photographs were made of the vegetation in each of the quadrats. The purpose of these photos was to serve as a fixed time reference for later comparisons. Beginning in 1958 invading trees were mapped and plotted on grid maps together with their height at the time (August-September).

Reports:

Auerbach, S. I. 1957. White Oak Lake bed studies. In Health Physics Division Annual Progress Report for period ending July 31, 1957, ORNL-2384.

Auerbach, S. I. 1958. White Oak Lake bed studies. In Health Physics Division Annual Progress Report for period ending July 31, 1958, ORNL-2590.

Auerbach, S. I. 1959. White Oak Lake bed studies. In Health Physics Division Annual Progress Report for period ending July 31, 1959, ORNL-2806.

Auerbach, S. I. 1960. White Oak Lake bed studies. In Health Physics Division Annual Progress Report for period ending July 31, 1960, ORNL-2994.

Auerbach, S. I. 1961. White Oak Lake bed studies. In Health Physics Division Annual Progress Report for period ending July 31, 1961, ORNL-3189.

Subproject 3.009 - Insect invaders of pioneer vegetation on White Oak Lake bed.

Date initiated: June 1956 Date completed: September 1958

Research personnel: H. F. Howden, D. A. Crossley, Jr., M. E. Pryor, and J. H. Marks

Objectives: To discover which species of insects would invade the lake bed vegetation and to estimate the biomass of these insects. The ultimate objective is quantification of the role of the insects in cycling of the radioactive fission products.

Procedures: Weekly random samples with a sweep net were taken in two replicates each of three vegetation types (smartweed, sedge-rush, and willow). A sample consisted of 10 rapid consecutive sweeps through the vegetation. Two sets of samples were taken weekly; one was used for a taxonomic survey and the other for determination of insect biomass.

Reports:

Crossley, D. A., Jr. and H. F. Howden. 1961. Insect-vegetation relationships in an area contaminated by radioactive wastes. Ecol. 42(2): 302-317.

Crossley, D. A., Jr. 1957. Insect fauna of White Oak Lake bed. In Health Physics Division Annual Progress Report for period ending July 31, 1957, ORNL-2384.

Crossley, D. A., Jr. 1958. White Oak Lake bed studies/insect studies. In Health Physics Division Annual Progress Report for period ending July 31, 1958, ORNL-2590.

Crossley, D. A., Jr. 1959. White Oak Lake bed studies/insect studies. In Health Physics Division Annual Progress Report for period ending July 31, 1959, ORNL-2806.

Howden, H. F. and D. A. Crossley, Jr. 1961. Insect species of the White Oak Lake bed, Oak Ridge, Tennessee. ORNL-3094.

Subproject 3.010 - Succession of soil mites on White Oak Lake bed.

Date initiated: October 1956 Date completed: August 1959

Research personnel: D. A. Crossley, Jr. and J. H. Marks

Objectives: To discover the succession of soil mites species in the

developing soils of White Oak Lake bed, to relate the soil mite succession to the vegetational succession, and to estimate population sizes for the various soil mite species.

Justification: The soil fauna is important in the reduction of fallen organic materials, but little is known of the precise contribution of the various animal groups to the reduction of litter. Although mites are the most numerous of the soil arthropods, the habits of most of the species are not known. By following the mite populations as they develop in the new soils of White Oak Lake bed, information on the roles of certain of the species may be gained. Most studies of soil arthropods have been conducted in mature soils, where the picture is complicated by the vast array of species present.

Procedures: Samples of soil ( 1 3/4 in. dia. and 1 to 2 in. deep) were treated in a modified tullgren apparatus for recovery of microarthropods. Sampling was systematic and was related to the 10-meter grid system in existence on the lake bed. Initially a monthly sampling interval was employed but later the time interval was increased to three months. The distribution of the mite species was analyzed for association with vegetation type and for lake shore to center gradients, as well as time sequence.

Reports:

Crossley, D. A.,Jr. 1957. Microarthropods of White Oak Lake bed. In Health Physics Division Annual Progress Report for period ending July 31, 1957, ORNL-2384.

Crossley, D. A., Jr. 1958. Soil microarthropod studies. In Health Physics Division Annual Progress Report for period ending July 31, 1958, ORNL-2590.

Strandtmann, R. W. and D. A. Crossley, Jr. 1962. A new species of soil-inhabiting mite, Hypoaspis marksi (Acarina, Laelaptidae) Jour. Kansas Ent. Soc. 35(1): 180-185.

Subproject 3.011 - Gross aspects of the movement of fission products in the soil to plant to insect food chain.

Date initiated: June 1958 Date completed: September 1959

Research personnel: D. A. Crossley, Jr., J. H. Schnell, J. H. Marks, and M. P. Hoglund

Objectives: To determine the concentrations of radioactive fission products in the insects inhabiting White Oak Lake bed vegetation, and relate these data to the information concerning soil and plant concentrations and biomass data.

Justification: Since insects are one of the major groups of herbivores on the White Oak Lake bed, an evaluation of the role of these animals in the cycling of radioactive fission products is essential to understanding the fate of radionuclides in terrestrial ecosystems. This information also has basic implications for the understanding of energy flow in terrestrial ecosystems.

Procedures: Large random samples of several species of insects were taken in four areas on White Oak Lake bed. The insect species sampled were limited to those which could be collected in numbers. The concentrations of radionuclides in the insects were measured.

Soil and plant concentrations of radionuclides in these areas are available from other studies. The relation of concentrations in insects to concentrations in plants, together with laboratory estimates of rates of elimination, provide a gross estimate of the radionuclide consumption by herbivorous insects.

Reports:

Crossley, D. A., Jr. 1959. White Oak Lake bed studies. In Health Physics Division Annual Progress Report for period ending July 31, 1959, ORNL-2806.

Crossley, D. A., Jr. 1960. Insect studies. In Health Physics Division Annual Progress Report for period ending July 31, 1960, ORNL-2994.

Crossley, D. A., Jr. and H. F. Howden. 1961. Insect-vegetation relationships in an area contaminated by radioactive wastes. Ecol. 42(2): 302-317.

Crossley, D. A.,Jr. 1962. Movement and accumulation of radiostrontium and radiocesium in insects. Radioecology. Proc. of First Nat. Symp. on Radioecology. Reinhold Publ. Corp., N. Y., N. Y. (In press).

Crossley, D. A., Jr. 1962. Consumption of vegetation by insects. Radioecology. Proc. of First Nat. Symp. on Radioecology. Reinhold Publ. Corp., N. Y., N. Y. (In press).

Subproject 3.012 -- $Sr^{90}$ and $Cs^{137}$ accumulation in plant insect-bird food chain (cooperative project with University of Georgia studies in radiation ecology).

Date initiated: June 1958 Date completed: June 1959

Research personnel: W. K. Willard, S. I. Auerbach, and E. P. Odum

Objectives: To determine the accumulation of $Sr^{90}$ and $Cs^{137}$ in summer and winter resident birds of White Oak Lake bed, and to evaluate these concentrations in terms of the bird species feeding habitats (i.e., place on the food chain) for summer and winter resident populations.

Justification: The determination of the fates and ultimate effects of radionuclides introduced into the environment requires knowledge of the fractions of total radioactivity entering the environment which may be expected to reach successive trophic levels in the major types of ecosystems.

Procedures: Birds were collected from various habitats of White Oak Lake bed by means of Japanese mist nets. Specimens were dissected into several components and these prepared for radioanalysis by the method of Krumholz and Emmons. Strontium-90 was determined by the method of Willard and Goodspeed. Cesium-137 was determined by Farabee's modification of the method of Mizzan.

Reports:

Willard, W. K. 1960. Avian uptake of fission products from an area contaminated by low-level atomic wastes. Science 132(3420):148-150.

Subproject 3.013 - Accumulation and elimination of $Cs^{137}$ by a grasshopper, *Romalea microptera*.

Date initiated: June 1957 Date completed: September 1957

Research personnel: D. A. Crossley, Jr. and M. E. Pryor

Objectives: To measure the rate of elimination of $Cs^{137}$ by *Romalea*

*microptera* and to follow the accumulation of $Cs^{137}$ in this insect, under conditions of known intake of $Cs^{137}$.

Justification: These studies are necessary to aid in the interpretation of field data collected from White Oak Lake bed, where $Cs^{137}$ and other fission products have become incorporated into the biological system. If the rate of elimination is known, the equilibrium concentrations may be predicted from the rate of feeding; or, equilibrium concentrations will provide an estimate of feeding rate.

Procedures: In laboratory experiments, adult *Romalea microptera* were arranged in three cages of 16 grasshoppers each. Each cage received a different level of $Cs^{137}$ in bean plants. Whole body counts on the animals were made every other day, so that the approach to equilibrium with food concentration could be followed. A biological half-life, obtained in separate experiments, was used to calculate expected values for the body burden in the three cages.

Reports:

Crossley, D. A., Jr. 1958. Accumulation and excretion of $Cs^{137}$ by grasshoppers. In Health Physics Division Annual Progress Report for period ending July 31, 1958, ORNL-2806.

Crossley, D. A., Jr. and M. E. Pryor. 1960. The uptake and elimination of Cesium-137 by a grasshopper: *Romalea microptera*. Health Physics 4: 16-20.

Subproject 3.014 -- Accumulation of $Sr^{90}$ and $Cs^{137}$ by grasshopper species on White Oak Lake bed.

Date initiated: June 1959 Date completed: September 1960

Research personnel: D. A. Crossley, Jr. and J. H. Schnell

Objectives: To measure the elimination rates for Sr and Cs by *Melanoplus differentialis*, *M. bilittoralis*, *Conocephalus fasciatus*, and *Conocephalus brevipennis*, to determine the rate of accumulation of $Cs^{137}$ and $Sr^{90}$ by these insects on White Oak Lake bed, and to estimate the equilibrium concentrations and their fluctuations in these insects, under field conditions.

Justification: The species listed are the more abundant grasshopper species on White Oak Lake bed. The data collected should provide an estimate of the role of these insects in fission product turnover.

Procedures: In the laboratory, rates of elimination of the nuclides were measured by whole-body counting of insects which have eaten food containing the nuclides. In the field, uncontaminated grasshoppers were caged on the White Oak Lake bed. Every other day a single cage of grasshoppers, constituting a sample, were analyzed for radionuclides. The elimination rates was used to calculate expected values for radionuclide concentrations in grasshoppers under field conditions.

Reports:

Crossley, D. A., Jr. and J. H. Schnell. 1961. Comparative elimination of radiocesium and radiostrontium by grasshoppers. Ann. Ento. Soc. Amer. 54(2): 459-461.

Fig. 12. Elimination rates of radionuclides for various insect species are determined directly in the field by means of portable sodium-iodide scintillation detectors and scalers such as the model shown (Subprojects 3.014, 3.016).

Subproject 3.015 - Radiation effects: biometrical analysis of two species of sedge growing on White Oak Lake bed.

Date initiated: June 1959 Date completed: August 1959

Research personnel: G. L. Plummer

Objectives: To determine if the sedge produced a measurable response which could be related to the dose rate from ionizing radiation in their habitat.

Justification: There is need to obtain quantitative data on the effects of chronic radiation upon organisms. In White Oak Lake bed there are species of annual and perennial plants which have been growing there for three years, subjected to ionizing radiation in all phases of their life cycle.

Procedures: Several hundred mature culms of both species were collected in mid-June from different stations on four kinds of sites--no radiation, 10, 20, and 40 mr./hr. Each collection site was sampled at not less than two stations. Each station within a site covered an area of about 50 square meters. Ten stations on the lake bed and ten stations in the central sites were sampled.

Analyses of variance were used to study differences between mean floral length in the four sites; differences were further evaluated by Tukey-Hartley tests.

Regression of mean length of floral parts against dose rate was fitted by least squares.

Reports:

Plummer, G. L. 1960. Biometric analysis of growth response of two plant species inhabiting a radioactive waste area. ORNL-2903.

Plummer, G. L. 1962. Growth responses of Helianthus Annuus to internal calcium-45. The Botanical Gazette 123(4): 272-278.

Subproject 3.016 - Turnover of $Cs^{137}$ by Chrysomela knabi.

Date initiated: May 1960 Date completed: September 1960

Research personnel: D. A. Crossley, Jr. and M. P. Hoglund

Objectives: To measure the rates of elimination of cesium by means of radioactive nuclides for adults and immature stages of this beetle species, to measure the $Cs^{137}$ concentration in these insects on White Oak Lake bed, as well as in their host plants (willows), to estimate the food consumption by these insects from the equilibrium for $Cs^{137}$ on the lake bed, and the elimination rates, and compare this estimate with food consumption as actually measured in the laboratory, and to investigate the effects that sex, size, and stage of life history have upon the elimination rate, and compare these data with those for other insects.

Justification: Previous work (subprojects 3.013 and 3.014) has shown the value of using the elimination rate of a radionuclide as a tool for determining the consumption of plants by insects. This work has two important implications for radioecology. From a basic viewpoint it offers a possibility not only for

measuring direct nutrient transfer from plant to insect, but also for future determinations of energy flow between these two food levels of an ecosystem. From an applied point of view it provides further information on the turnover of radioactive materials in a natural system. All stages of Chrysomela knabi feed readily upon willow leaves and their life history is completed in a matter of weeks so that the beetles are ideal for the investigation of the relation of bionomic parameters to elimination rates.

Procedures: Experience with this species has shown that the beetles can be reared in small containers with willow leaves as the only food source. For estimation of the elimination rates, immature stages and adults will be fed willow leaves tagged with $Cs^{134}$. After the initial contamination of the beetles, whole body counting of individuals will be done at frequent (4-6 hr.) intervals. For independent estimation of food consumption, individual beetles were reared upon weighed amounts of willow leaves. The weight of food actually consumed and feces production was estimated. Insects and willow leaves collected on White Oak Lake bed were analyzed for $Cs^{137}$ for an estimation of the field equilibrium values in the insects. By means of the laboratory data an expected value for concentration of $Cs^{137}$ was obtained. This concentration will be compared with those of the field populations.

Reports:

Crossley, D. A., Jr. 1961. Studies on Chrysomela knabi. In Health Physics Division Annual Progress Report for period ending July 31, 1961, ORNL-3189.

Subproject 3.017 - Studies on the summer birds of the AEC controlled area.

Date initiated: June 1957 Date completed: August 1957

Research personnel: J. C. Howell

Objectives: To obtain ecological information about the birds and to select sites suitable for future studies on birds in radioactive areas.

Justification: Basic knowledge for the avian component of the fauna is especially needed for planning studies in radioactive areas in view of differing mobilities, varied feeding habits, and diverse habitat preferences of birds.

Procedures: Visual and auditory observations of birds were made within a 1,000-ft. grid covering Melton Valley. At each observation station, records were kept of the birds identified by sight or song, time of day, type of plant successional stage, and other pertinent information. Time spent at a station was 15 minutes, after which the observer moved to the next station. A population index (number of individuals of each species) was used to indicate relative abundances. The index was employed to relate the species to four plant successional stages. Supplemental observations of birds were also made in other parts of the AEC controlled area.

Reports:

Howell, J. C. 1958. Long-range ecological study of the Oak Ridge area: I. Observations on the summer birds in Melton Valley. ORNL-CF-58-6-14.

Howell, J. C. 1960. The birds of a valley. Audubon Magazine 62(6): 283-286.

Subproject 3.018 - Survey of mammals in the AEC controlled area.

Date initiated: June 1958 Date completed: August 1958

Research personnel: J. C. Howell and P. B. Dunaway

Objectives: To ascertain (1) the mammal species present on the area, (2) the relative abundance of each species, and (3) the areas that would be suitable for future population studies.

Justification: This study was a necessary basic prerequisite for planning future studies in the AEC controlled area.

Procedures: Records for mammals were compiled by trapping in various parts of the area, by using data from the subprojects on the population studies of small mammals, from a rabies control program in the area in 1958, and from several other qualified sources. Data on the trapped small mammals included species, date of capture, location, type of trap, sex, age, weight, and length measurements of hind foot, tail, and body plus tail. Trapping success was evaluated in terms of mammals caught per "trap night". Areas trapped were classified as to plant communities and soil characteristics.

Reports:

Howell, J. C. and P. B. Dunaway. 1959. Long-term ecological study of the Oak Ridge area: II. Observations on the mammals with special reference to Melton Valley. ORNL-CF-59-10-126.

Subproject 3.019 - Early mammal succession on White Oak Lake bed.

Date initiated: October 1956 Date completed: April 1960

Research personnel: P. B. Dunaway, V. I. Dodson, J. A. Payne, and S. V. Kaye

Objectives: To follow the succession of mammalian species during the first years after the draining of White Oak Lake.

Justification: Knowing the length of occupancy by a species in a radioactive area is important in understanding the effects of long-term chronic radiation on the populations.

Procedures: Records were kept of the small mammals trapped on the lake bed and in similar or differing habitats. Observations were also made of large mammals that were present in these areas.

Reports:

Dunaway, P. B. 1958. Small-mammal program. In Health Physics Division Annual Progress Report for the period ending July 31, 1958, ORNL-2590.

Dunaway, P. B. 1959. Small-mammal program. In Health Physics Division Annual Progress Report for period ending July 31, 1959, ORNL-2806.

Dunaway, P. B. and S. V. Kaye. 1961. Studies of small-mammal

populations on the radioactive White Oak Lake bed. Trans. N. Amer. Wildl. Conf. 26: 167-185.

Dunaway, P. B. and S. V. Kaye. 1962. Cotton rat weights in relation to season, breeding, and environmental radioactive contamination. Submitted to Amer. Midl. Nat.

Subproject 3.020 - Home ranges and territories of small mammals.

Date initiated: October 1956 Date completed: April 1960

Research personnel: P. B. Dunaway, V. I. Dodson, J. A. Payne, and S. V. Kaye

Objectives: To determine the home ranges and territories occupied by resident small mammals.

Justification: It is necessary to know what part of a contaminated habitat is occupied by an animal through time in order to calculate the dose received by the animal.

Procedures: Live-traps were set in 10-meter grids on an area contaminated with radioactive wastes and on an uncontaminated area. During a trapping period, traps were placed in one of 12 non-random patterns. The capture-recapture method was used whereby individuals were marked by toe-clipping and recaptured for as long as they reappeared. Home ranges were plotted around the points of capture after 10 or more captures. Territories were inferred by the amount of overlap of the home ranges. Each area was trapped at least once a week (when possible) all through the year.

Reports:

Dunaway, P. B. 1959. Small-mammal program. In Health Physics Division Annual Progress Report for period ending July 31, 1959, ORNL-2806.

Dunaway, P. B. and S. V. Kaye. 1961. Studies of small-mammal populations on the radioactive White Oak Lake bed. Trans. N. Amer. Wildl. Conf. 26: 167-185.

Subproject 3.021 -- Population ecology of small mammals.

Date initiated: April 1957 Date completed: continuing

Research personnel: P. B. Dunaway; V. I. Dodson, J. A. Payne, and S. V. Kaye

Objectives: To determine the ecology of natural populations in a radioactively-contaminated area and in several uncontaminated areas and to make biostatistical studies of pertinent population phenomena, comparing the populations of contaminated areas with those in uncontaminated areas.

Justification: The ecology of mammal populations must be known in order to assess the role of mammals in the cycling of radioactive elements and to test for the effects of low-level chronic radiation.

Procedures: The small mammals are captured in live traps in radioactive and nonradioactive areas marked off in 10-meter grids. The capture-recapture procedure is used, with trapping on a weekly basis the year round. Captured individuals are examined in the field for species, weight, sex, age, place of capture,

reproductive condition, etc. Some individuals are brought to the laboratory and caged, usually to check size and sex ratio of litters, weight increase of the young, or abnormalities. These specimens are usually returned to the populations when observations are completed. Laboratory studies of dead individuals include examinations of reproductive tracts, gross and microscopic examinations of tissues, weighing of organs and tissues, and anatomical measurements. Information on the individuals is transferred from field and laboratory records to IBM cards for machine sorting and tabulation of population data such as densities, biomass, weight classes, sex and age ratios, and natality and mortality rates.

Reports:

Dunaway, P. B. 1958. Small-mammal program. In Health Physis Division Annual Progress Report for period ending July 31, 1958, ORNL-2590.

Dunaway, P. B. 1959. Small-mammal program. In Health Physics Division Annual Progress Report for period ending July 31, 1959, ORNL-2806.

Dunaway, P. B. and S. V. Kaye. 1960. Small-mammal program--population studies. In Health Physics Division Annual Progress Report for period ending July 31, 1960, ORNL-2994.

Dunaway, P. B. and S. V. Kaye. 1961. Mammalogy--dynamics of cotton rat populations. In Health Physics Division Annual Progress Report for period ending July 31, 1961, ORNL-3189.

Dunaway, P. B. and S. V. Kaye. 1961. Cotton rat mortality during severe winter. Jour. Mamm. 42(2): 265-268.

Dunaway, P. B. and S. V. Kaye. 1961. Studies of small-mammal populations on the radioactive White Oak Lake bed. Trans. N. Amer. Wildl. Conf. 26: 167-185.

Dunaway, P. B. and S. V. Kaye. 1961. Effects of industrial oil disposal on wild mammals. Jour. Mamm. 42(4): 554-556.

Dunaway, P. B. and S. V. Kaye. 1962. Litter-size record for eastern harvest mice. Jour. Mamm. 43: 428-429.

Dunaway, P. B. and S. V. Kaye. 1962. Effects of ionizing radiation on mammal populations of the White Oak Lake bed. Radioecology. Proc. of First Nat. Symp. on Radioecology. Reinhold Publ. Corp., N. Y., N. Y. (In press).

Dunaway, P. B. and S. V. Kaye. 1962. Cotton rat weights in relation to season, breeding, and environmental radioactive contamination. Submitted to Amer. Midl. Nat.

Subproject 3.022 -- Body burdens of radionuclides in vertebrates from areas contaminated with radioactive wastes.

Date initiated: September 1959 Date completed: September 1960

Research personnel: S. V. Kaye and P. B. Dunaway

Objectives: To identify and quantify the radionuclides in various tissues of vertebrates from areas with known radioactive contamination.

Justification: Data on the specific radionuclides present, location as to tissue, and the amounts present are prerequisite for calculations of internal dose as well as for determining the part

that vertebrates play in the cycling of radionuclides through the environment.

Procedures: The desired organs or tissues were dissected from the specimens (in many cases the entire carcass was prepared), wet and dry weights of the samples were taken, and then the samples were radionalyzed.

Reports:

Dunaway, P. B. and S. V. Kaye. 1960. Small-mammal program--radioassay studies of small mammals. In Health Physics Division Annual Progress Report for period ending July 31, 1960, ORNL-2994.

Dunaway, P. B. and S. V. Kaye. 1961. Mammalogy--Bioaccumulation. In Health Physics Division Annual Progress Report for period ending July 31, 1961, ORNL-3189.

Dunaway, P. B. and S. V. Kaye. 1961. Studies of small mammal populations on the radioactive White Oak Lake bed. Trans. N. Amer. Wildl. Conf. 26: 167-185.

Kaye, S. V. and P. B. Dunaway. 1962. Estimation of dose rate and equilibrium state from bioaccumulation of radionuclides by mammals. Radioecology. Proc. of First Nat. Symp. on Radioecology. Reinhold Publ. Corp., N. Y., N. Y. (In press).

Subproject 3.023 -- Integrated doses to mammals from external radiation.

Date initiated: April 1960 Date completed: October 1960

Research personnel: S. V. Kaye and P. B. Dunaway

Objectives: To calculate the integrated doses received by mammals living in areas with known radiation fields.

Justification: Knowledge of radiation dose is required before

evaluations can be made of population phenomena and histopathological findings in relation to ionizing radiation.

Procedures: Calculations were made which considered (1) the intensities of the external radiation fields, (2) the home ranges occupied by the individuals, and (3) the amount of time spent by the individuals in the home ranges. Applicable equations were used to calculate the absorbed radiation dose from the external sources.

Reports:

Dunaway, P. B. and S. V. Kaye. 1961. Mammalogy--bioaccumulation. In Health Physics Division Annual Progress Report for period ending July 31, 1961, ORNL-3189.

Kaye, S. V. and P. B. Dunaway. 1962. Bioaccumulation of radioactive isotopes by herbivorous small mammals. Health Physics 7: 205-217.

Kaye, S. V. and P. B. Dunaway. 1962. Estimation of dose rate and equilibrium state from bioaccumulation of radionuclides by mammals. Radioecology. Proc. of First Nat. Symp. of Radioecology. Reinhold Publ. Corp., N. Y., N. Y. (In press).

Subproject 3.024 -- Effects of sociopsychological (crowding) stress on the whole blood and plasma components of small mammals.

Date initiated: June 1960 Date completed: September 1960

Research personnel: J. A. Sealander, Jr.

Objectives: To study the effects of crowding and other stresses in small mammals by means of electrophoresis of whole blood.

Justification: At present there are no rapid or completely satisfactory methods in use for ascertaining the presence of or the

degree of stress existing in a mammal population or for following the time course of stress in individuals or populations. Analysis of serum protein patterns in individuals and populations of mammals by electrophoretic methods holds forth considerable promise as a useful technique for analysis of the alterations in internal equilibrium caused by various sorts of stress.

Procedures: The technique used was electrophoresis of whole blood in starch gels. Electrophoretic determinations were made on whole blood from several species of mice and rats soon after capture. These animals were then confined in groups of various sizes. Repeat measurements were then made on these same individuals at approximately 10-day intervals during the course of their confinement to determine whether any significant alteration of blood components occurred that could be related to "crowding" stress.

Reports:

Sealander, J. A., Jr. 1960. Subproject 4.010. The effects of sociopsychological and radiation stress on the serum proteins of the white-footed mouse (*Peromyscus leucopus*). Prog. Rept. on Summer Research. multi.

Subproject 3.025 - Pathological conditions exhibited in chronically irradiated and natural populations of small mammals in the AEC-controlled area at Oak Ridge, Tennessee.

Date initiated: June 1960 Date completed: September 1961

Research personnel: H. E. Childs, Jr., G. E. Cosgrove (Biology Division), P. B. Dunaway and S. V. Kaye.

Objectives: (1) To determine the occurrence of pathological conditions in native small mammals under natural conditions, (2) to determine whether the incidences and type of pathological conditions in small mammals resident in an area contaminated by radioactive waste varies significantly from that of nonirradiated populations, and (3) to evaluate the role of disease as a mechanism governing the size of mammalian populations.

Justification: Considerable concern has been expressed about the possible harmful effects of radiation from nuclear weapons and waste products from power plants. This concern will increase as greater utilization of nuclear power is made. Much effort is being expended on the effects of long-term exposure to various types of radiation on laboratory animals under laboratory conditions. Of possible greater importance is the need to know what happens in nature from chronic low-level irradiation from external as well as internal sources.

Procedures: Small mammals on a gridded radioactive area were live trapped, marked and released, and retrapped for several weeks for residency information. Blood samples were taken for microhematocrits and differential counts. After sufficient residency information were accrued, each animal was autopsied and gross lesions noted. All major organs and gross lesions were sectioned and examined histologically. Each residual carcass was radioassayed for determining the body burden of radionuclides which was used in calculating the whole-body dose rate from internal emitters. Small mammals from areas not contaminated

with radioactive wastes were treated similarly, except that radioanalyses were not made.

Reports:

Dunaway, P. B. and S. V. Kaye. 1961. Mammalogy. In Health Physics Division Annual Progress Report for period ending July 31, 1961, ORNL-3189.

Dunaway, P. B. and S. V. Kaye. 1962. Effects of ionizing radiation on mammal populations on the White Oak Lake bed. Radioecology. Proc. of First Nat. Symp. on Radioecology. Reinhold Publ. Corp., N. Y., N. Y. (In press).

Childs, H. E., Jr. 1960. Subproject 4.011. The effects of chronic irradiation in nature on wild small mammals in the AEC-controlled area at Oak Ridge, Tennessee. Prog. Rept. on Summer Research. multi.

Subproject 3.026 -- Blood studies of wild small mammals resident on the radioactive White Oak Lake bed.

Date initiated: July 1961 Date to be completed: continuing

Research personnel: P. B. Dunaway, S. V. Kaye, L. L. Smith, and R. J. Pryor

Objectives: To determine the effects of chronic ionizing radiation on (1) the numbers of leucocyte types, total leucocytes, and total erythrocytes in the blood of wild mammals on White Oak Lake bed, (2) the abundance of the circulating elements of blood in relation to species, age, and sex of White Oak Lake bed mammals, (3) blood cell count changes in relation to accumulated dose by using uncontaminated animals introduced in

Fig. 13. View of 10 m x 10 m pens used for field experiments on small mammals. In the above photo are shown two pens located in an uncontaminated area. Similar pens are located on the radioactive White Oak Lake bed (Subproject 3.026).

pens established on White Oak Lake bed. To evaluate the effects of seasonal and population fluctuations on the blood picture of wild small mammals.

Justification: The circulating cells of the blood are among the most sensitive indicators of ionizing radiation effects on the hematopoietic system. Many previous laboratory studies have shown that the effects of radiation can be measured in the peripheral blood of animals receiving chronic low-level doses. Leucocytes are the most sensitive of the blood cells and, of these lymphocytes are more radiosensitive than granulocytes. Lymphopenia and morphological alterations of leucocytes, particularly the lymphocytes, are probably the most sensitive indicators of radiation damage (Ingram 1956).

Resident mammals and introduced mammals on White Oak Lake bed are constantly irradiated with beta and gamma radiation from both internal and external sources. Strontium-90 and $Ru^{106}$ are the chief contributors to internal exposure, whereas $Cs^{137}$, $Co^{60}$, and $Ru^{106}$ are the contributors to external exposure. Almost all of the $Sr^{90}$ is located in bone, the principal site of hematopoiesis. Ingested $Ru^{106}$ primarily irradiates the gastrointestinal tract. External exposure to $Cs^{137}$, $Co^{60}$, and $Ru^{106}$ would result in total body doses. Whereas responses of laboratory animals are well documented, we have little information on the responses of wild animals to ionizing radiation delivered continuously over a major part of the animal's life.

Fig. 14. Subcutaneous insertion of metaphosphate glass rod dosimeter in cotton rat (Sigmodon hispidus). Dosimeter is contained in nylon protective shield which can be seen in the tip of the needle. Two rods, one dorsal and one ventral, are placed in each animal (Subproject 3 026).

Procedures: Blood samples are taken from animals in 100 square meter pens on White Oak Lake bed, unconfined animals from White Oak Lake bed, and penned and unpenned animals from nonradioactive areas. During warm weather the animals are sampled in the field, but during cool weather the animals are bled in the laboratory where circulation is increased with artificial heat. The following tests are run on each blood sample: hematocrit, differential white cell count, Coulter electronic counter red cell and white cell total counts and cell size distributions, total solids, protein concentration, refractive index, and mean cell volumes. Radiation doses are calculated from silver metaphosphate glass dosimeters and radioanalyses of animals.

Reports:

None

Fig.15. Electronic particle counter which is used for erythrocyte and leucocyte counts and for blood cell size distributions of native small mammals subjected to chronic ionizing radiation (Subproject 3.026).

## PROJECT IV: AQUATIC STUDIES

Over-all Objectives:

To characterize the biological populations and their environment.

To determine and understand the biological mechanisms which affect the distribution and fate of radionuclides in the Clinch River and associated fresh-water aquatic systems.

To determine the effects of radioactivity as an environmental factor in such systems.

Justification:

Long-lived fission products are being released continuously into the Clinch River by ORNL. Those presumed to be of greatest biological importance are $Sr^{90}$, $Co^{60}$, $Cs^{137}$, $Ru^{106}$, transuranic elements, rare earths, and $Zr^{95}$-$Nb^{95}$.

These studies are needed to determine the role of specific organisms in the uptake, accumulation, and cycling of the various radionuclides (biogeochemical cycles) in the river ecosystem. Information is needed regarding food chain relationships and the quantitative transfer of isotopes to succeeding trophic levels.

The future development of nuclear power depends in part on cheap methods of disposal of long-lived fission waste materials. The initial release in most cases will be made to fresh-water streams. Therefore, the Clinch River is a unique stream on which to evaluate the effects of these waste products.

Subproject 4.001 - The suspended microbiota of the Clinch River and adjacent waters, in relation to radioactivity in the summer of 1956.

Date initiated: June 1956-1957 Date completed: September 1956-1957

Research personnel: J. B. Lackey

Objectives: To collect, identify, and enumerate the planktonic algae and protozoa found in the Clinch River and adjacent waters, to determine the algae and protozoa of the Clinch River with those found in the Columbia and Ottawa Rivers, and to evaluate the role of algae in the transport of radionuclides.

Justification: A knowledge of the kinds and abundance of planktonic organisms in the Clinch River is needed as a basis for ecological studies of these organisms and an understanding of the river ecosystem.

Procedures: Water samples were collected, planktonic populations were concentrated by centrifugation, and counted by standard procedures. Blooms were studied with regard to species and concentration of radioactivity.

Reports:

Lackey, J. B. 1957. The suspended microbiota of the Clinch River and adjacent waters, in relation to radioactivity in the summer of 1956. ORNL-2410 .

Lackey, J. B. 1958. The suspended microbiota of the Clinch River and adjacent waters, in relation to radioactivity in the summer of 1956. Florida Eng. and Indust. Expt. Sta. Tech. Paper 145: 1-26, Append.

Subproject 4.002 - Uptake of cesium-137 by cells and detritus of *Euglena* and *Chlorella*.

Date initiated: June 1957 Date completed: August 1957

Research personnel: L. G. Williams and H. D. Swanson

Objectives: To determine some factors controlling the fate of cesium in biological systems: (1) a comparison of uptake of *Chlorella* with a cellulose wall, and *Euglena* with a protein pellicle, (2) a comparison of uptake with age of cells, age of culture medium, influence of bacteria, population density, concentration of stable cesium, influence of stable sodium and potassium.

Justification: Algae are able to concentrate radioisotopes by many orders of magnitude from water in their natural environments. A fundamental understanding of factors which affect the concentration of radionuclides by algae may be obtained in the laboratory through controlled experiments.

Procedures: *Euglena intermedia* (Klebs) and *Chlorella pyrenoidosa* Chick were cultured in aquaria under varying environmental conditions. The uptake of $Cs^{137}$ by the cells from experimental solutions was measured for each variable.

Reports:

Williams, L. G. and H. D. Swanson. 1958. Concentration of cesium-137 by algae. Science 127(3291): 187-188.

Williams, L. G. 1960. Uptake of cesium-137 by cells and detritus of *Euglena* and *Chlorella*. Limnol. and Oceanogr. 5(3): 301-311.

Subproject 4.003 - The role of the fresh-water crayfish as an agent in strontium concentration.

Date initiated: June 1959 Date completed: September 1959

Research personnel: M. N. Stamper and J. M. Schurr

Objectives: To determine the uptake of strontium by the fresh-water crayfish, to determine the role of gastroliths in the conservation of calcium and strontium from the exoskeleton at ecdysis, and to determine the uptake of radionuclides by crayfish from a contaminated stream.

Justification: The crayfish, because of its calcium carbonate exoskeleton is potentially an organism capable of concentrating strontium from its environment. Crayfish are scavengers in the aquatic environment and are utilized as food by vertebrates such as fish, birds, and mammals. Consequently, crayfish provide a trophic link within the aquatic environment and between aquatic and terrestrial environments.

Procedures: Strontium$^{85}$ was used in the laboratory because of ease of detection of its gamma emission.

Berried female crayfish were caught in Bear Creek and carried into the laboratory where hatchings of the eggs occurred. Immediately before the hatch, the berried female was placed in a $Sr^{85}$ solution. After hatching, the young and mother were left in the solution until they had a sufficient concentration of $Sr^{85}$ for analysis. Newly ecdysed crayfish were found to take up Sr very rapidly according to an exponential function,

that could be explained by first order kinetics, filling a limited number of sites. The radioactive crayfish were transferred to 25 ml. flasks containing 10 ml. of creek water for daily observation and strontium elimination studies.

In field studies crayfish from Bear Creek were placed in wire cages in White Oak Creek and the uptake of radioactivity was measured.

Reports:

Schurr, J. M. and M. N. Stamper. 1962. Model for the accumulation of strontium and calcium by recently molted crayfish Cambarus longulus longerostris Ort. Limnol. and Oceanogr. 7(4): (In press)

Subproject 4.004 - Analysis of the bottom organisms from Clinch River mile 23 to Tennessee River mile 560.

Date initiated: February 1960 Date completed: July 1960

Research personnel: D. J. Nelson

Objectives: To determine kinds, relative abundance, and gross gamma activity of bottom organisms in reaches of the Clinch and Tennessee Rivers of immediate interest in connection with this study.

Justification: This survey is needed to describe the river bottom fauna to insure that representative sampling sites are chosen for an anticipated intensive study.

Procedures: Dredges will be used to collect bottom sediments. The organisms will be washed from bottom materials, sorted, identified, and radioactivity levels determined by gross gamma counts or preferably by gamma scans if facilities are available.

Reports:

Nelson, D. J. 1961. Bottom organisms in the Clinch River. In Health Physics Division Annual Progress Report for period ending July 31, 1961, ORNL-3189.

Subproject 4.005 - The role of heterotrophic microorganisms in the concentration of specific radionuclides on organic matter.

Date initiated: October 1959 Date completed: continuing

Research personnel: D. J. Nelson and E. W. Bowen

Objectives: To separate the effects of physical sorption of radionuclides on organic matter from uptake associated with biological action on organic matter.

Justification: The bottom sediments of the Clinch River contain an admixture of organic matter which is contaminated with significant amounts of $Cs^{137}$, $Ru^{106}$, and $Co^{60}$. The organic matter is available as food for sediment-ingesting organisms such as chironomid larvae and oligochaete worms. In considerations relating the food chain to transfer of radionuclides, it is desirable to know whether biological or physical processes are functioning at this level. A logical extension of this study would be to determine whether the organisms were actually obtaining their food from the organic detritus or from bacteria or bacterial products associated with the detritus.

Procedures: Laboratory experiments have been designed in which finely ground organic matter (leaf material) was included in sterile and nonsterile solutions in flasks. The uptake of

specific isotopes was determined at intervals by filtering the solutions through millipore filters and measuring the dissolved and particulate activity. A third sterile flask without added organic matter was used to determine isotope adsorption by the filters.

Reports:

None

Subproject 4.006 - Uptake and elimination of cobalt-60 by a species of crayfish, *Cambarus longulus longerostris*.

Date initiated: June 1960 Date completed: September 1960

Research personnel: C. W. Wiser and D. J. Nelson

Objectives: To determine if crayfish accumulate $Co^{60}$, to determine the rate of uptake and loss of $Co^{60}$ by crayfish, to determine the effect of varying concentrations of $Co^{60}$ in water upon uptake, to determine the effect of size upon uptake and loss of $Co^{60}$, and to compare the accumulation of $Co^{60}$ with that of $Sr^{85}$.

Justification: Cobalt$^{60}$ is one of the radionuclides resulting from weapons explosions and frequently is a constituent of radioactive wastes. It has a relatively long half-life. Data on $Co^{60}$ accumulation by marine organisms suggest that significant bioaccumulation of this radionuclide may occur in the natural environment. The purpose of this work was to obtain data on the accumulation of this radioactive nuclide by representatives of a common and abundant fresh-water organism.

Procedures: The effect of different levels of concentration upon

uptake was determined by placing small crayfish of approximate equal sizes in $Co^{60}$ solutions of varied levels of concentration, ranging from 2.56 dpm./ml. to $2.56 \times 10^5$ dpm./ml. To show rates of uptake, small crayfish were placed in a known volume of $Co^{60}$ solution of medium level concentration. Counts for accumulation were made at frequent intervals, especially in the initial period of uptake. These radioactive crayfish were then removed to fresh water and counts made at frequent intervals to determine the rates of loss or excretion of $Co^{60}$. The effects of size upon uptake and loss were determined by this same procedure using several different sizes of crayfish. Evidence of the mode of accumulation was acquired in a number of ways. Uptake by way of the digestive tract was determined by feeding uncontaminated animals upon contaminated food, such as beans germinated in a $Co^{60}$ solution. Absorption and adsorption of the isotope was differentiated by comparing uptake by dead and living crayfish of equal sizes from solutions of the same initial concentration. The site of concentration of $Co^{60}$ was determined by dissecting radioactive crayfish and counting the activity per unit of weight in the various organs. Also any loss of $Co^{60}$ at the time of molting indicated accumulation on or in the exoskeleton.

Reports:

Wiser, C. W. and D. J. Nelson. 1961. Uptake of $Co^{60}$ by crayfish. Status Report No. 1 on Clinch River Study, ORNL-3119.

Wiser, C. W. and D. J. Nelson. 1961. Crayfish studies. In Health Physics Division Annual Progress Report for period ending July 31, 1961, ORNL-3189.

Subproject 4.007 - The role of tubificid worms in the transport of radioactive bottom sediments in the Clinch River

Date initiated: June 1960 Date completed: September 1960

Research personnel: D. J. Nelson and R. C. Early

Objectives: To determine the quantity of radioactive mud which is transported downstream as a function of feeding activity by tubificid worms and low velocity water currents.

Justification: Water currents with velocities up to 0.2 ft./sec. are created in TVA reservoirs as a result of water control activities in this hydroelectric system. Tubificid worms are the most abundant organisms in the Clinch River. Since these worms feed with their heads in the mud and their tails projecting into the overlying water, their fecal pellets may contribute significantly to the downstream transport of radioactive bottom sediments.

Procedures: Establish laboratory cultures of tubificid worms to determine rate of feeding, height worm tail is held above substrate, and depth in mud at which worms feed. Determine the rate of fall and diameter of fecal pellets and mud density. The following mathematical model describing the downstream transport of bottom sediments as a function of feeding activity of the worms and current velocity has been formulated with the assistance of M. A. Kastenbaum:

Height variable $y = a - rt$,

where a, is the height of the worm tail above the sediment surface; r, is the rate of fall of fecal pellets (acceleration due to gravity is not considered), and t, is time.

Distance variable $x = vt$,

where v, is current velocity. Then,

$$y = a - rx/v,$$

where a, r, and v are determined experimentally. For $y = 0$, $x = av/r$, or distance downstream; $t = a/r$, time to travel distance x. For purposes of simplicity, the worm is considered to be feeding in an area of 1 $cm^2$. of bottom surface with its head at a depth h cm. in the mud. In order for a worm to ingest a pellet on the surface of the mud, we assume it must eat the entire volume of mud. Therefore the worm will displace h $cm^3$. When w is the rate of feeding in g./hr. and s is the specific gravity of the sediment, it will take hs/w hours to move the volume of h $cm^3$. The concentration of the various radionuclides sorbed onto the mud is known. Therefore, using physical half-lives of the nuclides, it will be possible to calculate the quantity of radioactivity being transported, and distance of movement.

Reports:

Nelson, D. J. 1961. Transport of bottom sediments by tubificid worms. Status Report No. 1 on Clinch River Study, ORNL-3119.

Subproject 4.008 -- The biogeochemistry of strontium and calcium in mollusks of the Clinch and Tennessee Rivers.

Date initiated: May 1960 Date completed: July 1961

Research personnel: D. J. Nelson, R. C. Early, and N. A. Griffith

Objectives: To determine the content of stable Sr and Ca, and $Sr^{90}$ as a function of species and location in the Clinch and Tennessee Rivers, and to obtain information on long-term changes in stable Sr to calcium ratios in clams by analysis of prehistoric indian midden, pre-TVA, and present-day clam shells.

Justification: Mollusks are abundant throughout the Tennessee River system. Preliminary analyses show that $Sr^{90}$ is concentrated in clam shells by a factor of $10^3$ or $10^4$ over that which is found in river water while recent data show that fish concentrate $Sr^{90}$ only by a factor ranging from 10 to 35. In addition the mollusks are relatively immobile in the river bed while fish are highly mobile. Clams may be a highly sensitive integrating sampler for $Sr^{90}$ in river waters. If this is the case, the $Sr^{90}$ content of clams can be utilized to describe the loss of $Sr^{90}$ from river water as a function of distance downstream from the source of release.

Procedures: Clams were collected from the Clinch and Tennessee Rivers and the shells analyzed for stable Sr and Ca, and $Sr^{90}$. Shells of some of the same species of clams have been obtained from prehistoric indian kitchen middens located where present-day collections have been made. Series of shells collected prior to the construction of TVA dams have been obtained for two species from the University of Michigan Museum of Zoology. Statistical analyses include regression analyses to determine if there is a variation of Sr and Ca with age. An analysis of

variance was used to compare Sr and Ca as a function of species, and collection site.

Reports:

Nelson, D. J. 1962. Clams as indicators of strontium-90. Science 137(3523): 38-39.

Nelson, D. J. 1962. Strontium, strontium-90, and calcium analyses of Clinch and Tennessee River clams. ORNL-TM-270.

Nelson, D. J. 1962. The strontium and calcium relationships in Clinch and Tennessee River mollusks. Radioecology. Proc. of First Nat. Symp. on Radioecology. Reinhold Publ. Corp., N. Y., N. Y. (In press).

Nelson, D. J. 1963. Deposition of strontium in relation to morphology of clam (Unionidae) shells. Proc. XV Intern. Cong. of Limnol. (In press).

Subproject 4.009 -- The effect of effluent atomic wastes on a *Chironomus tentans* Fabricius population as determined by salivary gland chromosome analysis.

Date initiated: July 1960 Date to be completed: June 1963

Research personnel: B. G. Blaylock, D. J. Nelson, and D. L. Lindsley

Objectives: (1) To determine the frequency of chromosomal aberrations in free living populations of *Chironomus tentans* Fabricius from uncontaminated areas and from areas which have been receiving liquid low-level radioactive wastes over a period of seventeen years. (2) To determine the degree of differentiation of an Oak Ridge population from Canadian and European populations by the frequency and kind of chromosomal aberrations.

Justification: Larvae of Chironomus tentans Fabricius live in the radioactive bottom sediments of White Oak Creek and the Clinch River. The radioactivity adsorbed on the silt results in a continuous dose of ionizing radiation to these larvae. This population has been subject to ionizing radiation significantly higher than background since the first release of radioactive waste material to this area in 1943.

In a survey of the invertebrates of the Clinch River, Chironomus tentans larvae were observed with unusual characteristics. A preliminary investigation was made in 1960 to determine whether C. tentans would be a suitable organism to use for a cytogenetic study. Besides having a life cycle which includes a relatively long aquatic larval stage, C. tentans has large polytene chromosomes in the salivary glands. Considerable background data are available on these easily identified chromosomes. For example, they have been mapped by Beerman in Germany, and the chromosomes of Canadian and European populations of C. tentans have been analyzed by Acton. The latter worker evaluated the degree of speciation in these widely separated population.

The preliminary investigation, besides revealing C. tentans as an ideal organism for cytogenetic studies, indicated that there was a high frequency of chromosomal aberrations in the population from the contaminated area. Related experimental studies on populations of Drosophila melanogaster have been

conducted by Bruce Wallace. Drosophila free of recessive lethal and semilethal mutant genes in their second chromosome were exposed to .9 r/hr. and 5.1 r/hr. of chronic gamma irradiation.

These Drosophila in population cages were exposed to chronic irradiation from external emitters for approximately three years. Populations greater than 10,000 were considered large populations. It was found that the irradiated populations accumulated recessive lethals in the second chromosome much faster than the control population and maintained them at a much higher frequency. In the proposed study, the Chironomus population in its natural environment is receiving from 4 to 200 rads/year from both internal and external emitters.

The frequency of mutations is considered to be directly proportional to the radiation dose, whereas, the frequency of chromosomal aberrations increases as the 1.5 power of the radiation dose. Since two hits are required for chromosomal aberrations and mutations may be produced by one hit, more mutations than aberrations are expected from equal doses of radiation. However, instead of testing for mutations in one chromosome, all chromosomes from each individual will be examined. The individuals analyzed will come from natural populations which are much larger than laboratory populations. In contrast to the Drosophila studies which determined mutations by genetical methods the chromosomes of Chironomus will be examined microscopically for aberrations.

Since many concepts of genetics have been formulated from

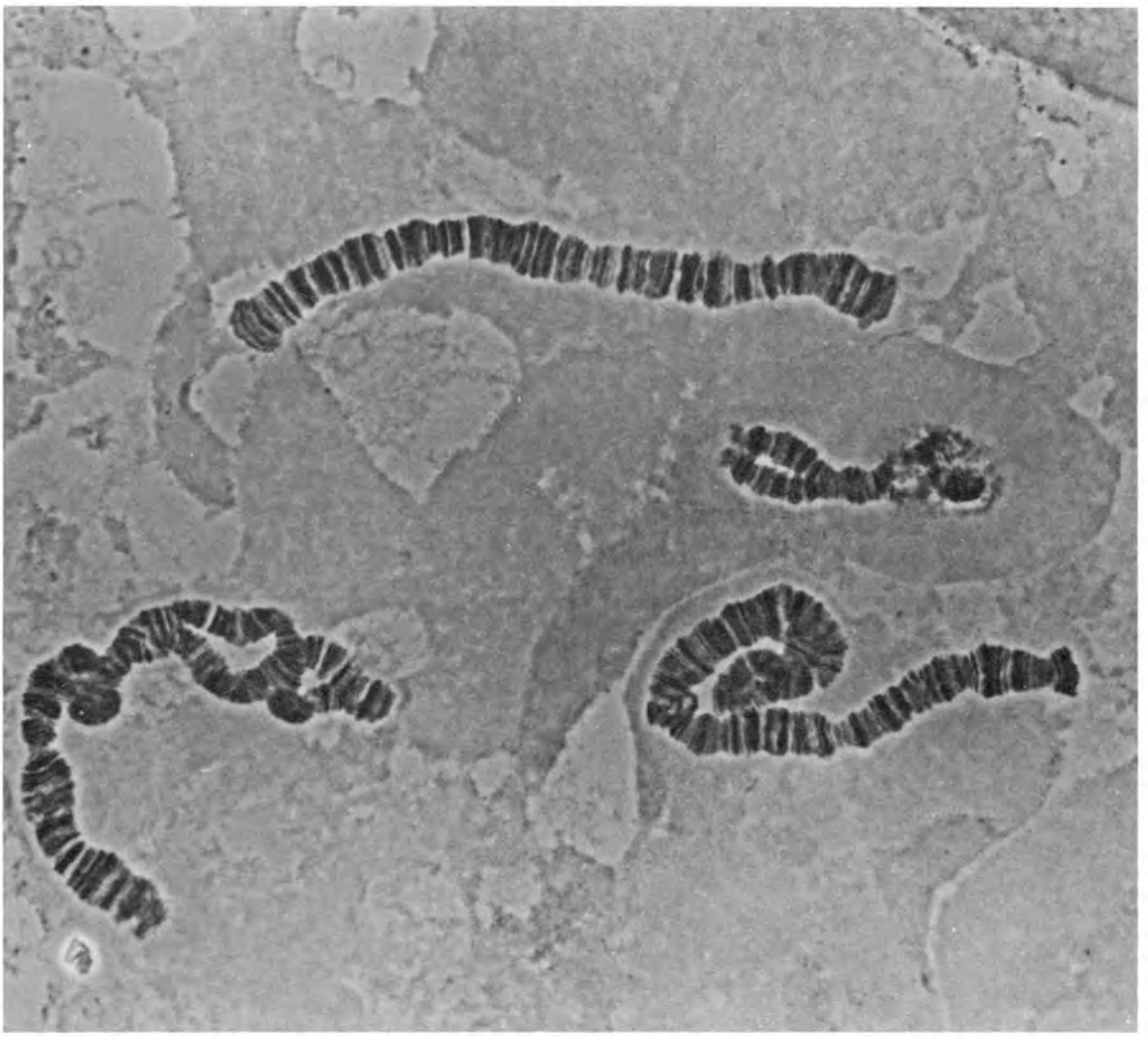

Fig.16. The salivary gland chromosomes of Chironomus tentans from the Clinch River. At the top is chromosome number three. Immediately below it is the short fourth chromosome which contains the Balbiani Rings. At the bottom left is chromosome number two which contains an inversion. Chromosome number one with a short inversion in the left arm is at the bottom right (Subproject 4.009).

experiments on Drosophila it is always necessary to test these in other organisms. Chironomus offers a good biological contrast to Drosophila in having a short adult stage with a long aquatic larval stage.

Since populations from uncontaminated areas will be studied this material can be used to compare the chromosomes of this area with those of areas already studied by Acton. This would then give additional information on the speciation of this organism.

Procedures: Chironomus tentans Fabricius larvae will be collected from the contaminated portions of White Oak Creek and the Clinch River and from other local populations living in noncontaminated areas. Salivary gland squashes will be studied and analyzed for chromosomal aberrations such as inversions, deletions, and translocations by means of phase-contrast microscopy. The number of chromosomal aberrations in organisms from contaminated and uncontaminated areas will be compared by means of a chi-square test. The inversions found in the population from the uncontaminated area also will be compared with the ones found in Canada and Europe.

Reports:

Nelson, D. J. 1961. Radiation effects on biota. Status Report No. 1 on Clinch River Study, ORNL-3119.

Nelson, D. J. 1962. Estimated radiation doses received by Diptera with life stages in bottom sediment. Status Report No. 2 on Clinch River Study, ORNL-3202.

Nelson, D. J. and B. G. Blaylock. 1963. The preliminary investigation of salivary gland chromosomes of Chironomus tentans Fabr. from the Clinch River. Radioecology. Proc. First Nat. Symp. on Radioecology. Reinhold Publ. Corp., N. Y., N. Y. (In press).

Subproject 4.010 - The biogeochemistry of strontium and cesium in the white crappie (Pomoxis annularis).

Date initiated: July 1, 1961 Date to be completed: June 1963

Research personnel: D. J. Nelson, R. C. Early, and N. A. Griffith

Objectives: To determine the uptake and metabolic turnover of Sr and Cs by fresh-water fish from a river environment contaminated with $Sr^{90}$ and $Cs^{137}$, to determine whether the ratio of radioactive atoms to stable atoms (specific activity) in the environment may be utilized to predict the body burden in fish subject to chronic releases of low-level radioactive wastes, and to determine if there is a seasonal change in the Sr and Cs concentration in fish tissues.

Justification: The Clinch River receives small quantities of low-level radioactive wastes, including $Sr^{90}$ and $Cs^{137}$, released by Oak Ridge National Laboratory. These released radionuclides are found subsequently in food and game species of fish which are consumed by humans. Because fish in the Clinch River may enter and leave areas contaminated by radioactive wastes, the body burden of $Sr^{90}$ and $Cs^{137}$ in these fish varies by several orders of magnitude. Thus, an evaluation of the potential hazard to humans may be made only within wide statistical limits.

By using the relationship of specific activities of $Sr^{90}$ and $Cs^{137}$ between water and fish tissue, radioecologists may be able to predict the potential radionuclide burdens in fish living in habitats receiving continuous releases of radioactive wastes.

Procedures: Recoveries of fish tagged in the Clinch River showed that white crappies tended to remain in the vicinity of original capture. Therefore, this species will be sampled because the relationship between radionuclide contamination of fish and water can be defined more clearly in a species of fish that is resident in the Clinch River. A minimum of 10 fish will be collected each month and flesh and bone samples will be analyzed for stable Sr and Cs by flame spectrophotometry. These same samples will be analyzed for $Sr^{90}$ and $Cs^{137}$.

The concentration of stable Sr in river water has been determined from water samples taken over the period of a year. Preliminary stable Cs analyses of river water will be augmented with the analysis of additional samples. Quantitative measurements of $Sr^{90}$ and $Cs^{137}$ released to the river are available from the Applied Health Physics Section. Therefore, it will be possible to compare the specific activity expected in fish from the relationship $\frac{r(\text{tissue})}{r(\text{water})} = \frac{R(\text{tissue})}{R(\text{water})}$, where $\underline{r}$ = total atoms of an element and $\underline{R}$ = radioactive atoms of the same element, with the specific activity observed by chemical and radiochemical analyses of fish tissue. It should be emphasized that this

relationship is valid only for continuous releases of radionuclides which are accumulated by organisms having a life span much less than the radioactive half-life of the radionuclide involved. This is true for fish, $Sr^{90}$, and $Cs^{137}$.

Deviations from expected specific activities in fish tissue may occur because releases of radioactive wastes are not constant. In order to evaluate the possible effect of varying environmental specific activities, the biological half-life of Sr and Cs in white crappies will be measured in fish held in laboratory aquaria. These fish will be contaminated in the laboratory by the feeding of contaminated food and the rate of loss of radioactivity can be ascertained by periodic counting of the live fish in a gamma scintillation detector. For these experiments $Sr^{85}$ will be used instead of $Sr^{90}$.

Reports:

Nelson, D. J. 1962. Strontium and $Sr^{90}$ in the white crappie. In Health Physics Division Annual Progress Report for period ending July 31, 1962, ORNL-3347.

Nelson, D. J. 1962. Strontium in white crappie flesh and bone. Status Report No. 4 on Clinch River Study. (In press).

Subproject 4.011 -- Application of radionuclide uptake and bioaccumulation data to the study of fish population dynamics.

Date initiated: September 1961 Date to be completed: March 1963

Research personnel: R. E. Martin and D. J. Nelson

Objectives: To test a new method of applying data on the uptake and

bioaccumulation of radionuclides as a tag for the study of fish populations, to survey the radionuclides accumulated in the scales of small-mouth buffalo, Ictiobus bubalus (Rafinesque), a commercially important food fish taken from Watts Bar Reservoir, and to determine the age and rate of growth for smallmouth buffalo in Watts Bar Reservoir and the Clinch River.

Justification: Fish living in the Clinch River below the mouth of White Oak Creek inhabit an area where they are exposed to low level radioactive wastes from Oak Ridge National Laboratory. These radionuclides are accumulated by the fish either directly from the water or through the food chain. Autoradiography of fish scales showed localized rings of radioactivity which presumably were associated with the growth of fish when they were in areas contaminated by radioactive wastes. The possibility appears that these rings may be used as a fish mark. Present-day methods of marking fish by the attachment of metal or plastic tags are used to study movement, but have a disadvantage in that they restrict the movement and inhibit the growth of the tagged fish. The development of a radionuclide tagging method where the fish would be marked without being handled or injured would provide a means of studying the animal without altering its behavior or growth.

Smallmouth buffalo are taken in quantities ranging from 50,000 to 60,000 pounds per year by commercial fishermen on Watts Bar Reservoir and sold as a food fish. A study of the

Fig. 17. Electro-fishing for smallmouth buffalo (Ictiobus bubalus (Raf.)) off ecology research boat in an embayment of the Clinch River. Electrodes are mounted fore and aft of boat. Man in forward part of boat is operating foot pedal control of shocker (Subproject 4.011).

radionuclides accumulated in the scales of these fish would provide an indication of their movement in and out of contaminated areas.

Few studies have been made on the age and growth of the smallmouth buffalo and the numbers taken in the radioactivity survey provide an excellent opportunity for learning more about this commercially important species.

Procedures: A laboratory experiment is in progress in an attempt to tag fish with $Cs^{134}$ through feeding. Through autoradiography of the scales proof is sought that these fish deposit a ring of radioactive cesium around the periphery of the scale when they are eating contaminated food. The presence and location of this ring will be used as an identifying mark. Correlation between autoradiographic rings and the growth phenomena exhibited on the scale surface would then be used in determining when the fish was in a contaminated area. Another phase of this experiment is attempting to determine if radioactive materials are translocated from other tissues to areas of new scale growth when contaminated fish are fed on noncontaminated food.

Smallmouth buffalo are being taken from White Oak Creek and the Clinch River to its confluence with the Tennessee River. Scales from these fish are being autoradiographed in an attempt to determine when they were in a contaminated area as an indicator of movement.

Large numbers of smallmouth buffalo taken from Watts Bar Reservoir are brought to a fish wholesaler in Knoxville. Scale

samples were taken from these fish over a period of four months during the growing season. The scales were surveyed to determine which contained enough radioactivity to give an autoradiographic exposure. These scales are now exposing in an attempt to determine when and for how long the fish were in areas of contamination. Measurements were taken on these commercial fish and will be used in conjunction with scale impressions in determining the age and instantaneous growth rate for this species. The radioactive rings will be correlated with age and growth of this fish in contaminated areas.

Reports:

None