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THE ECOLOGY OF A SMALL FORESTED WATERSHED TREATED WITH THE INSECTICIDE MALATHION-S^{35}

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of the Ohio State University

By

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*****

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JUSTIFICATION: NEED FOR RESEARCH ON THE ECOLOGICAL EFFECTS OF INSECTICIDES

THE PROBLEM

One of man's greatest problems is mistaking the perceptual world for total reality. Man easily perceives the economic importance of insects - their benefits and destruction. He encourages or manages the beneficial ones for increased benefits, destroys harmful ones in order to decrease economic loss and annoyance. Insecticides, along with cultural and biological controls, have been a major effective tool in reducing effects of harmful insects. They have, however, produced many obvious effects on other forms of animals and on plants essential to satisfactory ecological function of communities and to man within these communities. Less obvious effects on plants and animals, long overlooked by competent ecologists, are being discovered at a rate which causes thoughtful people to be alarmed over the remaining unknown. English (1955:280) stated that "while we may deplore mistakes that have been made, we cannot join wholeheartedly those who are always afraid of upsetting the 'balance of nature'.... Man is interested in upsetting the balance of nature in his own favor." Such a statement may be as argumentative as real. Nevertheless, it is imcompatible with Aldo Leopold's (1949) concept of a land ethic, of man living <u>in harmony</u> with nature; it denies the interdependence of man with his total natural environment, and in part explains the ecological unawareness and disregard that has characterized some use of insecticides.

The effects of pest control, besides spectacular expected ones, have been subtle and have indirectly touched almost everyone. The ecologist, unable to maintain current studies on the effects of insecticides since the explosive use of DDT and other organic compounds beginning in the mid 1940's, has generalized on the effects of insecticides from existing ecological principles. Economic facts and ecological generalities have been in opposition, and the latter have met with little acceptance.

There is widespread speculation among ecologists and conservationists that elements of nature and their dynamic interrelationships may be so adversely affected by insecticides that problems even greater than the one for which the toxicant was used may arise. Aldo Leopold (1953:147) presented the wise saw that "To keep every cog and wheel is the first precaution of intelligent tinkering." This attitude is also reflected by Cottam (1960:45) who said, "Preservation and conservation is far better than costly restoration." These attitudes are in direct opposition to that of F. C. Bowden (Worthington, 1959:1100) who, unable to quote any case where the secondary trouble had resulted in greater economic loss than the primary one, concluded that, on the whole, there was always a net gain from insecticide use.

Objectivity in resolving a problem which deals with aesthetics as well as economics is very difficult. Emotionalism seems to be one of the metabolites of applied insecticides. Such disquiet is, in large part, fear of the unknown immediate and after effects of the use of an apparent boon to mankind. The results of studies to date help little; they ask more questions than they answer. Insecticide use, as yet, has few answers that fall into categories of black or white - use or do not use; insecticide effects on the natural ecosystem are a zone of gray. It is obvious that much research is needed, but it is unrealistic to expect a moratorium on insecticide use until man's scientist-servants have provided all answers to questions about safe use. It is realistic to require that all available knowledge be used to decide whether an insecticide is needed, and if so, which one; that caution be used in balancing known effects with potential hazards; and that use be in a method, area, season, time, quantity, and formulation that will provide minimum hazards to man and his environment. Such an idealistic situation seems only possible in a well-informed, conservation-educated, democratic society that has provided for itself a technically competent managerial organization with policy and authority.

Widespread and intensive programs of insecticide use have caused many people concern over their possible ecological effects. It is difficult to appraise whether an application is widespread when an aircraft sprays a ton or when 1000 people each use 2 pounds of poison in their gardens. Nevertheless, within undetermined limits, any insecticide use creates a new environment proportional to the rate and area over which it is used. Results have been and will continue to be chemical pollution of man's environment.

Most ecologists will admit their inability to say whether the well-documented reports of wildlife losses from insecticidal programs are significant or not. The immense problems of detecting and measuring effects of insecticides are compounded by problems of evaluation of significance of changes in terms that can be equated to human mental and physical health, to economics, to recreation, and to aesthetics. The expression of significance will be produced by experienced scientists working with wise specialists in the fields to which ecological loss must be equated. Ecological techniques, knowledge, and wisdom are not yet available for the task.

George (1957:11) explored the problem of the relationship and expression of ecological effects of insecticides on humans. He stated (1957:39) that so far as he knew,

> ...there has been no thorough study done on any single area over a reasonably long period of time. A fundamental population study dealing with known marked individuals over a period of time is long overdue. Until a study of this nature is undertaken, there will be no answer to charges that the biotic balance of an area is being seriously upset. The interaction of biotic changes in habitat, population density, biogeochemical cycles, food change, etc. is not an easy matter to study. Results will not be forthcoming in a single year or even, in some cases, several years. Yet eventually fundamental studies of this nature must be done unless we are to continue in a state of ignorance, possibly dangerous ignorance.

George (1957:35 ff) and Leedy (1959) outlined research needs that combine laboratory and field studies.

Hickey (1961), Glen (1954), Beard (1960:21), Wallace (1960:67), DeWitt and George (1960:4), Hoffman and Drooz (1953:187), and Linduska (1952:147), pointed out the major problems of diagnostic work on the effects of insecticides. "Attempts to work out the effect of an insecticidal treatment on a whole ecological system have seldom been carried out. This type of research requires highly organized team work, and it is beset by sampling problems that are often quite difficult to resolve" (Hickey, 1961:25). Yeager (1956:264) stated that even though several thousand titles are available on effects of insecticides on wildlife, evaluation of such losses is presently impossible. He attributed inability of the analyst to "the lack of results from controlled experimentation."

The possible detrimental ecological effects of an insecticide application gleaned from the literature and based on ecological principles is amazingly complex; few effects are mutually exclusive. The basic effect is the reduction in efficiency of the ecosystem following reduced energy and nutrient cycling rates. Animal population changes, increases in some, decreases in others, occur from direct and secondary mortality; reduced reproductive potential and survival of young; increased susceptibility to predation; selection for insecticide resistance; and altered behavior patterns including feeding. Elton (1958:142) has reported reduced crop productivity following use of insecticides, probably due to altered metabolic activity of the soil community.

These effects clearly indicate the complexity of the problem, the needs for confirmation or refutation of each item, and the gaps in knowledge of natural field biology and the effects of unnatural factors upon life.

Among the least known effects of insecticides are chronic or long-range effects of repeated sublethal exposure of animals to insecticides (George, 1957:24). Little is known about the real difference between toxicity and hazard. The problem is especially acute in aquatic environments where low dilutions of leached and eroded poisons enter the streams (Tarzwell, 1959:139). Animals tend to suffer morbidity, not mortality, and the aftereffects on growth, reproduction, and normal behavior and later death are unknown. See Cottam (1960:44), DeWitt, et al. (1960:2), Zavon (1958:12), and DeWitt and George (1960:16).

A. C. Worrell (Conservation Foundation, 1959:22) stated that:

> ...there is no valid way to resolve these conflicting valuations in a quantitative measurement of the public interest....It may not always be possible to show that a particular use of pesticides is completely beneficial or even that it represents the best compromise of interests.

He contended that major pesticide decisions are likely to be satisfactory if the process followed in making them conforms to the following criteria: (1) bringing all available facts to bear on the decision, (2) considering the value preferences of the public, (3) considering legitimate public interests, (4) protecting resources and organisms that will be harmed needlessly, (5) investigating the the pesticides and their total effects, (6) educating the public to dangers and proper use of poisons, and (7) protecting the public from worthless pesticides or misrepresentation of effects.
Additional limitations might be that applications be made with the safest available materials and methods where there is an emergency need, and where less drastic controls cannot be reasonably employed. Though some alternate methods may be more costly than insecticides, George (1959:253) contended that the public will generally support the safer operation. Hickey (1961:18) said that if all hypotheses

about an insect control program are true, then wildlife loss may simply be a part of the price that society has to pay in the long run.

Many questions have been raised about the uses and effects of insecticides.

> These are questions which an enlightened citizenry is entitled to ask but not always able to judge. In the modern technological world, we still want the technical efficiency of managerial government with the traditional responsibilities of public servants in a democracy. What appears to be lacking in insect-control machinery of some states is an administrative realization of the emotional impact of modern insecticides on the public mind, an alertness to all the questions that are puzzling conservation minded people, a willingness to admit that wildlife losses are taking place under certain conditions, and a sense of responsibility to show exactly how these losses are being kept to a minimum and why these losses are justified. When these are lacking, public fears regarding an entrenched bureaucracy are bound to mount (Hickey, 1961:15).

Only within the atmosphere of these traits can sound, progressive research on insecticides and ecology be conducted.

OBJECTIVES AND SCOPE

The objectives of this study were (1) to determine the effects of an aerial application of malathion to one of two adjoining, forested watersheds, and (2) to develop techniques for the field study of an isotope-labeled insecticide that will permit (a) the rapid location of the insecticide and its metabolites in lethal and sub-lethal quantities within the ecosystem; (b) the determination of the effects of the insecticide on species interaction such as predator-prey relations; and (c) the study of soil-water-insecticide relations within the watersheds.

Fulfillment of these objectives was sought in three project subdivisions. Sub-project I, Faunal Studies, concentrated on measurement of population differences before and after spraying. Sub-project II, Preparation and Application of an Isotope-Labeled Insecticide, dealt with the methodology of preparation, acquisition application, and measurement of quantities of malathion-S^{35}. Sub-project III, Fate of the Insecticide Following Application, studied the distribution and re-distribution of malathion and its effects within the ecosystem.

The field and laboratory project was conducted during 2 years. Some factors which limited the scope of the project were funds; time required to cover the study area; availability of competent, qualified assistants; and limitations in existing techniques and the ability to develop new ones to obtain needed information.

Criteria for the study area were: remote, forested, essentially natural, having streams, and preferably with stream gaging and

and meteorological equipment in operation. A study of two 20-acre watersheds with their inclusive populations was adopted. The reasons for this selection were: (1) the cost and adequate supervision of intensive studies on small plots over a large area were prohibitive, especially in light of existing, questionably-accurate faunal sampling and census techniques; (2) the watershed was believed to be the smallest practically manageable natural land unit with some faunal isolation and potentially-measurable faunal interactions; (3) the watersheds selected were the largest areas practical for study with the time and funds available to meet the objectives of the project; (4) these areas were considered the smallest suitable for a typical aerial application from which might be detected the possible cumulative or inter-related effects on an insecticide on wide-ranging mammals, birds, and reptiles. The treated area probably responded to the insecticide treatment more nearly like a small public or private woodland park than a forest.

Justifications for adjoining treatment and non-treatment areas were: (1) generally, the closer the areas were to each other, the more homogeneous they were, (2) travel time between areas was minimal allowing observations to be made on both areas without time-lapse variables, (3) if contamination of the check area occurred in amounts decreasing with distance from the treated area, and if effects were observed in decreasing magnitude with distance from the area, further credibility would be lent to conclusions about effect, however slight. Both spatial gradients as well as temporal gradients of measurable influence were theoretically possible.

The general approach to the problem was, therefore, to study as intensively as possible the ecology of two adjoining, forested watersheds for 1 year; to spray aerially one of these in the spring of the second year with malathion-S^{35}; and observe the effects of the insecticide and its distribution within the ecosystem as compared to the untreated watershed.

MALATHION IN FOREST INSECT CONTROL

Forest insects account for a loss of one-fourth of the annual production of forest products in the United States (National Academy of Science, 1962:30). Benedict (1959:245-46) described the broad types of losses. Craighead and Hutchins (1951:5), Prebble (1959:255) and Yuill and Isler (1959:263) outlined the forest insect threat and the history of the development of forest insect control. Forbes and Meyer (1956:8:3) and Benedict (1959:247) discussed costs. Insect damage has begun to take increasingly realistic form as human demands for forest products and consequent cost per forest-product unit, including recreation, have increased; as new forest composition has developed following harvest; and as forest management, especially on the small forest, has fallen behind utilization. Losses are great because insect attacks occur over wide areas involving many ownerships that are not united in a cooperative control effort.

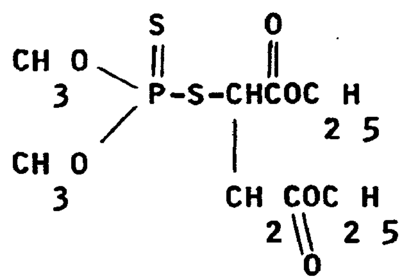
As late as 1959, little work had been done with malathion in forests and the compound was considered only promising (Yuill and Isler, 1959:264). Though less toxic to insects than DDT, the most widely used forest insecticide, it has the advantages of being essentially nontoxic to warm blooded animals at prescribed application rates. It has most frequently been used on conifers. Schuder (1960) reported on its use against the bagworm (*Thyridopteryx ephemeraeformis*). Stephenson (1958:210) using it against Nantucket pine moths (*Rhyacionia frustrana*) attacking loblolly (*Pinus taeda*) and short leaf pine (*Pinus echinata*) plantations, found trees protected with a water emulsion of 0.5% malathion grew 2.1 ft taller than controls. Michelbacher (1954) found that nicotine rather than malathion allowed natural agents to function in walnut aphid (Chromaphis juglandicola) control in California. Four per cent dust of malathion gave control of rose chafer (Macrodactylus subspinosus) on walnut trees (Juglans sp.) during June 1953 and a rapid kill of a heavy blister beetle (Epicauta sp.) infestation on mimosa trees (Acacia sp.) (Tinger, J. H., pers. correspondence, 1960). Used as a dip to prevent deterioration of pulp sticks at the mill, it was not as effective as other insecticides tested (Neel, 1958:8). When malathion is applied to pecan (Juglans sp.) trees at no more than 12.5 lbs. actual per acre, pecans can be marketed immediately. As with most foodstuffs, an 8 ppm tolerance is set by the U. S. Department of Agriculture (1963a and 1963b).

MALATHION

CHARACTERISTICS

Malathion, 0, 0-dimethyl dithiophosphate of diethyl mercapto-succinate, ($C_{10}H_{19}O_6PS_2$) is a broad spectrum, general purpose, residual organo-phosphate insecticide and acaricide. It is a contact and stomach poison and kills by some vapor or fumigant action (Robb, 1958:14). It was originally called compound 4049 and malathon. Malathion was introduced in 1950 (Martin, 1953) and released to the general public in 1953 by American Cyanamid Company after tests beginning in 1949 (Anon., 1953a).

The chemical structure is:



(Fisher, 1956:73).

Some of the physical and chemical properties of malathion which bear on its use in this study are: yellowish to brown oil with a strong garlic odor, water solubility of 145 ppm, vapor tension of about 10^{-5} mm/30^{o}, miscible with most organic solvents, technical grade of 95 to 98% active ingredients, rapid hydrolization above pH 7.0 or below pH 5.0 and relative stability between pH 4 and 7 (Norris et al., 1954:572), stability in aqueous solution buffered at pH 5.26 (Martin, 1953:152), and compatibility with most spray materials. Its molecular weight is 330.3. Extensive reviews have been prepared by Frear (1955), Metcalf (1955), Negherbon (1959), and Spiller (1961). Spiller's review based on 658 cited technical references is most complete.

Malathion ranks second only to parathion in world production of organo-phosphates (Metcalf, 1959).

CHEMICAL BREAKDOWN

Krueger and O'Brien (1959) stated that there are 12 *in vivo* malathion metabolites, only eight of which have been described. Perry (1960:220) found 10 *in vivo* metabolites; 6 were named. *In vitro*, there are 10 metabolites (Seume and O'Brien, 1960:37). Spiller (1961:251) reported seven distinct compounds, products of breakdown, found in urine and droppings. *In vitro* or *in vivo*, the picture of insecticide breakdown is yet incomplete. This hiatus presents critical limitations to the tracing of malathion and its byproducts throughout an ecosystem. At present, the known natural *in vitro* byproducts appear to be nontoxic; there is no evidence that unknown metabolites are any different. These compounds and their present and future role in the ecosystem can only be studied as a group.

In vitro malathion is hydrolized readily by acid (widespread in the forest ecosystem) chiefly to 0, 0-dimethylphosphorothionic acid (Mattson and Sedlak, 1960:110). Cook and Yip (1958:411) explained that the mono-acid derivative is formed by the removal of one alcohol group from the diethylsuccinate portion of the malathion molecule.

Infrared heat causes isomerization of malathion to S-methyl thiolphosphate (isomalathion) (O'Brien, 1956:489).

CHROMATOGRAM STUDIES

Using the methods of Cook (1954), paper chromatograms were prepared of 4 µg of malathion-S^{35} after subjecting the liquid to ultraviolet light. Table 1 presents the results.

Table 1. - Radioactivity of 4 µg of malathion and its metabolites as measured from 25 mm diameter discs from a paper chromatogram.

Sample Number	Location on Strip in mm	Approximate R_F Value	Radioactivity in net cpm
1	0-25	0.14	1.7
2	25-50	0.28	1.6
3	50-75	0.43	1.5
4	75-100	0.57	1.8
5	100-125	0.71	2.7[1]
6	125-150	0.86	4.1
7	150-175	1.00[2]	1.7

[1]Significant at the 10% level of confidence
[2]R_F of malathion is 0.93

Another chromatogram was prepared of a 40 µg malathion-S^{35} sample exposed to a sunlamp for 42 hr. The results are in Table 2.

These studies indicate that the sulfur-35 was distributed throughout the metabolites but that certain of them had higher quantities of the radionuclide than others. The measure of radioactivity in segments of the ecosystem therefore may vary due to adsorption and absorption as well as to differential uptake or

Table 2. - Radioactivity of 40 µg of malathion and its metabolites as measured from 25 mm diameter discs from a paper chromatograph.

Sample Number	Location of disc on strip in mm	Approximate R_F Value	Radioactivity in Net cpm
1	0-25	0.08	1.5[1]
2	25-50	0.16	10.6
3	50-75	0.24	128.3
4	75-100	0.33	147.7
5	100-125	0.41	61.5
6	125-150	0.49	1996.0
7	150-175	0.57	4998.0
8	175-200	0.65	2494.0
9	200-225	0.73	163.5
10	225-250	0.82	3.5

[1]Not significant at the 10% level of confidence; all others significant.

segration of metabolites. For example, 10 volumes of metabolite A having a unit cpm of 10 are equivalent to 1 volume of metabolite B having a unit cpm of 100.

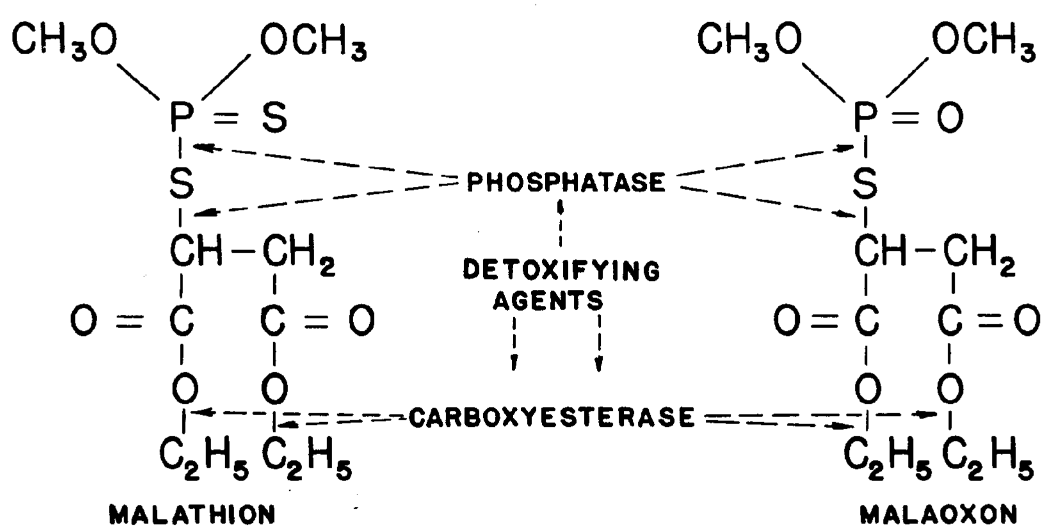
MECHANISMS OF ACTION

The mechanism of action of malathion *in vivo* has received intensive study. Malathion is generally considered an anti-cholinesterase but as Spiller (1961:257) explained: "It is now clear that pure malathion itself is not an anticholinesterase; rather it is converted to an active anticholinesterase by the animal." Before discussing this transformation and action, the following brief review of the cholinesterase system may be of value in understanding the mode of action of the poison.

In mammals stimulations from an external source (e.g. touch) or internal source (muscle or gut) are relayed by the somatic and autonomic nervous systems respectively to the central nervous system. This relay is accomplished electrically along an axon by interchange of positive sodium ions on the outside and negative potassium ions on the inside of the axon sheath. This wave of reverse polarization progresses to a neuromuscular junction, the end-plate, between the axon and the effector cell, e.g. a muscle, and releases acetylcholine (ACh). ACh diffuses across the gap and causes the muscle to contract. (See O'Brien, 1960.)

ACh crosses the gap and initiates events leading to a fresh impulse. If allowed to persist, it would continue its stimulation and destroy the required proportionality between input stimulus and event. ACh is rapidly hydrolized by the specific catalyst acetylcholinesterase (AChE) to choline and the acetate ion. AChE is universally present in nerve and muscle tissue and is localized at neuronic surfaces. Normal synaptic functioning depends on its presence. The presence of an anti-AChE interferes with acetycholine from hydrolysis by attachment of a phosphoryl group to AChE. ACh accumulates and its action is thereby prolonged and intensified. Expected symptoms of this prolonged action in mammals are characteristic of cholinergic intoxication, namely: Muscarinic and nicotinic reactions, vaso-dilatation, feeling of warmth, bounding pulse, profuse sweating, salivation and lacrimation, nausea and vomiting, inspiratory difficulty and slight cough, with death resulting eventually from respiratory failure. O'Brien (1956:489) noted anomalies in his experiments on the effects of malathion on cockroach cholinesterase activity. Cholinesterase showed a preliminary sharp depression and then rose until death of cockroaches.

Malathion itself, once believed to be an anticholinesterase, is now known to be converted _in vivo_ to an anticholinesterase, malaoxon (Spiller, 1961:257) the oxygen analog of malathion 0,0 dimethyl S-(1,2 dicarboethoxyethyl phosphorathiolate) (O'Brien, 1957:79). Matsummura and Brown (1961:1177) showed the points of enzymatic hydrolysis of malathion to malaoxon as:



Spiller (1961:257) suspected previous erroneous conclusions were due to malathion impurities, aging of stock solutions, and other causes.

Brown and Brown (1956:679), O'Brien (1956:489; 1957:79), and Spencer and O'Brien (1957:265) report on the effects of malathion on carbohydrate metabolism. The results are not conclusive.

ACTION WITH INSECTS

Malathion, invarying degrees, is toxic to insects. _Per se_ it is generally a poor inhibitor of cholinesterase but its high insect toxicity is the result of metabolism to malaoxon within insects, particularly in midgut and fatbody (O'Brien, 1957:162). Spencer and O'Brien (1957:265) reported that malathion initially produced a marked inhibition of cholinesterase in poisoned flies, but by the time death occurred the cholinesterase was largely reactivated, and therefore inhibition of this enzyme may not be the cause of death. They noted a lack of evidence on time-course of cholinesterase inhibition in poisoning insects by typical anticholinesterases. Respiratory patterns in malathion-poisoned insects are also unlike those obtained with use of other organo-phosphates. Other unexplained malathion activity and insect conversion mechanisms are reported. Lewallen (1959) found that resistant strains of *Culex tarsalis* mosquitoes converted malathion to malaoxon as did the susceptible strain (Lewallen 1959:767). Weidhaas (1959:445-46) failed to show that a strain of malathion-resistant houseflies had developed a mono-acid derivative conversion mechanism for detoxifying malathion. Malathion had no striking effect on housefly or cockroach glycolysis nor did it have inhibitory effect on acidogenesis, lactate production, or phosphorous incorporation (O'Brien, 1957:80-81).

Perry (1960:270) accounted in part for some of these anomalies. In the American roach enzymatic hydrolysis is less rapid than activation or oxidation, thereby allowing the lethal accumulation of malaoxon. Degradation of malathion is extensisve in the roach, however, through the pathways of phosphatases attacking P-S-C bonds which are predominant and carboxyesterases attacking the $COOC_2H_5$

moiety. More metabolites are produced by houseflies than cockroaches in their degradation of malathion. This degradation and rapid excretion involves hydrolysis of the diethyl succinate nucleus and of the P-S and S-C bonds.

ACTION WITHIN MAMMALS

Metcalf (1955:297) stated that "unfortunately, the biochemical processes in the Insecta and Mammalia, insofar as they affect the action of the organic phosphorus compounds, are remarkably similar." In the case of malathion, there is a difference in the metabolism of malathion by insects and mammals which makes it toxic to the former, virtually non-toxic to the latter. The carbohydrate cycle does not seem to be related to the insecticidal action of the compound. Malathion was altered quickly by rat liver homogenates whereas none of 11 other phosphates was altered under the same conditions. Cook *et al*. (1958:399) commented that this detoxification of malathion by liver may make it unavailable to the blood plasma cholinesterases.

Studies with P^{32} labeled malathion have shown that large amounts of malathion-derived material are excreted by mice in the urine and are largely compounds not extractable by chloroform. The per cent of the administered dosage recovered in the urine ranged from 73 to 20 (average 46%) for the 5 day period of daily dosage of the animals. No great efforts were made to obtain quantitative recovery of all the urine. Urinary excretion of P^{32} falls off rapidly after interperitoneal injection but is measurable after

96 hr (Mattson and Sedlak, 1960:107).

Malathion is activated in the mouse liver, less in the heart, and perhaps in the testis, by oxidation to malaoxon. Malaoxon hydrolizes or degrades vigorously in the liver, kidney, and lung. The low toxicity of malathion to small mammals is due to

> ...the vigorous hydrolytic degradation of either malathion or malaoxon at the carboxylic ester link. This hydrolysis outstrips the process of malaoxon accumulation caused by oxidation of malathion. In the cockroach, activation is much more rapid than hydrolysis, and malaoxon presumably accumulates steadily to a lethal level (O'Brien, 1957:163).

The presence or absence of balanced activating or degrading systems within these organisms therefore seems to account for the much greater toxicity of malathion to insects than to mammals.

In summary, some of the reasons presented for the low mammalian toxicity of malathion even though activated to malaoxon are (1) rapid detoxification by the liver, kidney, and lung making it unavailable to the blood cholinesterases, and (2) extensive metabolic hydrolysis and rapid excretion proceeding faster than oxidation and lethal accumulations.

MALATHION LONGEVITY

Gunther and Blinn (1956:168-69) explained that residues tend to disappear or lose their analytical identity at a constant rate which is a function of concentration. The fractional exponential decreases with time, or half-lives, are independent of initial concentration or magnitude of the deposit and are a fixed characteristic of the insecticide on or in a particular substrate.

They also commented (1956:176) that in general, the fates of persisting residues seem to involve simple mechanical losses as well as degradation losses. Such mechanical losses include volatilization, sloughing off, washing off by rainfall, and others. Shankland (1958:70) listed possible insecticide fates: adsorbed and deactivated, retained and activated later, washed off, blown away, evaporated, or metabolized.

Hornstein *et al*. (1955) stated that "the lower the vapor pressure of the insecticide, the more slowly it is replaced at the surface, and in turn the greater the fraction of the insecticide that may be rendered unavailable by decomposition." Malathion had the lowest vapor pressure of the insecticides studied by these men and the least toxicity of the compounds tested. They found that the addition of chlorinated terphenyls prolonged the effectiveness of the residues.

Since most studies of malathion residues have been made with fruits and vegetables they will not be presented here. Negherbon (1959) and Spiller (1961:275-280) presented summaries of longevity studies. Westlake and Butler (1953) reported, for instance, that no more than a trace of malathion was found on snap beans, cucumbers, and broccoli 6 days after being sprayed with from 2.5 to 9.75 lb/acre of malathion.

Matsumura (1960:453) reported that dissipation of malathion on the kidney bean, *Phaseolus vulgaris*, took place very rapidly during the first 3 days and reached a plateau of persistence. The rapid degradation in the first 3 days was due to evaporation of the initial deposit. In the persistence stage, dissipation was not due to migration, metabolic alteration, or plant growth but to evaporation.

Smith et al., (1954) found that on lettuce, residue declines were due to chemical changes as well as volatilization.

In general, the longevity and consequently the effectiveness of malathion varies widely depending upon factors of the environment, all of which can have some effect on this characteristic (Shankland, 1958:70).

EFFECTS OF WEATHER ON MALATHION

Malathion is recognized as being a very unstable insecticide. In general it may be said that residues fall to less than 1 ppm in 7 to 14 days under normal weather conditions and dosage levels (DeOng, 1956). The speed with which this occurs and consequently the effects of the insecticide are directly related to weather, particularly to extremes. Mistric (1954) has made comprehensive studies of the effects of weather on malathion in cotton insect pest control. Generalizations from his results must be made cautiously for interpreting weather effects in the forest environment. Mistric (1954:187) believed that climatic conditions are one of the most important forces affecting insecticide toxicity.

Weather effects vary with the other factors affecting toxicity: method of application; dosage; formulation; timing of application; variations in insect life stage, morphology, physiology, and genetics; rate of plant growth; chemical nature of the surface to which applied; and methods of recording infestation following a treatment

(Mistric,1954:187). Increased dosages of insecticides above those required to give good immediate control do not increase the period of residual effectiveness (Mistric, 1954:184).

If insecticidal spray applications have become thoroughly dry before it rains, the effectiveness of the application is not greatly reduced by rainfall (Mistric, 1954:19). Smith _et al_. (1954:184) reported that a heavy rain 2 days after application greatly reduced malathion residues on the vegetables they were studying.

Dew alone did not greatly reduce the toxicity of insecticides. Dew may have a relation to action of other climatic factors such as sunlight, high temperatures, wind, and rain (Mistric, 1954:194). Smith _et al_. (1954:184) seemed to suspect dew decreased poison residues.

Atkins and Anderson (1954:971) stated that "a high temperature (90° F) and a low humidity (30%) will substantially increase the mortality interval."

Malathion is most effective in the relative humidity range of 35 to 85%. The humidity range is not as critical as that of temperature (Mistric, 1954:194).

Malathion is most effective in the range of 70° to 90° F (Mistric, 1954:194). Sunlight effects are difficult to separate from temperature effects. Intense sunlight reduces the effectiveness of insecticides (Mistric, 1954:194). Hightower's studies (1959:840) indicated heat alone (100° F and over for 4 to 6 hr in absence of light) has little or no effect on residual toxicity of malathion for boll weevils. Heat plus natural light (approximately 13,000 ft-candles) adversely affected residue toxicity. Chemical additives are available to reduce ultraviolet detoxification (Shankland, 1958:12), though they were not used in this study.

SYSTEMIC ACTION

Malathion exhibits characteristics of only a mild systemic poison. Negherbon (1959:452) stated that the poison penetrates through plant leaves; when leaves are sprayed on one side, insects are killed on the other. There seems to be a 50% reduction in effectiveness incurred with passage through leaves. Solubility in water appears to be the limiting factor in efficient translocation and consequently in determining systemic poisoning activity. Malathion has been shown to penetrate into bean stems but with only a limited degree of absorption and translocation (Metcalf, 1960:405). It may be said, therefore, that malathion is mildly systemic and that in this role its effectiveness is reduced.

PHYTOTOXICITY

There is some evidence of phytotoxicity of malathion to a limited number of crop and ornamental plants (Hard and Ross, 1954; Martin, 1953:152; Clower and Matthysse, 1954:735; Schuder, 1960:33; and Spiller, 1961:271).

APPLICATION CONSIDERATIONS

The principle malathion formulations are 5 lb/gal emulsifiable concentrate, 25% wettable powder, and 4 to 5% dusts (Anon., 1957).

Multiple applications, as might be expected from the short life of malathion, provided better control. Clower and Matthysse (1954) presented results of experiments with the formulation selected for use in this project -- malathion, water, xylene, and emulsifier Triton X-100.

ASSAY

Determinations of the presence of malathion are made by gas and paper chromatography, an infrared method (Upham, 1960), and by colorimetric techniques for plants (Norris, *et al*., 1958). Measures of isotope-labeled malathion are made by standard radiological instruments.

REASONS FOR SELECTION

The Division of Entomology, Central States Forest Experiment Station, U. S. Forest Service, Delaware, Ohio, was requested to suggest a typical insecticide that was being used and would likely continue to be used in forest insect pest control. Malathion was selected as best meeting the objectives of this study. It has been extensively investigated and is well known. Malathion has been successfully labeled with P^{32} and S^{35}. It has acaricidal as well as broad insecticidal properties and is potentially useful as an aerial broadcast spray for large areas for control of the gypsy moth (*Porthetria dispar*) (Brown, 1961). Its low mammalian toxicity permits evaluation of the impact of its effects on lower animal forms on the ecosystem. This characteristic has also been responsible for conservationists recommending it to replace more toxic compounds. Its short residual toxicity allows evaluation of acute toxicity and secondary poisoning only, eliminating the many variables of chronic effects. DeWitt et al. (1960:5) stated that "...the quantities of malathion required to produce death from chronic poisoning were appreciably greater than those required to produce immediate kills." Its short toxic life presents possibilities for measuring and projecting or predicting effects on populations which would be impossible in the period of study with more stable compounds. Its short effective span also simplifies the measurement of the effects since long-residue compounds have cumulative effects that become more complex with duration of influence. It permits study of effects that do not have death as an end point. Study of the breakdown products, the nontoxic residues or metabolites and their effects, is also possible.

DESCRIPTION OF STUDY AREA

LOCATION

The 41.5 acre study area is located within a 160 acre U. S. Forest Service watershed management research area owned by the Muskingum Watershed Conservancy District. It is in unglaciated Tuscarawas County, Ohio, Sections 4 and 5 in Fairfield Township, Township 9 North, Range 1 West of the U. S. Military Survey (Peters, 1930) on land formerly known as the Krantz property (Fig. 1). The approximate center of the area is intersected by parallel

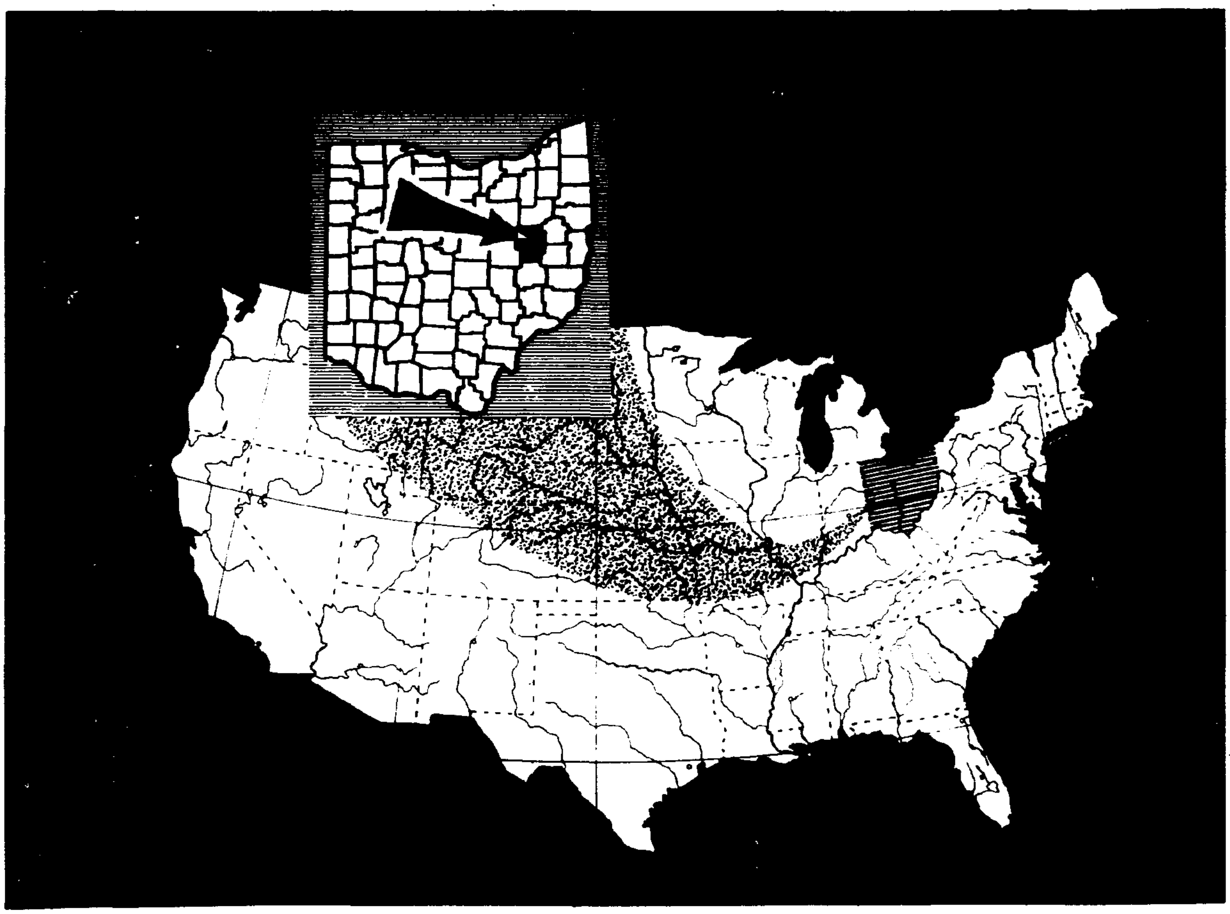


Fig. 1. Location of the study area in Tuscarawas Co., Ohio, U.S.A.

40° 34' 75" and meridian 81° 24' 75" and is 6.5 miles from Dover, Ohio, and 1.5 miles by road northwest of Dover, Dam.

SIZE AND TOPOGRAPHY

Two watersheds constitute the study area. Watershed no. 1 contains 23.4 acres; no. 2 18.1 acres. The small perennial streams of these lands join and flow into the Tuscarawas River 0.5 mile below their junction. Slopes of the watershed range from 5 to 50%. The range in elevation is from 290.2 m to 354.5 m (952 to 1163 ft), 64.3 m (211 ft). Fig. 2 shows the relations of the forest to other land uses. A topographic map is shown in Fig. 3.

Fig. 2. Aerial photographs of the 160 acre forest tract in which the study area was located. Watersheds are left of center; the Tuscarawas River flows southward in the right half of the pictures: Zoarville is in the upper right corner. The photo, CNK-2V-22, is by the Soil Conservation Service, taken 12 September 58. Scale: 1:20,000 or 1 in. equals 0.32 miles.

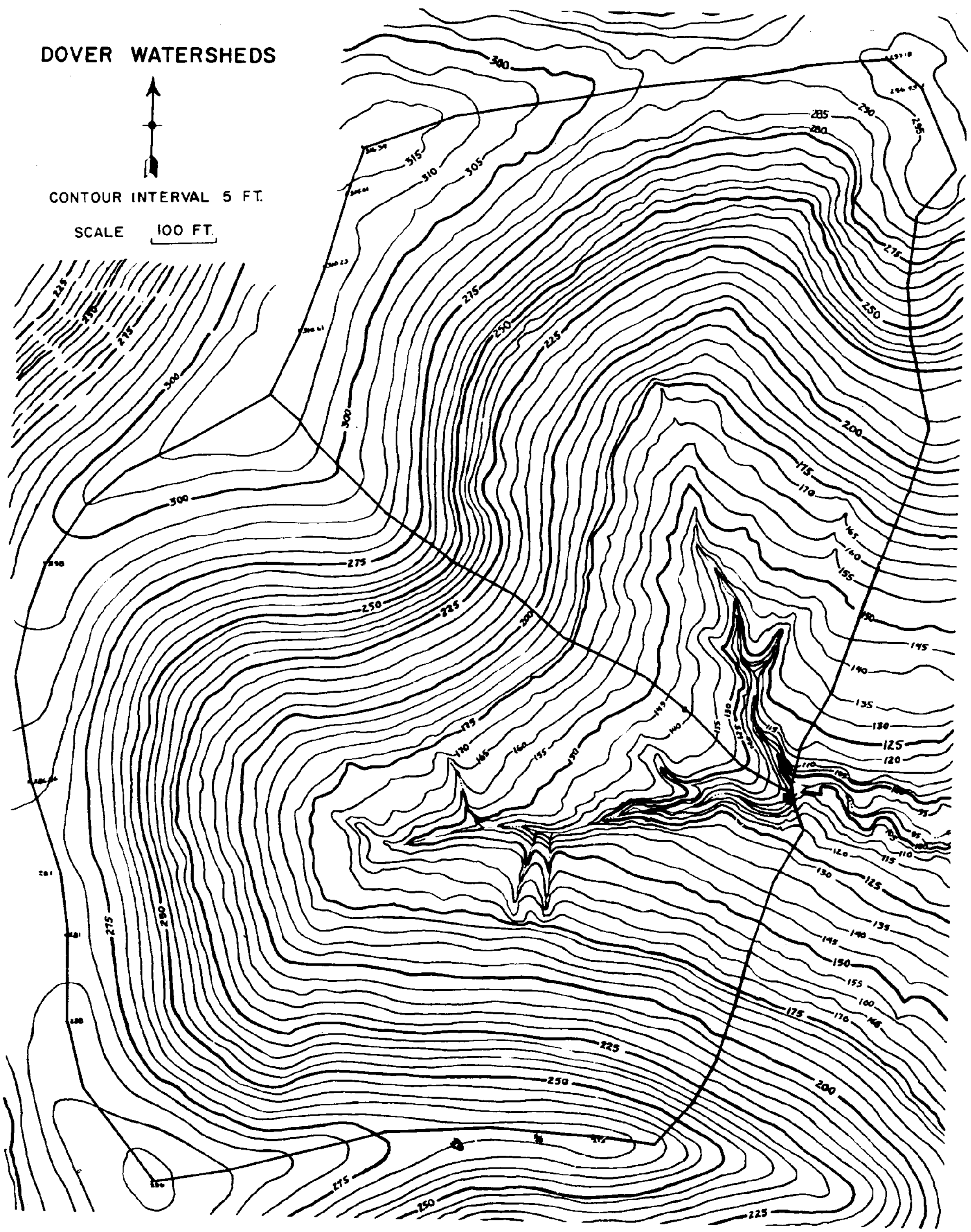


Fig. 3. Topographic map of the Dover, Ohio, study area. Watershed no. 1 is within the southern boundary; no. 2 within the northern boundary.

The watershed boundary map in Fig. 3 used throughout the study, was prepared by R. E. Marston, L. W. Hill, and R. Z. Whipkey of the Central States Forest Experiment Station, U. S. Forest Service, in January 1960 using a transit and steel tape. The two major water channel lines were added in July 1961 from data obtained by using a staff compass, steel tape, and range pole.

PRECIPITATION AND TEMPERATURE

A summary of the total precipitation and average temperature record for 1961 obtained from the Dover Dam and New Philadelphia, Ohio, stations (U. S. Weather Bureau, 1961) are as follows; January, 1.45 in., 21.9° F; February, 3.44 in., 32.0° F; March, 3.38 in., 41.2° F; April, 5.96 in., 43.3° F; May, 2.06 in., 59.8° F; June, 5.05 in., 67.2° F; July, 7.46 in., 72.1° F; August, 1.83 in., 72.4° F; September, 2.12 in., 68.5° F; October, 2.16 in., 54.1° F; November, 3.34 in., 41.5° F; and December, 2.18 in., 30.2° F. The total annual precipitation was 40.44 in. and the average temperature was 49.9° F.

VEGETATION

The study area is in the deciduous forest biome and Carolinian biotic province. It is covered by a vegetatively complex second growth, advanced hardwood forest composed of yellow poplar[1], white

[1]Scientific names of trees and plants not reported in Table 4 are reported in Appendix Table 1.

oak, red oak, red maple, black oak, and others. See Table 3.

Table 3. - Square feet of basal area per acre for species of trees on the Dover, Ohio, watersheds no. 1 and 2, winter, 1959 and 60, as measured by personnel of the Central States Forest Experiment Station, USFS.

Species	Watershed No. 1	Watershed No. 2
Yellow poplar	20.38	14.14
White oak	11.48	10.12
Red oak	10.26	9.99
Red maple	9.68	4.77
Black oak	6.81	8.22
Beech	3.74	5.77
Sassafras	3.45	4.29
Cherry	2.26	2.39
Elm	0.33	0.43
Miscellaneous	3.84	6.53
Total	72.23	66.65

The area is in a zone that was once largely an oak-hickory-chestnut forest, though chestnuts largely disappeared in the early 1940's due to chestnut blight (Endothia parasitica). Timber stocking is about 70 ft^2 of basal area/acre and about 6500 fbm/acre. Site indices for oaks and yellow poplar in the area are: black oak - Wellston silt loam, 18% slope, 76; Keene silt loam, 10% slope, 76; Muskingum silt loam, 22% slope, 69; red oak - Muskingum silt loam, 24% slope, 71; yellow poplar - Wellston silt loam, 28% slope, 98 (H. P. Garritt, Soil Conservation Service, pers. correspondence 24 June 1963).

Figures 4 and 5 depict the tree and shrub layers of a transect observed between watershed no. 1, stake 23, and watershed no. 2, stake 8. Hereinafter, locations will be designated by hyphenated

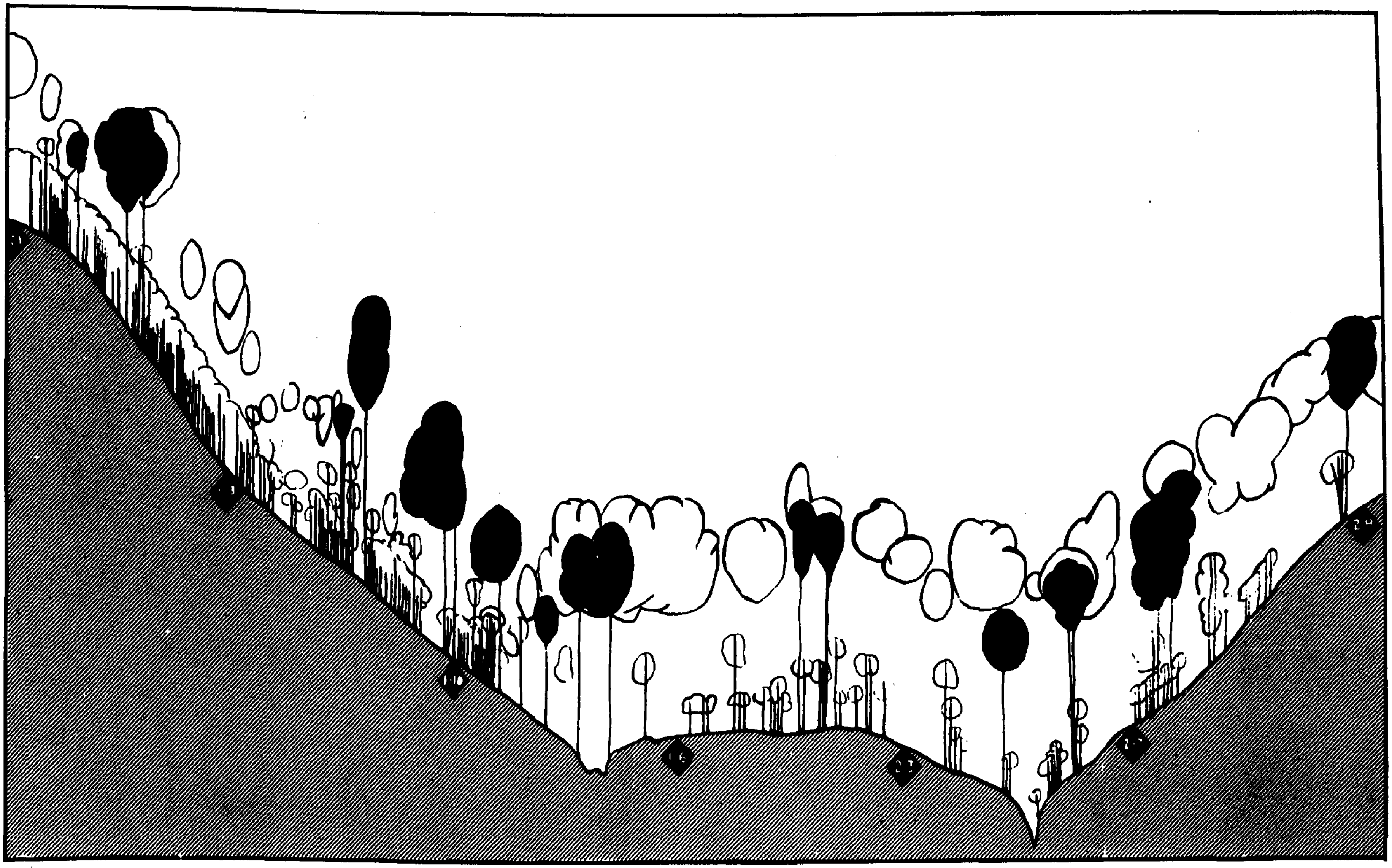


Fig. 4. A vegetative transect from grid stake 1-23 to 2-8 showing all woody stems 10 or more ft tall. Trees within transect 35 or more ft tall are shown with black crowns; crowns interesected by the transect are shown in white. Horizontal scale - 1 in.:160 ft; vertical scale - 1 in.:40 ft. Species composition is shown in Fig. 5.

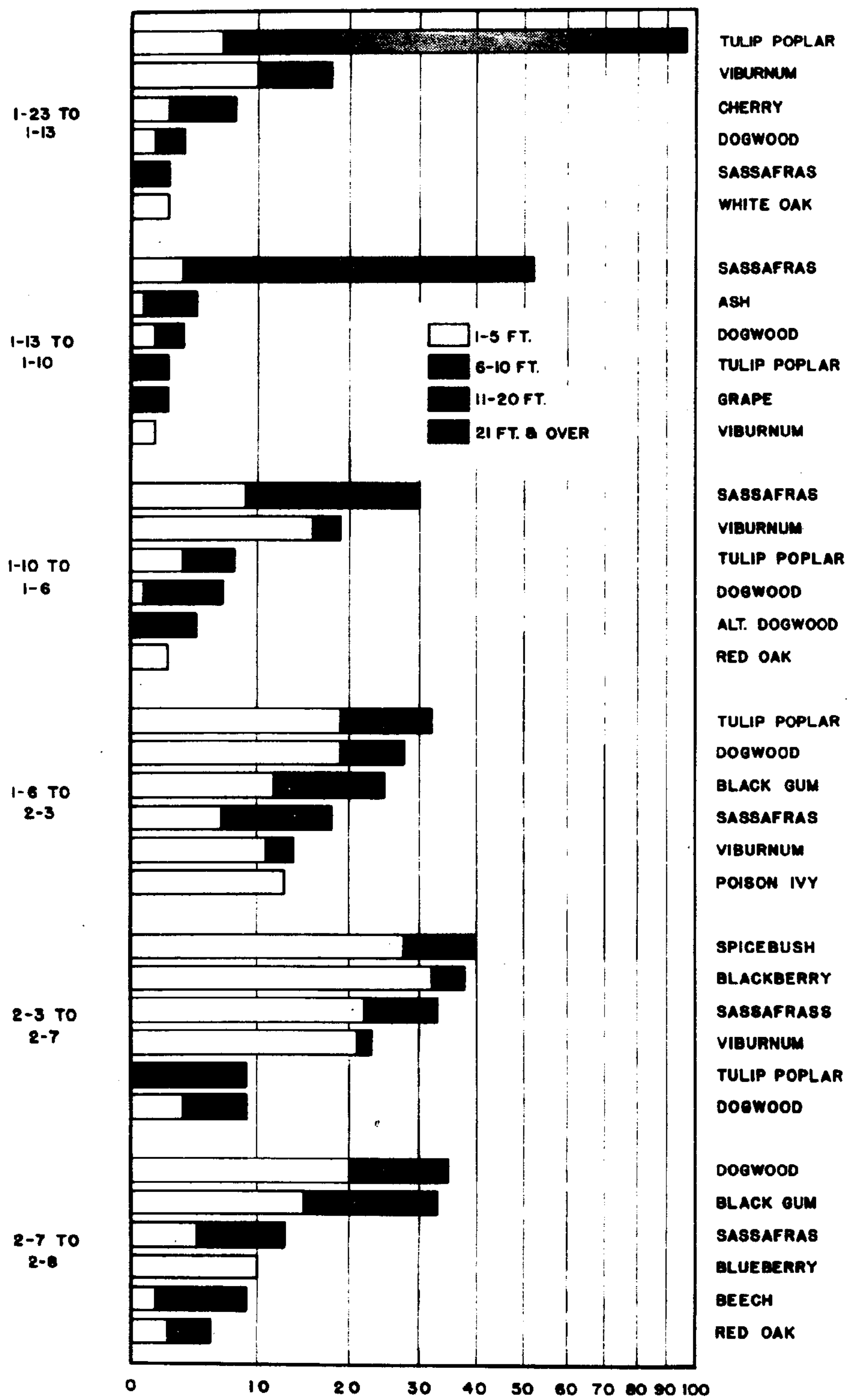


Fig. 5. Frequency and height of trees and shrubs in the transect shown in Fig. 4. Numbers to the left indicate location; those at the bottom represent numbers of stems (logarithmic scale).

numbers, e.g. 1-23, meaning watershed no. 1, grid stake 23. Stake locations are shown in maps throughout this publication. The transect which was 1 m wide and included 0.13 acres was taken not only to describe the vegetation of the area, but also to show the possible effects of the canopy density on the quantity of spray reaching the forest floor.

The shrub layer consists primarily of sassafras, spicebush, saplings of the species in Table 3, and maple-leaf viburnum. A system of permanent picture points was established. Figures 6, 7, and 8 taken from these points show major ground and shrub-layer vegetative characteristics and seasonal changes.

Ground cover varies widely throughout the area from dense fern and May apple to complete absence of herbaceous plants. The ferns, grasses, and seasonal wild-flowering perennials present are common to northeastern hardwood forests. Some of the more common herbs and shrubs are listed in Table 4.

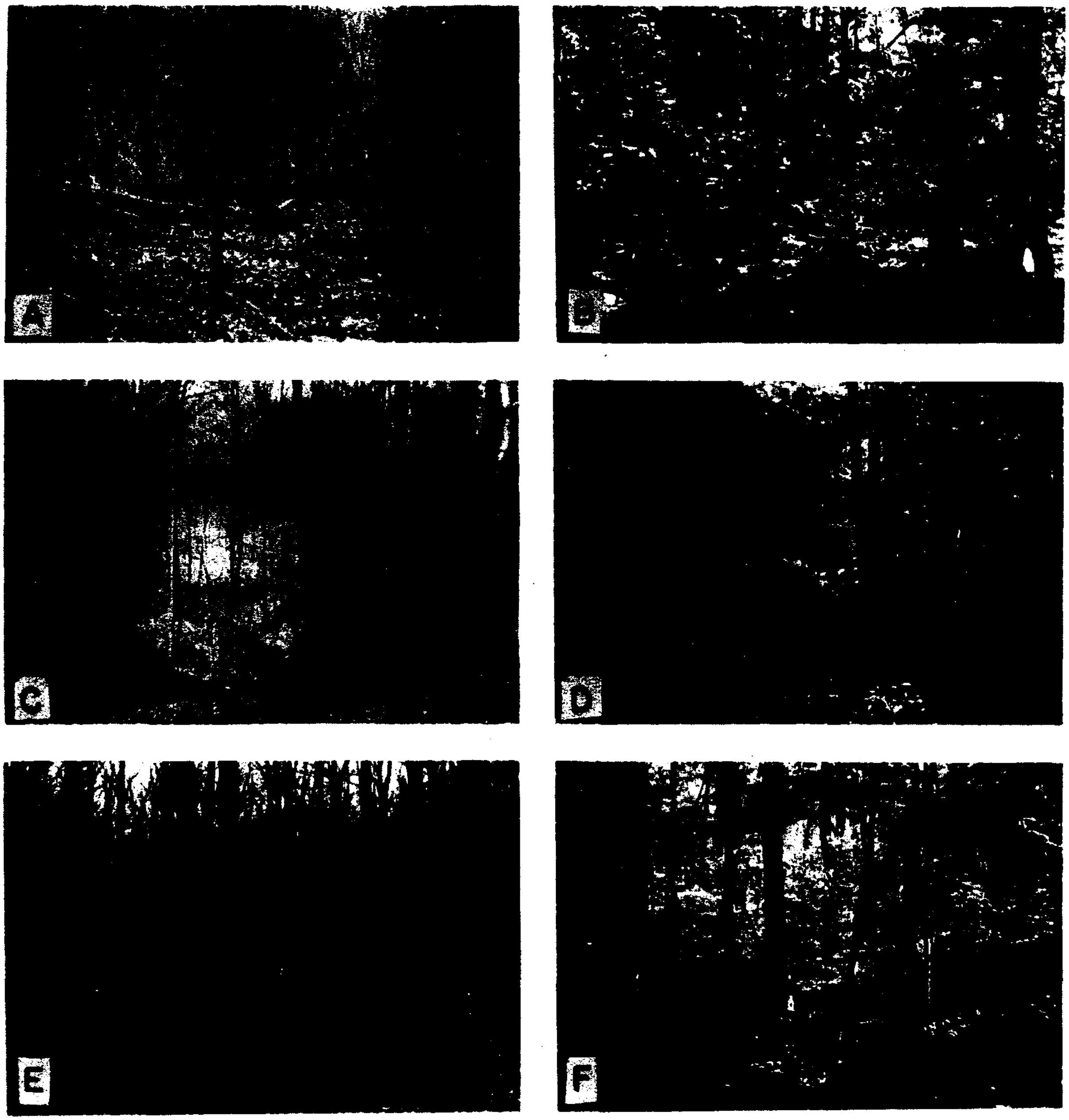


Fig. 6 A. Xeric ridge, northeastern boundary of Dover, Ohio, watershed no. 2, April 1961. B. Same location, July 1962. C. Watershed no. 2, April 1961. D. Same location, September 1961. E. Watershed no. 2, February 1962. F. Same location, July 1962.

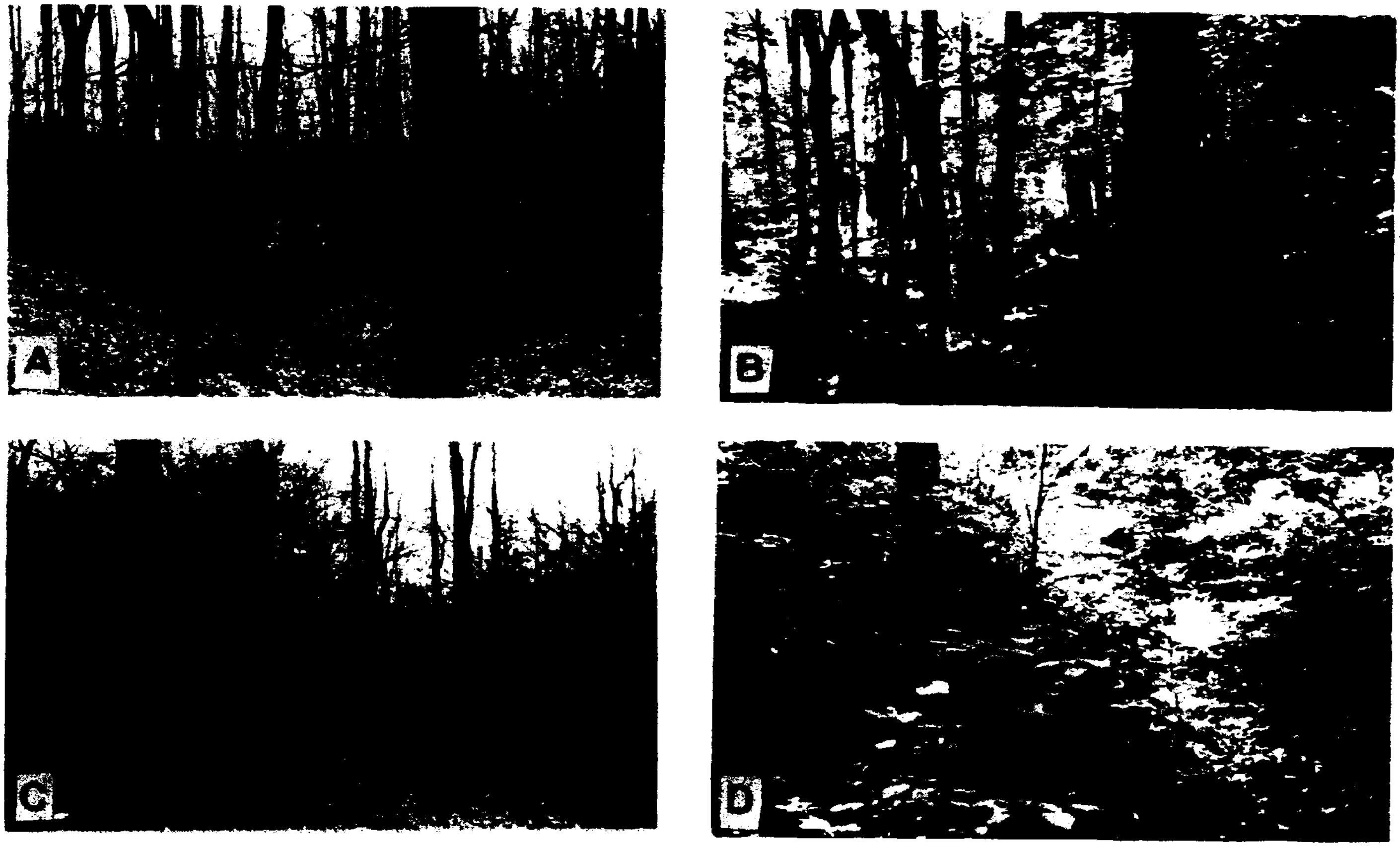


Fig. 7. A. Dover, Ohio, watershed no. 1, April 1961. B. Same location, August 1961. C. Western ridge boundary of watershed no. 1, April 1961. D. Same location, July 1962.

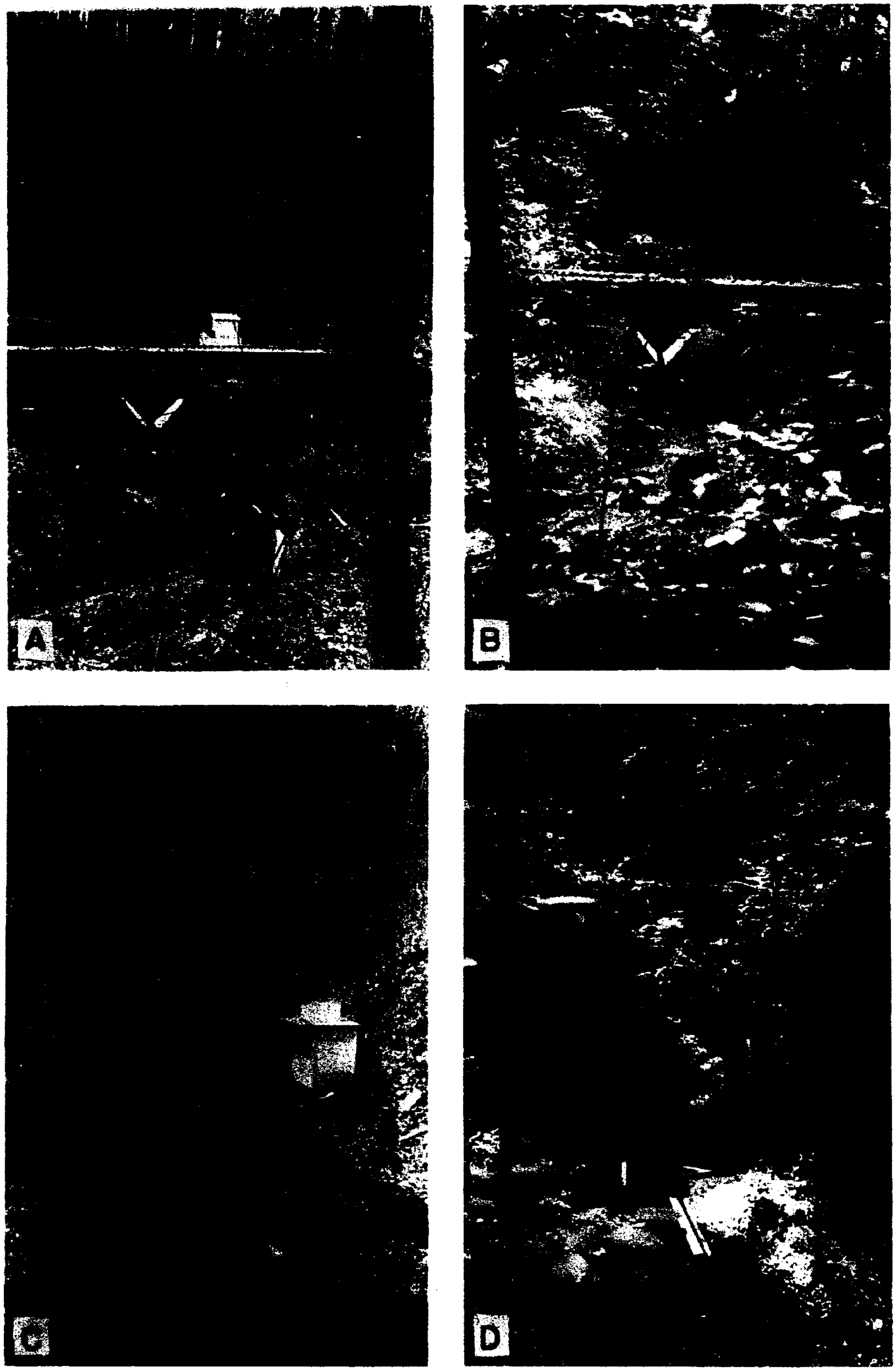


Fig. 8. A. Stream, bridge and gauging stations on Dover, Ohio, watershed no. 1, February 1962. B. Same location, July 1962. C. Watershed no. 2, April 1961. D. Same location, August 1961.

Table 4. - A partial list of herbaceous and shrub vegetation on the Dover, Ohio, watershed, 1961-62 (Based on Fernald, 1950).

	Common Name	Scientific Name
Abundant	May Apple	Podophyllum peltatum
	Wild Sasparilla	Aralia nudicaulis
	Great Trillium	Trillium grandiflorum
	Wild Ginger	Asarum canadense
	Poison Ivy	Rhus toxicodendron
	Virginia Creeper	Parthenocissus quinquefolia
	Greenbriar	Smilax sp.
	Christmas Fern	Polystichum acrostichoides
Moderately abundant	Jack-in-the-pulpit	Arisaema atrorubens
	Cinquefoil	Potentilla sp.
	Bedstraw	Galium sp.
	Wild Geranium	Geranium sp.
	Violets	Viola sp.
	Rue Anemone	Anemonella thalictroides
	Jewelweed	Impatiens sp.
	Black Cohosh	Cimicifuga racemosa
	Bloodroot	Sanguinaria canadensis
	Bur Marigold	Bidens sp.
	Maidenhair Fern	Adiantum pedatum
	False Solomon Seal	Smilacina racemosa
	Blackberries	Rubus sp.
	Raspberries	Rubus sp.
	Grape	Vitis sp.
Present	Royal Fern	Osmunda regalis
	Spleenwort	Asplenium sp.
	Bottlebrush	Hystrix patula
	Yellow Bead Lily	Clintonia borealis
	Eastern Trout Lily	Erythronium americanum
	Solomon Seal	Smilacina trifolia
	Downy Rattlesnake Plaintain	Goodyera pubescens
	Pokeweed	Phytolacca americana
	Hepatica	Hepatica americana
	Wood Anemone	Anemone quinquefolia
	Saxifrage	Saxifraga sp.
	Gooseberry	Ribes sp.
	Wild Virginian Strawberry	Fragaria virginiana
	Queen Ann's Lace	Daucus carota
	Wild Indigo	Baptisia tinctoria
	Yellow Oxalis	Oxalis stricta
	Wood Sorrel	Oxalis sp.
	Indian Pipe	Monotropa uniflora
	Blue Berry	Vaccinium sp.
	Deer Berry	Vaccinium stamineum

Table 4. - Continued

	Common Name	Scientific Name
Present	Lousewort	Pedicularis canadensis
	Partridge Berry	Mitchella repens
	Bluet	Houstonia caerulea
	Cardinal Flower	Lobelia cardinalis
	Goldenrod	Solidago sp.
	Asters	Aster sp.
	Yellow Star Flower	Trientalis borealis
	Carolinian Rose	Rosa carolina
Shrubs and Small Trees	Dogwood	Cornus florida
	Alternate Leaf Dogwood	Cornus alternifolia
	Spicebush	Lindera benzoin
	Maple Leaf Viburnum	Viburnum acerifolium
	Elderberry	Sambucus sp.
	Witch Hazel	Hamamelis sp.
	Hornbeam	Carpinus caroliniana

GEOLOGY

The study area is immaturely dissected by headwaters stream erosion and is typical of the unglaciated section of the Alleghany Plateau of eastern Ohio. It is underlain with the Alleghany series of the Pennsylvanian system and is 11 miles from the edge of Pleistocene glaciation.

The composition of the endemic aquatic fauna of the study area and lower regions is a reflection of the effects of Pleistocene glacial barriers and barrier changes that supported, isolated, and then mixed the fauna of the prehistoric stream and river environs.

SOILS

The soils are those commonly of the forests of eastern Ohio and include those of the Johnsburg, Muskingum, Wellston, and Zanesville series.

Two 1 in. diameter 3 in. core samples were taken at each grid point in 1961. A Beckman portable pH meter was used to make determinations of the pH of the top one-fourth inch and the 1.75 to 2 in. layer of the horizon. These observations are presented in composite Tables 5 and 6. In 1962 five trowel scoops were collected 5 ft from around each numbered stake, mixed thoroughly in a plastic bucket and a 0.24 l (0.5 pint) sample collected in a plastic bag. These samples were processed by the Soils Testing Laboratory, Department of Agronomy, Columbus, Ohio. Results of some of the tests are also shown in Tables 5 and 6.

Color and texture are coded in Tables 5 and 6 as follows:

Color Code No. 1. light grayish brown
2. grayish brown
3. very dark grayish brown
4. very dark gray

Texture Code No. 1. silt loam
2. sandy loam
3. silty clay loam

Aspect or direction which a slope faces is coded as 1, north-facing, 2, south-facing.

Correlations between soil factors and other factors of the environment and biota will be presented in later sections. Correlation coefficients of pairs of 46 observations of soil, topography, and physiography and vegetative density (Table 77) are shown in Appendix Table 3. Since 1.0 indicates perfect positive correlation, the farther the r values are from 1.0, the less is the

Table 5. - Results of analyses of soil samples taken from Dover, Ohio, watershed no. 1 in 1961 and 1962.

Location number	Elevation in ft.	Soil pH	pH Upper 0.25 in. of A Horizon	pH 1.75-2.0 in. of A Horizon	Per cent organic matter	lb/acre available phosphorus	lb/acre available potassium	Distance from water in ft.	Aspect	Soil color	Soil texture
1-1	136	6.5	60	54	35	18	165	10	2	1	1
1-2	158	12.0	41	40	55	10	165	170	1	4	2
1-3	213	10.0	46	42	70	120	177	360	1	3	2
1-4	223	10.5	44	48	50	80	160	350	1	2	2
1-5	160	10.5	43	41	40	47	135	10	1	1	2
1-6	154	9.0	43	45	45	18	174	60	2	2	2
1-7	192	11.0	43	43	50	20	132	240	2	1	2
1-8	220	9.0	40	41	86	15	120	270	2	4	2
1-9	171	12.0	40	43	78	9	156	10	2	2	2
1-10	175	5.0	58	52	79	90	384	10	1	4	2
1-11	225	8.0	37	40	45	49	120	340	1	2	2
1-12	286	6.5	52	50	86	80	270	590	1	3	2
1-13	227	5.5	53	51	69	125	324	400	1	3	3
1-14	198	5.0	56	53	64	80	378	240	1	2	2
1-15	193	2.0	55	55	66	35	414	210	2	3	2
1-16	232	2.0	63	54	68	24	363	340	2	3	2
1-17	290	10.0	47	45	55	24	258	510	2	3	2
1-18	298	7.0	55	44	35	35	246	630	2	2	1
1-19	267	2.0	57	63	89	52	444	480	2	4	2
1-20	250	2.0	54	54	55	54	354	410	2	4	2
1-21	263	2.0	56	47	74	54	384	410	1	4	2
1-22	273	5.5	54	53	35	54	282	530	1	2	2
1-23	295	5.0	51	50	69	25	300	660	1	3	2

Table 6. - Results of analyses of soil samples taken from Dover, Ohio, watershed no. 2 in 1961 and 1962.

Location number	Elevation in ft.	Soil pH	pH Upper 0.25 in. of A Horizon	pH 1.75-2.0 in. of A Horizon	Per cent organic matter	lb/acre available phosphorus	lb/acre available potassium	Distance from water in ft.	Aspect	Soil color	Soil texture
2-1	110	7.5	4.5	4.4	35	30	141	10	2	2	1
2-2	125	9.0	4.5	4.1	35	12	217	50	2	2	2
2-3	152	5.5	4.8	4.5	35	17	264	160	1	2	1
2-4	282	6.5	5.1	5.2	68	34	222	510	1	4	1
2-5	214	2.0	6.0	5.4	55	37	330	310	1	4	2
2-6	157	7.5	4.5	4.6	50	47	258	110	1	2	2
2-7	159	9.0	4.4	4.2	50	20	147	100	2	1	2
2-8	218	10.0	4.0	3.8	35	9	96	330	2	2	2
2-9	191	8.0	4.7	4.5	40	30	141	160	2	2	2
2-10	173	5.5	5.3	4.9	50	27	336	60	2	2	1
2-11	219	2.0	4.7	4.5	92	210	462	260	1	3	1
2-12	287	0.0	6.2	6.3	69	100	480	460	1	3	1
2-13	311	5.5	5.7	4.5	88	65	369	650	1	3	1
2-14	300	2.0	6.0	5.6	64	100	369	500	1	2	1
2-15	272	2.0	5.7	5.8	74	120	288	330	1	3	1
2-16	223	7.0	5.3	4.3	57	65	264	220	2	3	2
2-17	223	10.0	4.1	4.2	55	37	240	260	2	2	2
2-18	272	14.0	3.7	4.0	78	29	141	400	2	4	2
2-19	294	5.5	4.5	4.5	59	34	252	550	2	2	1
2-20	273	4.0	5.9	5.5	66	40	369	450	2	3	1
2-21	283	4.0	5.7	4.8	65	100	306	420	2	2	1
2-22	302	2.0	5.9	5.8	66	100	462	490	1	3	1
2-23	316	4.0	6.0	5.2	79	57	276	620	1	4	1

correlation (Snedecor, 1956:162). Outstanding is the lack of strong positive correlations in any of the factors except between elevation and distance from permanent water. There is a strong negative correlation of pH and lb/acre of available phosphorus. Lack of correlation may be simply a reflection of inadequate or abnormal sampling. However, it may reflect the true conditions within the forest. If such is the case, readily measured soil factors are independent of each other.

DISSERTATION ORGANIZATION

When coordinate relationships are recognized between members of the fauna or of several ecological factors, the impossibility of a satisfactory 1, 2, 3; a, b, c, manuscript organization is evident.

The methods and procedures in preparing and treating the tracer plots and the study areas are described separately although they apply to all aspects of the project. Methods and procedures used in studying individual faunal segments or ecological factors will be described under the total treatment of each segment or factor and are listed in Table 7. Some sampling methods were used for two or more faunal groups.

Faunal segments are considered separately for ease of presentation, coherency, and brevity. They are presented in the order of increasing consumer level and the generally conceived lower-to-higher life forms.

Chronologically, the organization used is unsound also, but it seems to most nearly satisfy the major purpose of the study--to view the integrated effects of the insecticide on the fauna.

A summary of sampling and observational techniques is presented in Table 7. Arthropods with their common or descriptive names (based on Laffoon, 1960) are presented in Appendix Table 4.

Table 7. - Summary of sampling and observational techniques used to study fauna of the Dover, Ohio, watersheds, 1961 and 62.

Soil micro and macro fauna	Dilution plate counts Soil CO_2 output Berlese funnel extractions from nylon bags Cryptozoan oak boards
Insects and Invertebrates	Light traps Sweep nets Earthworm $KMnO_4$ extractions Molasses traps Suspended sticky boards Stream drift nets Stream bottom quadrats Cryptozoan oak boards Tree trunk sticky bands Random collecting
Mollusks	Random collecting Quadrat counts
Amphibians	Roofing felt quadrats Random observations Stream side counts Sherman trap counts
Reptiles	Random capture and marking of turtles Snake and turtle drift fence traps Random capturing and marking of snakes Roofing felt quadrats Electric blanket
Birds	Early morning transects Breeding bird observations Random observations
Mammals	Cat traps Sherman live traps Transect wires for mammal holes Mammal holes under drop boards Plastic tile dropping boards Feeding activity along hole transects Large mammal burrow counts
Fish	Cages and buckets

PHENOLOGY

Phenology is a study of the seasonal recurrence and natural phenomena. The vegetative changes of the study areas were examined in order to derive an index for comparing faunal observations not chronologically but phenologically, *i.e.*, as related to the annual vegetative stage of growth and development of the forest. Plants, considered good integrators of the abiotic factors, were selected as the best indicators of between-year seasonal similarity.

In 1961, 2 milacre plots were established near points 2-4 and 2-3 on the divide between the two watersheds. Measurements of plants within these plots were made throughout the summer. On seven dates, measurements in mm were made of the leaf or center leaflet blade lengths of sasparilla, maple leaf viburnum, red oak, white oak, American elm, beech, Virginia creeper, and great trillium. Also measured were Christmas fern frond length, May apple leaf diameter, and new stem growth of red and white oaks.

Approximately 30 leaves or stems of each species in each plot were selected for measurement. Natural variation within the sample and failure to select exactly the same items each time produced only four items that seemed meaningful and worthy of continuing in 1962. The results are shown in Fig. 9. The 1961 red oak measurements in Fig. 9 show erroneously decreasing stem size as the season progressed. This was caused by improper sampling. In 1962, measured items were marked either with white strings (stems and leaves) or ribbon-and-wire flags (May apples) so the same population

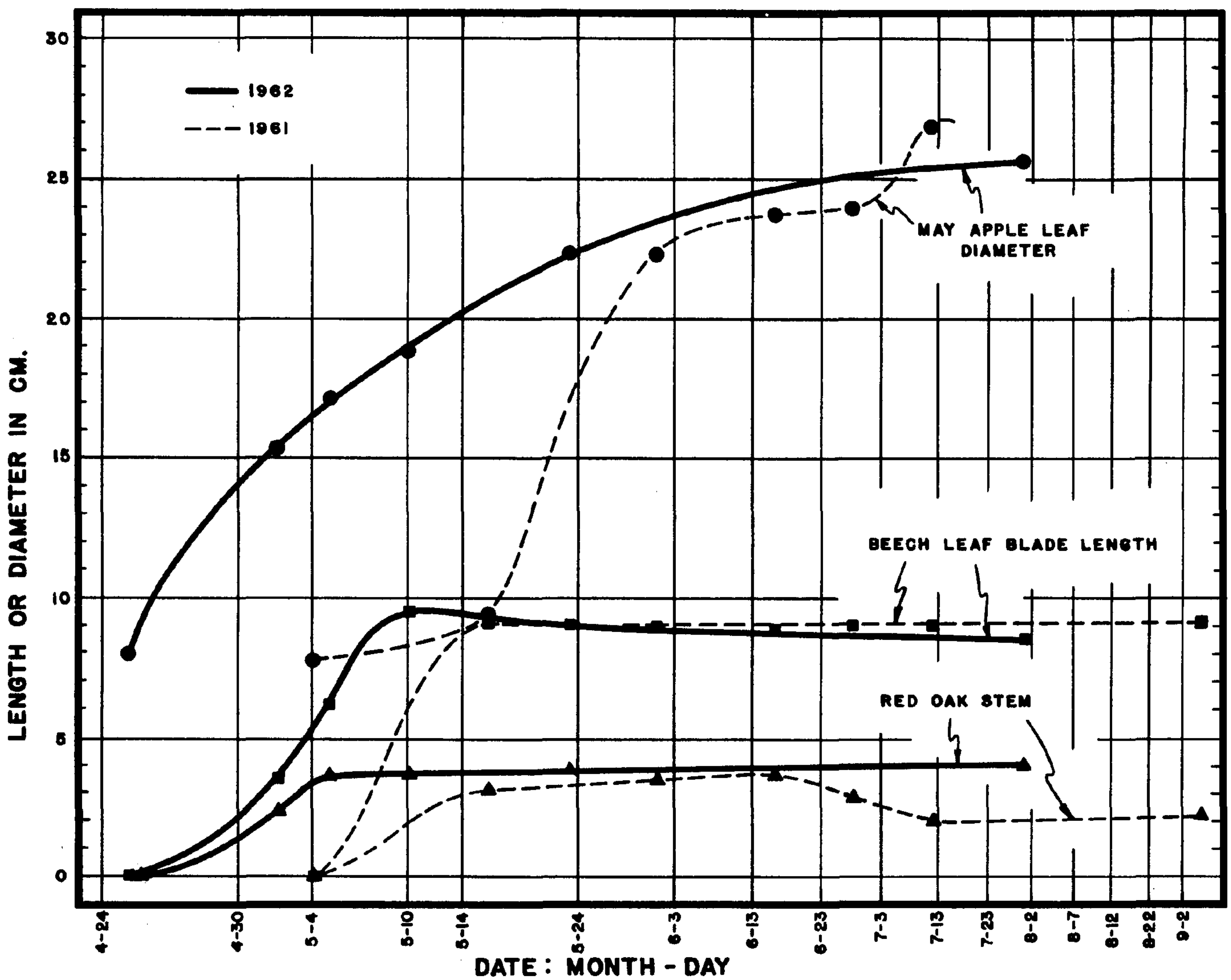


Fig. 9. A between-year comparison of the growth of three plant parts on the Dover, Ohio, study area, 1961 and 1962. Phenological corrections for the time were based in part on this drawing. Date is on logarithmic scale.

same population could be repeatedly observed.

Throughout both years of study, random field notes were taken on vegetative events. After eliminating questionable observations, a sequence of events for which there were observations in both years was developed. This is shown in Table 8. Prior to 8 May 1962 the season was late, *i.e.*, similar events occured about 4 days later than they had the previous year. After 8 May 1962 (the first rain of a dry spring season) the season was about 12 days earlier than in 1961. This is shown graphically in Fig. 10. Fig. 9 indicates an earlier initiation of stem growth in 1962 than in 1961. This does not refute the data of Fig. 10 but is believed to be the result of slow, gradual growth of only 3 species as compared to the more spectacular changes of over 13 major vegetative events. Based on Fig. 10 between-year comparisons of comparable dates were made. A chronological-phenological calendar was constructed (Appendix Table 5). This table was used throughout the study for between-year faunal population comparisons.

Table 8. - Dates and between-year time differentials of comparable biological events in 1961 and 1962 on the Dover, Ohio, study area. See Fig. 16.

	Observation	Date 1961	Date 1962	1962 Season Days Earlier	1962 Season Days Later
1.	Profuse bloodroot bloom	4-25	4-29		4
2.	First tent caterpillars	5-1	5-5		4
3.	Bloodroot bloom fall	5-3	5-7		4
4.	Morell mushroom decline	5-3	5-7		4
5.	Anemone bloom abundant	5-4	5-8		4
6.	Phlox blooming	5-15	5-10	5	
7.	Tulip poplar "flower" fall	6-11	5-25	17	
8.	Cardinal flower bloom	6-17	6-5	12	
9.	Hickory nut fall (2-19)	7-10	6-21	20	
10.	Sasparilla fruiting	7-10	7-2	8	
11.	Blackberries ripening	7-31	7-15	16	
12.	Sasparilla on wane	8-5	7-24	12	
13.	Blueberries ripening	8-4	7-26	9	

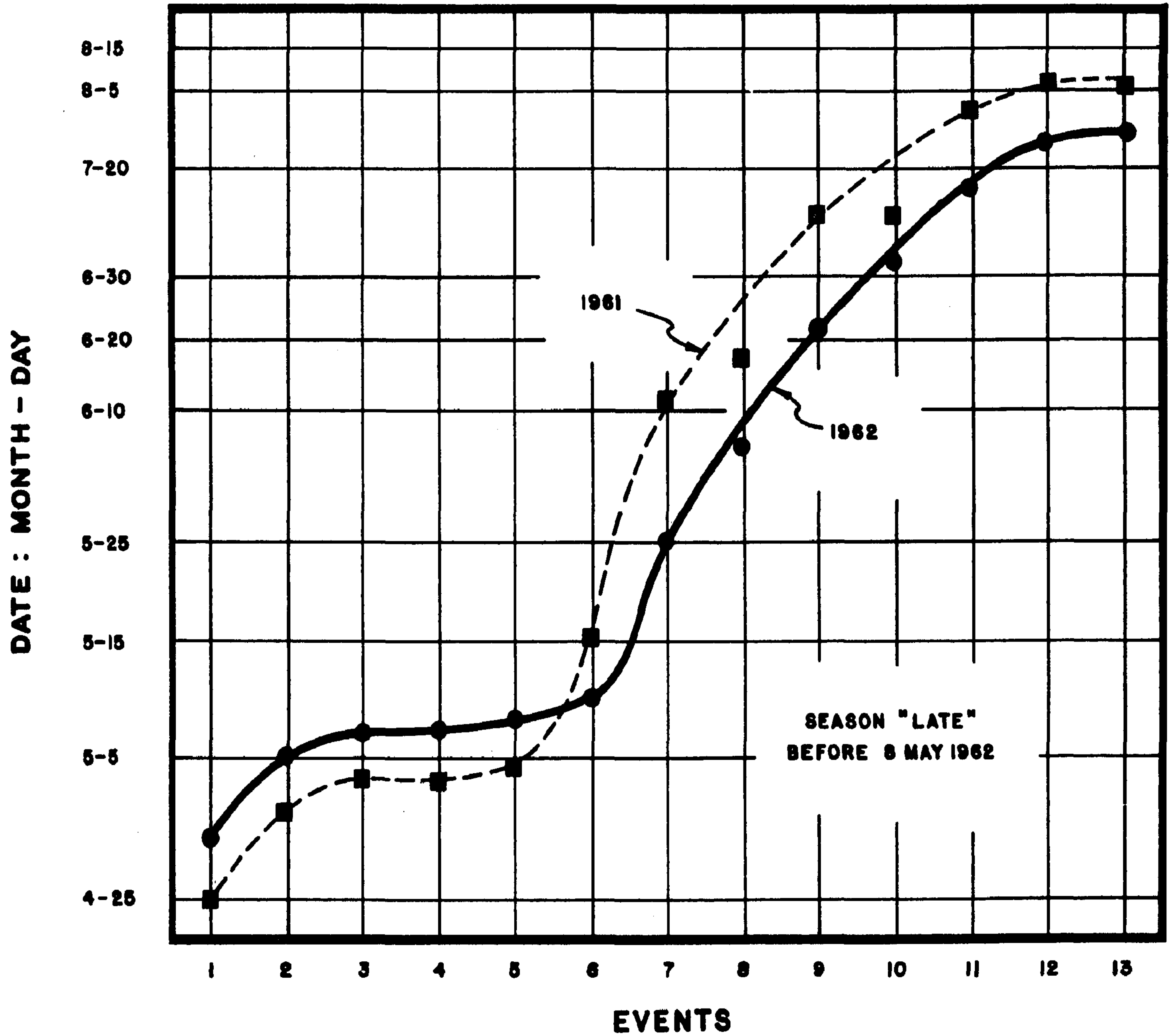


Fig. 10. A comparison of the dates on which a series of biological events occurred on the Dover, Ohio, watersheds in 1961 and 1962. Events are identified in Table 11. Date is on logarithmic scale.

TRACER PLOT STUDIES

In order to develop techniques for use of the isotopic tracer method on the large watershed, four plots, each 0.1 acre in size, were selected for hand application of malathion-S^{35}. Fifty yards from the eastern boundary of watershed no. 1 four plots each 6.1 x 66.5 m (20 x 218 ft) were staked out as shown in Fig. 11. The area was encircled with 16 ga. wire to which were fastened radiation warning signs and red plastic ribbons.

The spray apparatus shown in Fig. 12 was developed to simulate as nearly as possible an aerial application. A Kim Fast-O-Matic pressure gauge, Marsh Instrument Co., Skokie, Ill., Range 0 to 160 psi, was installed in a Hudson Climax 335-B sprayer. The 11.3 l (3 gal) brass tank could be charged to 40 psi. The 1.82 m (6 ft) long sprayer extension and boom were made of 1.6 cm (0.625 in.) galvanized iron pipe. The boom was fitted with a T and 2 L joints and two Teejet 8001, 1/4 T nozzles. All joints were caulked. A pressure release was operated at the junction of the 0.9 m (3 ft) long corrugated rubber hose and the pipe. The nozzles were 50.8 cm (20 in.) apart and at a height of 40 cm cast a swath 1.65 m (65 in.) wide. A string was dangled from the boom to indicate the proper spray height of 40 to 45 cm (16 to 18 in.) above the canopy. Wires were strung throughout the areas to indicate the center of each spray swath. The application could have been made more uniform by using only one nozzle though it would have taken longer. The undergrowth frequently caused pauses that produced over-concentrations of spray although the spray release was closed.

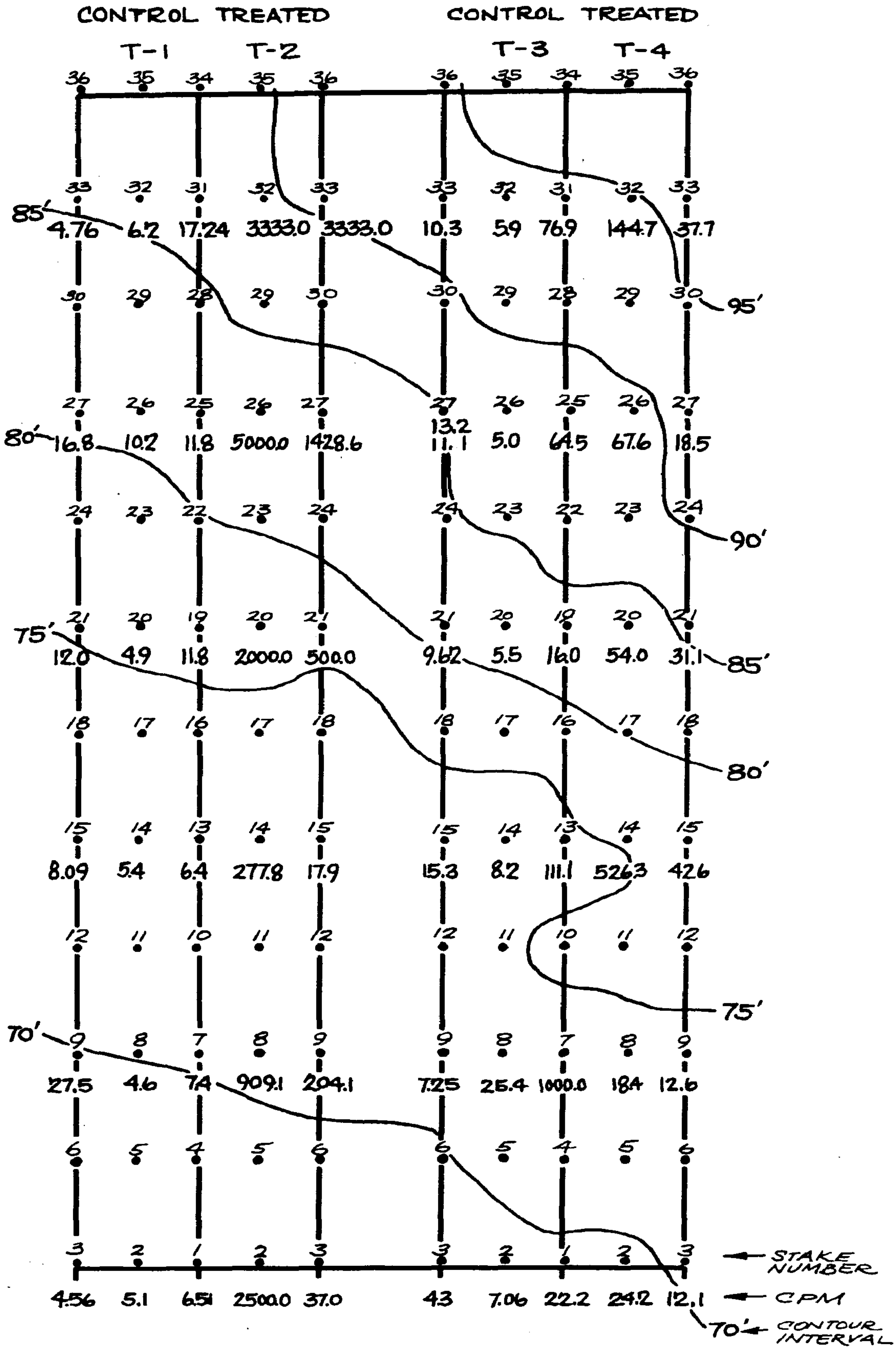


Fig. 11. Topographic map of the Dover, Ohio, tracer plots sprayed with a hand sprayer in 1961. Activity and amount of insecticide applied at numbered stakes was measured from one frosted glass disc. Plots were 20 x 218 ft.

Fig. 12. The writer in protective clothing used while spraying the tracer plots with the apparatus shown in the insert.

The data on the tracer plot applications is presented in Table 9. Spraying time was about 15 min per plot. The results are shown in Fig. 11.

The appropriate formulations (exclusive of malathion-S^{35}) were mixed the night before and stored 8 hr in gallon size glass jars. The radioactive insecticide had been emptied from its sealed glass shipment vials into small glass jars. Each was poured into the tank just prior to application. The writer used protective clothing shown in Fig. 12 during the application.

Cages and faunal sampling devices were placed throughout the plots near numbered stakes. One frosted glass disc was placed at each stake to measure spray distribution. Portable G-M tube readings were taken post-spray on the vegetation and ground. These observations

Table 9. - Summary of information on the tracer plot spray treatments, Dover, Ohio, 1961.

Tracer Plots	
Date and time:	29 August 1961, 8:40 a.m. EST
Area treated:	2, 0.1 acre plots
Dimensions of each plot:	218 x 20 ft
Rate-malathion:	2 lb/acre
Volume of formulation per plot:	1 gallon
Volume of formulation per acre:	10 gal/acre
Spray tank pressure:	20 psi
Plot 1 - control - carrier	
Xylene:	45.4 g
Triton X-155 emulsifier:	11.8 g
Distilled water:	up to 1 gal
pH:	6.5
Per cent of area sprayed:	92%
Plot 2 - Treatment	
Malathion:	91.4 g
Xylene:	45.4 g
Triton X-155:	11.8 g
Distilled water:	up to 1 gal
pH:	5.8
Activity of malathion-S^{35}:	3.0 mc
Per cent of area sprayed:	100%
Plot 3 - Control - no treatment	
Plot 4 - Treatment	
Malathion:	91.4 g
Xylene:	45.4 g
Triton X-155:	11.8 g
Distilled water:	up to 1 gal
pH:	6.1
Activity of malathion-S^{35}:	14.8 mc.
Per cent of area sprayed:	85%

are reported under the sections on dispersal and effects on vegetation.

Desirable results were obtained with both 14.8 and 3.0 mc applications, but the latter was considered low for definitive work. Based on this, 1.0 curie of malathion-S^{35} was ordered to apply at the rate of 4.5 mc/acre to watershed no. 1 in 1962.

An attempt was made to trap a number of mammals and reptiles from the forest around the study area, place them in wire cages on the tracer plot and observe them there after the plot treatment. This approach was very unsatisfactory because of the problems of uncertainty of the dates of isotope arrival and treatment, undesirability of holding animals a long time before spray, uncertainty of trapping success, uncertainty of wild animal response to confinement and treatment, constant possibility of escapes, and logistical problems including supplying cages for all animals caught and watering and feeding animals spread over a 0.5 acre area.

Many cages were built of 0.63 cm (0.25 in.) hardware cloth, 0.91 x 0.91 x 0.30 m (3 x 3 x 1 ft) for mice, chipmunks, and box turtles. The opossum cages had dimensions of 1.83 x 0.91 x 0.46 m (6 x 3 x 1.5 ft), with all edges fastened by hog-rings. For the most part the cages were satisfactory. They were made larger than laboratory cages to allow free movement, space for natural litter nests and water, and movement over variable spray distribution.

Free-running dogs and a falling tree collapsed four cages and allowed escape of the test animals. Two others escaped due to carelessness of the caretaker. All together the caging experiments were an expensive and only slightly satisfactory endeavor to conduct a laboratory study in the field. They were of value, however, in allowing the problem of biological sample preparation to be investigated before the final treatment, and provided direction for labeling, taxonomic work, safety precautions, and additional faunal sampling precautions to be taken. They did provide the directive for the amount of malathion-S^{35} for the final spray.

LABORATORY PROCEDURES FOR BIOLOGICAL SAMPLE PREPARATION

Specimens collected in the field were put in preservative, air dried (e.g. insects), frozen (e.g. birds), or brought to the laboratory in plastic bags and processed within 8 hr after collection. Each collection was given a number corresponding to the month, day, and collection number for that day. A 21.6 x 7.6 cm (8.5 x 3 in.) mimeographed form was prepared for each numbered sample. The form included number, location, description, date received by taxonomist, date received by laboratory, date counted, and disposal of sample. These were accumulated chronologically on a pin file.

Malathion-S^{35} was prepared by the method described in the Appendix.

The techniques given by Jeffay *et al*. (1960) were used with the following modifications to determine the radioactive sulfur in the sample. Because of weak beta emission of S^{35}, low amounts of S^{35} used and high background counts, all materials had to be processed within the laboratory. Readings with the Geiger-Mueller (GM) survey meter were gross. A Nuclear-Chicago[1] Model 2612P Alpha-Beta Portable Survey Meter with P16 probe having a D35 mica end window was used.

The collection, e.g. a shrew, was posted in the lab, and costal cartilage and tibial caps removed. These were placed separately on a numbered piece of paper towel and placed in the fume

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[1]Nuclear-Chicago Co., Des Plaines, Illinois.

hood on fiber glass screen drying racks. When judged air dry (24 hr or longer) the sample was removed, placed in a weighed 50 ml Pyrex beaker, and the two re-weighed to the nearest 0.1 mg. To this about 5 ml Pirie's reagent was added, at least to cover the sample. Pirie's reagent was prepared as other reagents by the O.S.U. Laboratory Supply by adding 3 volumes of concentrated nitric acid to 1 volume of 60% perchloric acid. One-fourth of this mixture was removed, saturated with 50 to 60 g of magnesium nitrate per 100 ml and mixed with the remaining acid mixture. The beaker was placed directly on a hot plate set at 121° (250° F) and evaporated to dryness. The beakers were swirled several times during evaporation. When dry, they were removed, cooled slightly, and enough distilled water (approximately 5 ml) added to dissolve the residue. Then 5 ml of hot 0.1 N Na_2SO_4 (sodium sulfate) was added, followed by 3 ml of hot 1.0 N BaCl (barium chloride). Automatic burettes were used. The solutions were heated to encourage large crystals. The resulting solution and precipitate of barium sulfate were filtered through Millipore[1] white filter papers (HAWPO 2500 25 mm diameter, pore size 0.45 micron). The beakers were rinsed and water poured into towers.

A battery of 5 filter towers (Millipore Filter Corp. Pyrex hydrosol microanalysis filter holder, No. XX100 2500.) with scintered glass bases and filter flasks (Fig. 13) were attached to a vacuum

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[1]Millipore Filter Corp., Bedford, Massachusetts.

pump. Filtration required approximately 3 min. Towers were rinsed while still under vacuum with distilled H_2O from a plastic wash bottle, then with 95% ethyl alcohol. When all the solution had been removed, the tower was removed and the damp filters and precipitates were partially dried on a 151 mm diameter Coors porcelain Buchner funnel lined with light weight clear sheet plastic in which small holes had been pierced to allow light suction from beneath for nine filters at once. When curling had just begun, the filter paper was removed with forceps and placed in an aluminum planchet. Precipitates were prepared for counting under the assumptions of absorption phenomena of infinitely thick samples.

Much difficulty was found in obtaining flat whole precipitates. The filters frequently curled and the dry precipitate cracked, chipped, and flaked off creating many counting irregularities ranging from irregular surface geometry to actual loss of small portions of the samples. All 1961 and early 1962 samples were counted under these conditions since no solution to the problem had been found or recommended by consultants.

The technique of Weatherford and Larson (1959:1931) was adopted as the best method for preparing a smooth, flat, permanent sample for counting. Aluminum planchets were nearly filled with acetone, the partially dried filter and precipitate were placed in the center of the planchet, and several more drops of acetone added. The cellulose acetate formed from the dissolved filter tightly adhered the precipitate to the planchet. Five planchets were dried for 1 hr or more under a Petri dish. The counting area was basically unaltered from the specific area developed during filtration. Final drying was done in an oven at 100^{o} C for 15 min.

Thickness of the cellulose acetate immediately below the precipitate may vary $\pm$ 0.7 mg/cm^2, an insignificant variation influencing back scatter in relation to the previously adopted techniques of dry, irregular filter and tape-held filter. The back scatter factor including absorption by the cellulose acetate, is 1.27 $\pm$ 0.02 (Weatherford and Larson, 1959) which is equivalent to an error of about 1.6% in the estimation of counting efficiency.

Prepared samples were placed on detergent-washed, precounted-for-background, aluminum planchet holders and placed in the loading magazine of a Nuclear-Chicago Inc. Model C-115 Low Background Automatic Sample Changer, having a thin end-window counter with cosmic-ray shield. The signals of the detector control unit were monitored by Nuclear-Chicago Model 181A Decade Scaler and recorded by Nuclear-Chicago Model C111B Time Interval Printer. There has been raised at the time of this writing some doubt of the accuracy of preset-for-time determinations of radioactivity of low-activity samples. Later workers should check the statistics of computation of cpm using this approach (Jarrett, 1946). The remainder of this dissertation is based on the assumption that the cpm readings obtained are sufficiently valid so as not to affect conclusions. Results obtained are believed to be conservative. The counting system used, not showing a Q-gas cylinder on the lower left, is shown in Fig. 13.

Operation of the system, based on plateaus obtained, was at

Fig. 13. A. Automatic sample changer, scaler, and timer used in determining sample radioactivity in the Dover, Ohio, laboratory; B. Arrangement of the battery of filter towers and apparatus for precipitate preparation.

1350 volts. A separate line for the system was run from the main electrical system of Dover, Ohio, to provide as nearly stable voltage as was obtainable.

Sample numbers and other descriptive data were written on the timer tape before each run. When complete, the data was entered with carbon copy in a data book and the tapes filed by date in separate envelopes. The carbon copy of each completed data sheet was sent to the Ohio Cooperative Wildlife Research Unit, the original retained for analysis. Over 3000 samples were thus processed.

TREATMENT OF THE STUDY AREA

To increase accuracy and safety of aerial spraying over forested, rolling terrain, four applications of approximately 50 gal each were planned for watershed no. 1. This deviates from the plan of having a typical forest application, but the differences did not appear to be significant in view of the advantages. The small atypical size of the forest, the need for complete coverage, the need for prevention of widespread contamination, especially of the adjoining untreated area, prevented an entirely typical application.

SELECTION OF TIME

A date was selected that approximated a typical early-foliage application of insecticide to a forest, and allowed a foliage condition that would permit enough sub-canopy application to allow assessment of effects in all strata of the forest. The tenth of May was selected as the most desirable date but delays occurred.

A very early application was desired to reduce thermal currents over the area. The 25 May 1962 flight was delayed over 1 hr because of fog at the airport. When all preparations were made, approval for the flights were radioed from the study area to the airport by Mr. W. D. Bates, an officer of the Ohio Division of Wildlife.

MARKING OF AREA

Markings for Trespassers

A month prior to the 1962 spray date, a 16 ga. galvanized wire was strung 8 to 10 m (20 to 30 ft) outside of the boundaries of watershed no. 1. Along the chest-high wire were hung, alternately, radiation hazard signs and no trespassing signs every 12 m (40 ft). Between the signs were attached plastic ribbons to increase the visibility of the wire. This marking and fencing was not adequate to prevent bold or non-observant mushroom hunters from entering the area before the spray. Under more hazardous conditions, where people might frequent the area, more elaborate marking and fencing would be required.

Markings for Pilot

A 127 cm x 15.2 m (50 in. x 50 ft) roll of blaze-orange paper was cut into ten 63.5 cm x 3.05 m (25 in. x 10 ft) panels. These were spaced evenly around the boundaries of the watershed to be treated. On the day of the flight, only one was seen from the air through the moderately dense foliage. Panels retained their color and strength through several rains but would only be of value as markers where there was little or no canopy foliage, or in very open forests.

Rubber 63.5 cm (25 in.) diameter paddle balloons[1] were used to mark the area. The morning of the application they were tied to lightweight cotton string, released to rise above the canopy and secured. Balloons could not be inflated more than 2 days prior to spray since helium escaped from them. Wind action above the canopy burst all test ballons within 36 hr of their placement

[1]No. 2516, Eagle Rubber Company, Ashville, Ohio

above the canopy. A cluster of four balloons was run up above the center of the area.

The pilot reported after the spray job that the area was very well marked; that red, yellow and orange balloons used to mark different boundaries were the most visible markers. Pink, blue, and green were unsatisfactory.

APPLICATION

A liner was sought to prevent the contamination of the tank of the commercial spray plane which was to be used with radioactive insecticide. Rubber, polyethelene, and vinyl-coated nylon liners were considered. None were suitable. Cost estimates were equal to those for replacement of a standard tank. The installation of a common 55 gal drum or barrel within the plane was considered practical.

Approval was gained to use a plane of the Ohio Agricultural Experiment Station. The existing installed tank was used without modification. Mr. Jack J. Eggspuehler, Director, The Ohio State University School of Aviation, was the pilot of the first flight. Mr. Vernon A. Vick, pilot with Aero Activities, Inc., Bowling Green, Ohio, flew the second, complete application using another Experiment Station plane.

The first flight was made at 8:32 am, 15 May 1962. The plane flew northwest over the dams along the northeast boundary of watershed no. 1 and crashed at the end of the first swath at a point near 1-17. (See Fig. 14) Pertinent data for the two flights

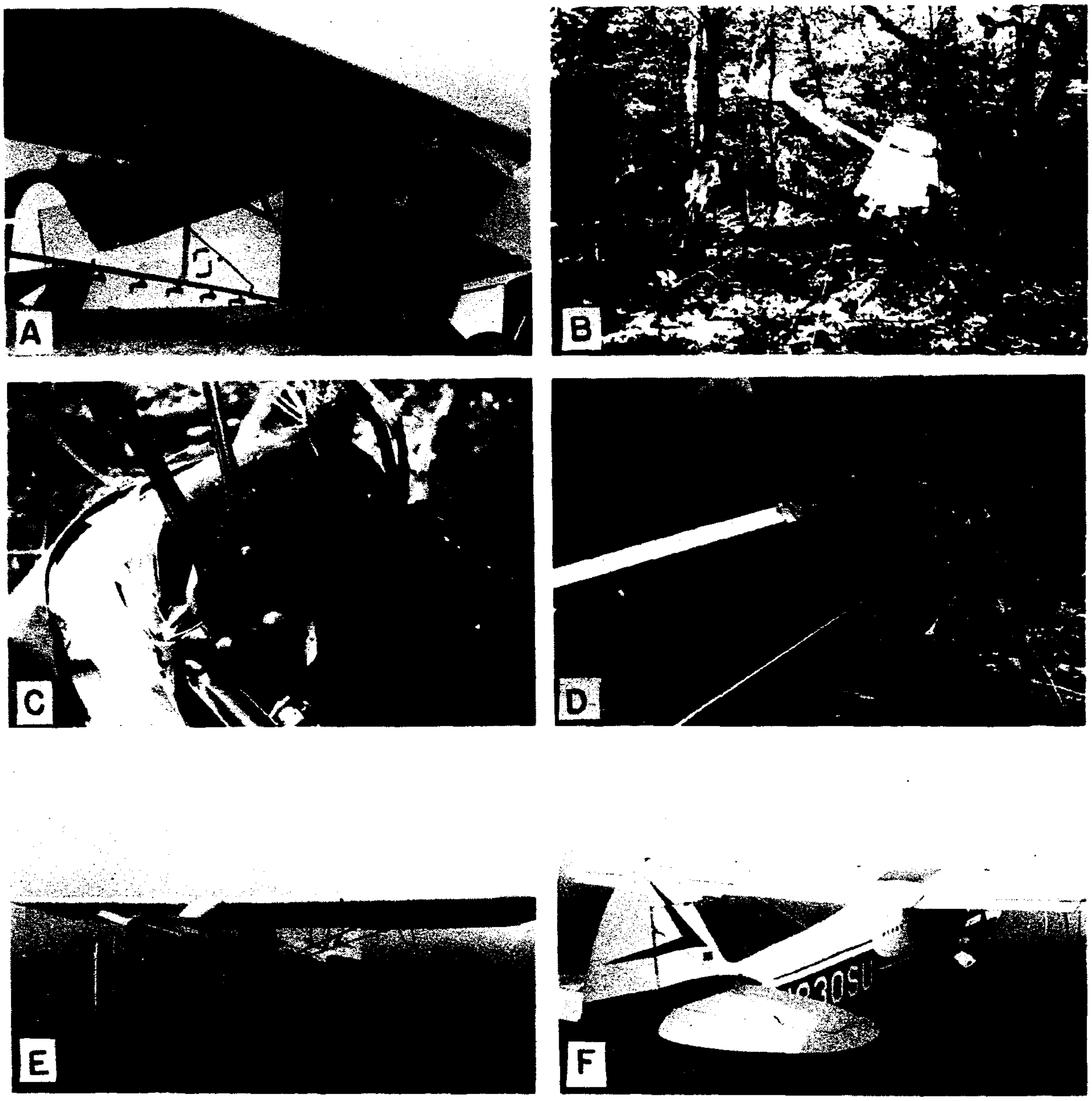

Fig. 14. A. A Piper J-3 was used for the initial spray flight. B. The plane crashed at the end of one swath flight; C. The cockpit was wrecked; D. Spray drained from the ruptured tank at this point; E. The final application was performed with a Piper P-18; F. Strippable paint was used to reduce the contamination from the malathion-S^{35}.

are shown in Table 10.

Table 10. - Summary of information of the two 1962 aerial applications of malathion-S^{35} to the Dover, Ohio, watersheds.

Crash Flight	
Date and time:	15 May 1962 8:32 am
Area treated:	1 spray swath (approx. 4 acres)
Rate-malathion:	unknown (2 lb/acre planned)
Volume of formulation per acre:	10 gal/acre
Number of loads:	4 planned, 1 executed
Total volume of each load:	58.5 gal formulation
Total volume to be applied:	234 gal formulation
Malathion per load:	11.7 lb (premium grade-actual)
Xylene per load:	1.41 lb
Triton X-155 emulsifier per load:	0.38 lb
Malathion-S^{35} in load:	4.98 g; 263 mc
Water per load:	55.1 gal
Estimated amount applied:	10 to 20 gal
Estimated amount entering soil at crash site:	30 to 40 gal
Type aircraft:	Piper J-3
Final Flight	
Date and time:	25 May 1962 6:40 am to 8:25 am
Area treated:	23.4 acres
Rate-malathion:	2 lb/acre
Volume of formulation per acre:	5 gal
Number of loads:	3 loads
Total volume of each load:	39 gal
Total volume to be applied:	117 gal
Malathion per load:	15.6 lb (premium grade-actual)
Xylene per load:	1.86 lb
Triton X-155 emulsifier per load:	0.5 lb
Malathion-S^{35} per load:	4.98 g, 263 mc; 4.92 g, 260 mc; 5.02 g, 265 mc
Total malathion-S^{35} applied final flight:	14.92 g, 788 mc
Water per load:	35.6 gal
pH:	Approx. 8
Grams malathion-S^{35} per acre:	0.638 g
Activity in cpm per 10 lambda (0.01 ml) formulation; 13 May 1962:	3500cpm
Activity in cpm per 0.01 ml formulation, 26 February 1963:	323 $\pm$ 6.44 cpm
Type aircraft:	Piper P-18, Lycoming 150 h.p.

Table 10. - Continued.

Tank description:	Aero-dynne 110 gal Fiberglass
Pump:	Geared-type, 3 bladed fan mounted on left side
Spray nozzles and lines:	20 nozzles on boom 8 to 12 in. spacing, 1/8 in. floating type K nozzle, diaphragm check, 466 valves, 0.75 in. boom diameter
Output:	2.5 gal/acre, 35 ft swath
Pressure:	25 lb psi
Speed:	about 75 mph

Most of the insecticide at the crash was confined to the airplane and to the ground under the plane. About 30 gal of formulation leaked from the cracked spray tank onto an area of about 1 m^2. Sampling activities within the area will be described under sections on soil, dispersal, soil fauna, and vegetation.

After the crash, three unused loads of malathion formulation were re-divided and additional components procured to allow application of 2 lb/acre of the remaining 788 mc of malathion-S^{35}. The volume of formulation applied was reduced from 10 gal/acre to 5 gal/acre to meet the capacity of the second plane.

The second flight was made in three runs from a local airport. Two men remained at the dams in protective suits to take needed samples. Electric air samplers were operated by project personnel at two nearby farm houses.

Safety Precautions and Equipment

All transfers of radioactive malathion were made or supervised by The Ohio State University radiation safety officers.

Protective clothing worn by personnel on the area during the application consisted of rubber coveralls, rubber gloves, gas masks, and plastic hoods or hats. Atropine syringes were available in a first aid kit.

Four hours after the application only coveralls were worn. These and all other field clothes were donned every morning in a shower room contructed for the project at the laboratory. Every evening all field workers came to the laboratory, took showers, and made complete clothes changes. Laundry of field clothes was done daily for 1 week after the spray; then, one to two times per week. In the field, precautions were taken to avoid ingestion of plants, soil, or water, and unnecessary skin contact with soil.

Throughout the 1962 study Nuclear-Chicago, Inc. film badges were worn for monitoring body exposure to radiation. All reports were for zero measured exposure.

No adverse public sentiment was against the treatment of the study area with an isotope. Project news releases were sent from The Ohio State University. A magazine article appeared in the Ohio Conservation Bulletin (Jan., 1962, p24-26 passim). Several pre-spray tours were conducted for high school science classes and other groups and individuals. Speeches were made to local citizen, school, and church groups and several newspaper articles with pictures were used to prevent local opposition to the research methods employed.

EFFECTS OF MALATHION ON SEGMENTS OF THE BIOTA

There are two purposes for the literature reviews preceding each faunal segment portion of this chapter. The first is the generally accepted attempt to have the work progress from past observations and knowledge. The second is speculative. Early in project planning it was considered that it might be possible to predict, by an intensive literature review, the results of a malathion application to the biota of a forested watershed. Malathion is one of the best studied insecticides, a fact which gave some credence to the proposal. If an accurate prediction could be made, the study would indicate that other insecticides might be evaluated similarly for great benefit. Correlations between certain reported with observed effects would strengthen the knowledge of insecticide action. Relationships might be found that would suggest research data most needed for appraisals of the ecological effects of insecticides. Indicator organisms might be found that would show average effects, others that would show extremes.

If an accurate prediction could not be made, the literature study would indicate that prediction of ecological effects following insecticide application based on current reported toxicological and entomological research is unlikely. If predictions by ecologists are unlikely for intensively studied insecticides such as malathion [a 3094 reference bibliography by R. A. Alverson(1957) and a 323 page bibliography, (Anon., 1960)] then predictions for less-well-known poisons would seem presently impossible. An attempt was made to verify or refute the possibility of an accurate prediction.

SOIL BIOTA

Bollen (1961:87) in a review of the effects of pesticides on micro-organisms, concluded that the effects of insecticides applied at normal or recommended field rates are not great enough to cause reduction in soil fertility. He suggested (1961:71) that results observed in the laboratory cannot be applied with confidence in the field.

Spiller (1961:316) reported that malathion was toxic to vegetative stages of four of five species of Entomophthora, a fungus harmful to spotted alfalfa aphids. Insecticides do not affect spores of E. virulenta and if other species are similar it is likely that present insecticidal programs do not have adverse effects upon survival of these beneficial fungi.

Microbes account for at least part of the rapid decomposition of the OP compounds like parathion (Bollen, 1961:72). Parathion has a stimulatory effect on soil bacteria nitrifier and nitrogen fixers. Excessive amounts, however, are inhibitory (Bollen, 1961:86).

In a letter dated 14 May 1962, Wayne I. Jensen, Bacteriologist, U. S. Fish and Wildlife Service, Brigham City, Utah, commented on studies with malathion saying, "...preliminary laboratory experiments [suggested] that 10 ppm of malathion (in a particular medium and under cont[suggested]itions) inhibited the germination of Closteridium botulinum [type C] spores."

Haddock's (1962) work on the effect of malathion on Tendipedidae larvae (midges) as they relate to botulism poisoning in waterfowl presented the possibility that malathion not only reduces one effective clostridium substrate but may also reduce the spore germination.

Bacteria and Fungi

In order to obtain measures of malathion effects on the microbiota and to correlate these effects with soil carbon dioxide output, eight soil and leaf litter cores were taken from the surface 3 to 5 in. at each of eight grid sites in both watersheds. Samples were taken with a 6 ft long 1 in. diameter King tube pressed into the ground by hand at the side of each small mammal trap at central grid sites. The post-spray sample was taken at the back of the trap, approximately 15 cm (6 in.) from the pre-treatment sample hole. Soils at approximately the same elevation were combined in 1 l (quart) polyethylene bags, tied and stored approximately 1 week before cultures were made. The pre-treatment samples taken May 23 were in storage 3 days longer than the post-treatment samples. The first dillution was made within 1 week after the spray application. Mr. Joseph T. Parisi, Graduate Assistant in Microbiology, The Ohio State University, was employed to process the soil samples, make plates, and count colonies.

Moisture content of samples was obtained by weighing three samples of 5 to 10 g each, placing them in a 175^{o} oven for 45 min. and reweighing.

The dilution-plate method described by Johnson, et al. (1959:5-6) was used with only minor modifications. Two media, Czapek-Dox agar and trypticase-soy agar were used.

There are several inherent weaknesses of this method, but it was judged best for the goals of the total project. Only 10 to 20% of the total bacterial soil population is normally obtained on dilution plates. Pelczar and Reid (1958:476) reported 19 x 10^5 bacteria present per g of deciduous forest soil. In this study populations ranged from 5.3 x 10^5 to 40.0 x 10^5 per g of soil. Fungi ranged from 15.7 x 10^5 to 35.0 x 10^5 per g of soil. Colonies were frequently indistinct. Bacteria and fungi have diverse cultural requirements, some perhaps incompatible with the two media used. Since fungi grow slowly, no attempt was made to distinguish between the bacteria and fungi on the trypticase-soy agar medium.

Two types of colonies were counted, typical bacterial colonies and typical mold colonies. The latter included both true, molds, e.g. *Penicillium* spp., and members of the Order Actinomycetales.

The dilutions of 1 x 10^{-6} produced so few colonies per plate (0 to 7) the results were considered insignificant and are not reported here. Table 11 presents the populations determined from a 1 x 10^{-5} soil dilution.

A formulation of malathion equivalent to that used in the spray was prepared from 6.20 g of 95% technical malathion, 3.00 g Xylene, 0.80 g Triton X-155, and 116 g distilled water. One ml of the 1:1000 dilution of each soil sample was placed in each of 2 sterile petri dishes. Fifteen to 20 ml of Czapek-Dox agar was poured into each plate. After the agar had solidified, a sterile filter paper disk was saturated with the malathion formulation and placed on the surface of one of the agar plates of each soil sample. These plates were incubated at room temperature for 4 days.

Table 11. - Bacterial and fungal populations in units of 100 thousand as measured in Dover, Ohio, soil plate counts on two media at a dilution of 1 x 10^{-5} in 1962.

Watershed Sample Number	Trypticase-Soy Medium Bacteria		Czapek-Dox Medium Bacteria		Czapek-Dox Medium Fungi	
	pre-spray	post-spray	pre-spray	post-spray	pre-spray	post-spray
1-1	16.5	23.0	27.0	29.3	26.0	11.3
1-2	12.0	5.0	11.3	9.3	20.3	7.7
1-3	13.3	17.3	29.3	35.0	19.0	23.3
1-4	5.3	13.7	40.0	33.3	26.5	18.0
$\bar{X}$	11.8	15.4	27.4	27.1	22.9	15.1
s^2	22.2	56.9	140.2	140.7	14.8	48.3
2-1	21.0	19.7	36.5	33.3	35.0	10.3
2-2	16.3	23.7	26.3	39.0	23.3	20.7
2-3	32.5	27.5	26.0	36.7	24.0	19.7
2-4	9.7	5.3	15.0	9.0	15.7	13.7
$\bar{X}$	19.9	19.0	25.9	29.5	24.5	16.1
s^2	92.3	94.2	77.1	192.3	63.1	24.5

F tests of differences between the means of areas, the means of pre- and post-spray colonies, and means of media used indicated no significant differences. Therefore, bacterial populations were not affected by the malathion.

There was some suggestion that there had been a reduction in the fungal population in the post-sprayed samples. However, the differences in the numbers of colonies in the pre- and post-sprayed samples were not significant and can be accounted for by plating and dilution errors. Also, the distinction between bacterial and fungal colonies was difficult to make, especially in the sub-surface colonies. As a result, the conclusion is that the malathion affected neither the bacterial nor fungal population. The results of the disk experiment further substantiate this conclusion.

Carbon Dioxide Output of Soil

The evolution of carbon dioxide from the soil was studied as a gross indicator of the effects of malathion on the microbiological activity of the soil. The effects of malathion on the soil fauna is a question pertinent to the energy base of the forest ecosystem. It possibly influences both present and future soil conditions and consequently soil, water, plant, and animal interactions within the study areas.

Early in the study the technique of Wallis and Wilde (1957) was attempted for measuring soil CO_2 output by collecting the gas in NaOH using a portable electric vacuum pump. A waterproof plywood two-wheel cart was built in which was mounted their suggested unit of an electric, battery operated, emergency automobile windshield wiper motor, 12 v storage battery, test tubes and glass funnels. The plan was to take the cart to several selected areas in each watershed, collect the CO_2 for approximately 1 hr, bring the test tubes to the lab and titrate to obtain quantities of CO_2 evolved. The technique was found unsatisfactory.

Two sites, one in each watershed, were selected on about the same elevation and in as nearly alike vegetation and soil as was possible from inspection. At each site five 14 in. long pieces of used 4 in. steel boiler pipe were driven with a hammer 12 in. into the ground 5 ft. apart along a line. The inside diameter of the tubes was 9.1 cm (3.6 in.). The area from which CO_2 was collected was 65.01 cm^2. Hammering the tubes into the ground cracked a small quantity of encrusted carbonates and iron oxide into the tubes. This was assumed equal in all cases since the pieces were cut from the same pipe. More accurate work could have been done with new pipe with a sharpened cutting edge. Within each tube a 25 mm x 50 mm plastic vial containing 10 ml of 1 N NaOH was placed with its plastic snap-on cap removed. The absorbing surface of the NaOH was 49 mm^2. A 17.5 x 17.5 cm (7 x 7 in.) square of polyethylene was placed over the exposed end of the pipe and secured with a heavy rubber band. The vial was left in the closed chamber for approximately 24 hrs, removed, capped, and titrated in the lab within 48 hrs following the procedures outline by Wallis and Wilde (1957:460). The five tubes were averaged to provide an estimate of the CO_2 from each watershed. No real difference was detected between areas.

After the field work on CO_2 was completed, Elkan and Moore (1962) reported a method of CO_2 measurement that appears very satisfactory and would have been suitable for the purposes of this study.

Fortunately, malathion, parathion, and other organic phosphorus compounds appear to involve no hazard through the accumulation of residues in the soil (Woodward, 1953). Mistric (1954:16) commented that the organic phosphorus compounds do not accumulate in the soil in any harmful amounts. He said, "It is interesting to note that these data on soil residues correspond closely to those obtained from the residual toxicities of these insecticides to insects." Solubility is a factor in percolation or transfer of insecticides into soils (Boswell, 1953:250). Malathion's low solubility would therefore decrease its zone of influence in the soil. Boswell (1953:249) also noted that when applied to aerial parts of plants, insecticides of low stability do not accumulate as rapidly in the soil as when applied directly to the soil. He suggested that even at 1 lb/acre every few years, it seems unlikely that even DDT (which is very persistent) sprayed on a forest will accumulate to a harmful degree.

Hartenstein (1959) studied the effects of 2 lb/acre of malathion of the soil fauna of a hardwood forest. He stated (1959:99) that "malathion was ineffective in regulating soil microarthropod density...The data indicated similar trends in population behavior in the plots which were treated with malathion as the diluent and the controls." He suspected that rainfall during the first month after spray caused the hydrolization of the malathion. Spiller (1961:289) reported complete Collembola control at 1 lb/acre.

Hartenstein stated (1959:93),

> there are many factors involved in the regulation of soil microarthropod density. The natural factors include rainfall, predation, inherent changes in fecundity, quantity and quality of food, and competition. The use of insecticides presents an additional factor that can be of importance.

Ripper's (1956:431) comments are of note: Collembola and acari play an important role in the production of humus and fertility of the soil. Most investigators agree that the balance of arthropod populations is considerably affected by insecticides; imbalances do occur. Though some workers conclude that cultivation has a much more permanent influence on the balance than insecticides do, others are concerned that adverse long-term effects on fertility might occur as a result of general decimation and imbalance.

Resistance within insects provides the possibility for the maintenance of a functional soil fauna.

Nylon Bags

The fauna of forest leaf litter has been studied intensively by many workers. Recently, effects of radioactive waste disposal and fallout on the soil ecosystem have been critically studied (Schultz and Klement, 1963). Since the importance of the soil fauna in litter breakdown, humus formation, and energy and nutrient cycling within an ecosystem is so great, knowledge of their populations and factors effecting populations is essential for the health of the ecological community upon which man is dependent.

Ripper (1956:404) stated that

> since the interdependence of the various arthropod species comes to an equilibrium, a 'balance', a removal of one or,

> more frequently, of several species by the application of pesticides can have profound influence on the other arthropods present.

Hartenstein (1960:360) reported that the use of DDT at 1 lb/acre, malathion at 2 lb/acre, and savicide (the vehicle used to carry DDT and malathion and representing a control) did not result in any population behavior different from that in the untreated control plots in a New York hardwood forest. He stated (1960:361) that malathion at a rate slightly higher than that used in practice is "ineffective in reducing or increasing beneficial soil microarthropods, i.e. ineffective in upsetting a 'balance of nature'".

Auerbach and Crossley (1960:282) concluded that since most arthropods are in the top portion of the soil (Hartenstein, 1959:13), weight differences in samples due to varying amounts of deeper soils have little effect. In their work they ignored differences between dry weight of samples since they were of similar volume and surface area.

Most methods for extracting animals from soil are inaccurate. Hartenstein (1959:29) reported that Berlese funnels were from 70 to 100% efficient. Auerbach and Crossley (1960:279-80) reviewed the developments in the use of Berlese and Tullgren funnels for quantitative soil arthropod studies. They concluded that for microarthropods, particularly mites and Collembola, a funnel device is most practical (1960:279).

Hartenstein (1959:29) stated that

> where comparative quantitative studies are being pursued, the percentage recovery is not as important as an accurate constant proportion recovery from each funnel at each collection date.

A homemade core sampler like that used by Hartenstein (1959:12) was unsatisfactory.

Like others who have studied soil arthropods the writer was unable to adequately handle mathematically, the non-randomly occurring aggregations with excessive variances (Hartenstein, 1961:191; Crossley and Bohnsack, 1960:635). Hartenstein (1961:194) found that Collembola and mite populations fit a negative binomial distribution in 28 out of 31 tests, but this treatment was inadequate to explain the cause of the spatial arrangement observed. He said: "The whole community is closely related and contagious distribution is evidenced." The writer was unwilling to accept the assumptions of Beall's (1954) transformations of data that would have been required to compare estimated means of populations in different localities or those exposed to different treatments, and to estimate population means and their experimental errors over the period of the study.

In order to sample the microfauna as accurately as possible and avoid some sampling problems, nylon bags were selected as a device that would provide a near-constant volume, area, location, and habitat.

In 1962, bags similar to those of Crossley and Bohnsack (1960), Shanks and Olson (1961) and Crossley and Hoglund (1962) were used to study the arthropods of the forest litter. Nylon net of the type used in dress-making was sewn into 23 x 23 cm (9 x 9 in.) bags. Shanks and Olson (1961:194) used 2.3 mm hole nylon mesh bags, 40 x 60 cm, with a 50g leaf litter sample. Crossley and Hoglund (1962) used a 1 dm^2 bag with 2.5 g (dry weight) of deciduous leaves.

The bag measured the arthropods that could be extracted from the 3 mm hole size of the mesh of the bags. After the first arthropod extraction, the bags essentially measured the "recharge" ability of the fauna surrounding each bag. Changes were expected to be evident in the recharge populations of the bag environment.

The modified Berlese funnels in Fig. 15 are 5.5 in. diameter galvanized funnels with a piece of 0.25 in. hardware cloth soldered at the top of the spout to hold samples. Forty watt lightbulbs 2 in. from the top of the funnel were used. Shell vials containing 95% alcohol and 5% glycerin were placed under the funnels and held in place with a piece of aluminum foil which also prevented the possible escape of arthropods moving downward away from the heat, light, and dryness. Samples were left under the lights continuously for 9 to 12 hr for extraction.

In early spring a 10.5 cm (4.12 in.) in diameter 17.8 cm (7 in.) tall fruit can was used to cut circular 85.2 cm^2 (13.2 $in.^2$) litter and soil cores (approximately 190 cc) to fill the bags. The hand was placed under the core which was removed with the can from the forest floor. The core was placed inside the bag as nearly undisturbed as possible. The bag was then sewn shut and replaced in the depression from which the sample came. Since cores were taken 15 cm (6 in.) from each of the 10 grid stakes in each study area, neither location of the bag nor replacing it was difficult. Bags were numbered most satisfactorily by ink on a thin square of wood tacked inside each bag. Some were identified with window screen numbered tacks driven through the net corner into a small block of wood.

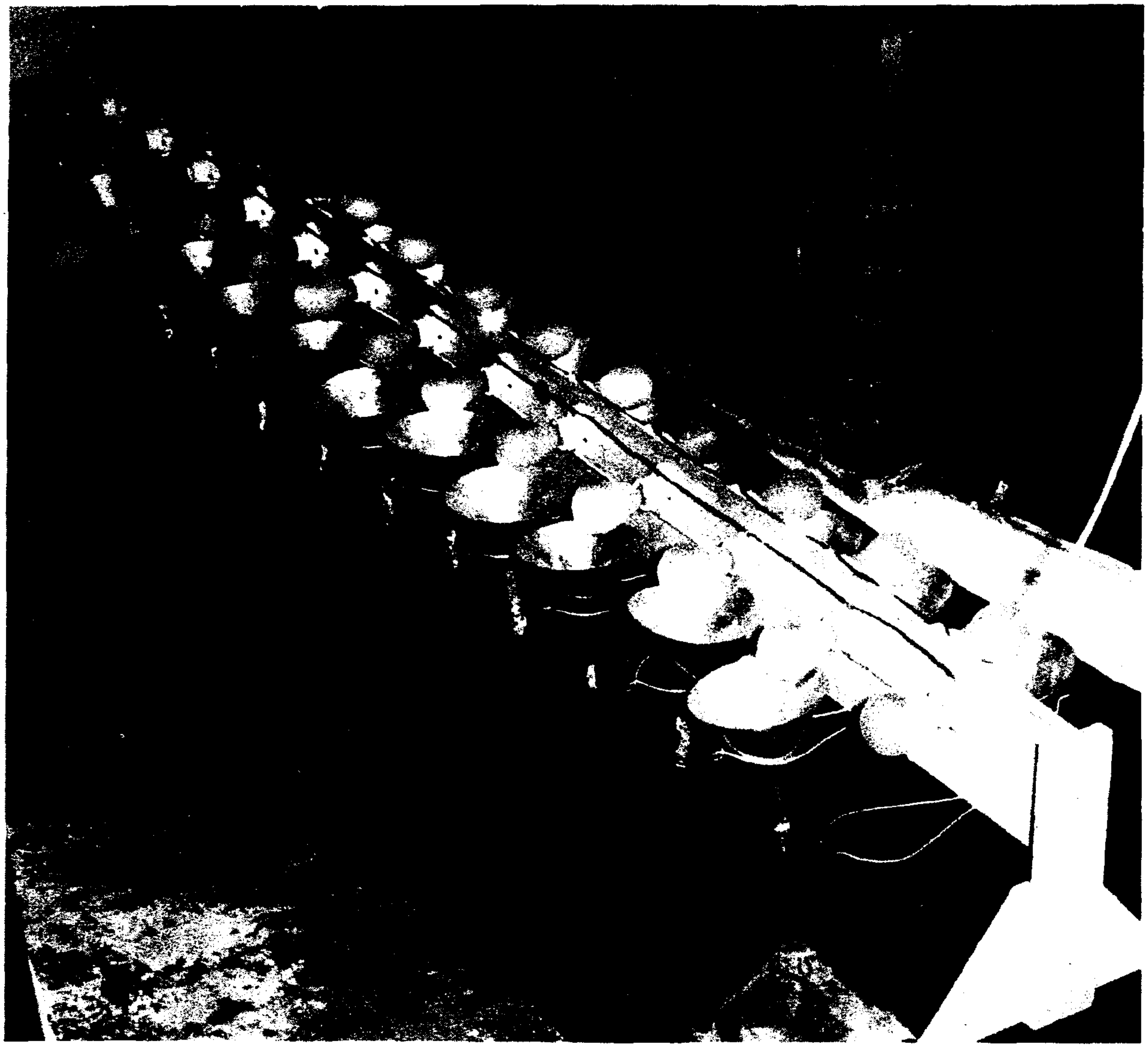

Fig. 15. A battery of Berlese-type funnels were used in Dover, Ohio, in 1961 and 1962 for extracting arthropods from samples of forest leaf litter enclosed in a nylon net bag such as shown in the lower right. Arthropods in bags moved into the funnels away from the light and heat and fell into the vials of alcohol and glycerine below.

Bags were removed periodically, placed in small polyethylene bags and carried in a pack to the lab where they were placed in the Berlese funnels within 12 hr from time of removal. They were "extracted" and vials containing arthropods were numbered, labeled, and sent to OSU where they were examined under a disecting microscope, identified by major groups, and counted. The nylon bags with their litter were returned within 24 hr to the exact spot from which they were removed.

Losses in weight of the litter within the bags, partially attributable to litter breakdown by arthropods, fungi, and other agents, are largely due to handling for extraction purposes--simply breaking of the leaves and the loss of small leaf particles and soil in the plastic bags and funnels. Regardless of the loss of suitable habitat for arthropods the rate of decrease is believed constant and comparisons of populations between areas would not be affected.

In 1961 on the tracer plots, only eight nylon bags were used, two at stake no. 11 in each plot. The results are shown in Table 12 and Table 13. The average number of animals extracted from each bag was 48. Inspection of the data indicates that there are real differences in the populations after spray, _i.e._, the spray had an effect. Although the data are not normally distributed, the variance ratios indicate highly significant differences in the populations.

Table 14 summarizes the forms collected and identified from nylon bags in 1962. From these a summary was prepared for 30 major groups. Of these groups only three had a total count of over 35 individuals and had individuals represented on all extraction dates.

Table 12. - Total number of arthropods and mollusks extracted by Berlese funnels from nylon net bags containing leaf litter, Dover, Ohio, tracer plots, 1961.

Species	Pre Spray (8-24-61) Plot Number				Post Spray (9-4-61) Plot Number			
	1	2	3	4	1	2	3	4
Collembola	9	11	19	8	57	12	53	21
Mites - Oribatids	2	17	6	7	14	5	3	4
Others	2	9	41	11	9	1	4	1
Diptera larvae	1		1		1	1	1	1
Hemiptera nymphs		1	3	8	2			
Coleoptera larvae	1		5	1	1	3	1	
Staphylinidae						1		
Pselaphidae						1	1	
Leoididae								1
Centipede			3	1		1	2	
Pseudoscorpion	1		3	1			2	
Protura	3			1				
Arachnida							1	
Psocioptera							1	
Diplopoda							1	
Psocoptera								1
Phalangida					1			
Snail	1							

Table 13. - Summary of Table 12 with sample characteristics.

	Pre Treatment		Post Treatment	
	Control Plots	Treatment Plots	Control	Treatment
Total number of taxonomic groups	10	8	13	10
Total number of individuals	101	76	155	54
Mean number of individuals per plot	10.1	9.5	11.9	5.4
s^2	195	100	895	32
Number of groups not present in same sample period	2	0	6	3

Table 14. - Summary of arthropods collected by modified Berlese funnel extractions from 10 nylon bags filled with leaf litter in each Dover, Ohio, watershed, 1962.

Order and Family
INSECTA
Protura
Thysanura
Collembola
Orthoptera
Gryllidae (nymphs)
Psocoptera
Thysanoptera
Hemiptera
Dipsocoridae (nymphs)
Homoptera
Aphididae
Coleoptera
Carabidae
Ptiliidae
Scaphidiidae
Staphylinidae
Scarabaeidae
Pselaphidae
Curculionidae
Larvae
Lepidoptera
Caterpillars
Diptera
Cecidomyiidae
Larvae
Hymenoptera
Chalcidoidea
Formicidae
Diplopoda
Chilopoda
Acarina
Oribatid mites
Non-oribatid mites
Pauropoda
Araneida
Symphyla
Phalangida
Pseudoscorpionida

The mathematics for handling such populations have not been adequately developed (Hartenstein, 1961). Because of these difficulties, the data from only the mite and Collembola collections are presented in Appendix Tables 6, 7, and 8 and Figs. 16 and 17.

The sharp drop in mite populations on the treated area that exceeds the drop in the control area 4 days after spray seems unlikely to be coincidental. Yet no sure statement of insecticide effect may be made since a similar drop, though not of the same magnitude, occurs on 19 July. The apparent natural differences between the mites of the two watersheds prior to treatment (watershed no. 1 supporting larger populations) are not present after treatment. Some suppression of populations in the treated area is indicated.

Collembolan populations show about a 75% reduction from a norm of approximately 200 individuals in the sample, but recover within 15 days after the insecticide treatment.

It is concluded that malathion does have some effect on the abundant micro fauna, the mites and Collembola, of the nylon bag environment, that almost complete recovery takes place within 2 weeks, and that there is inconclusive evidence that populations on the treated area remain slightly suppressed for about 2 months.

In order to study the possible correlations of soil arthropod abundance with factors of their environment, Table 15 was prepared. The lack of large positive or negative correlations indicates their population size as measured from nylon bags is independent of the measures made of their habitat characteristics. Multiple regression

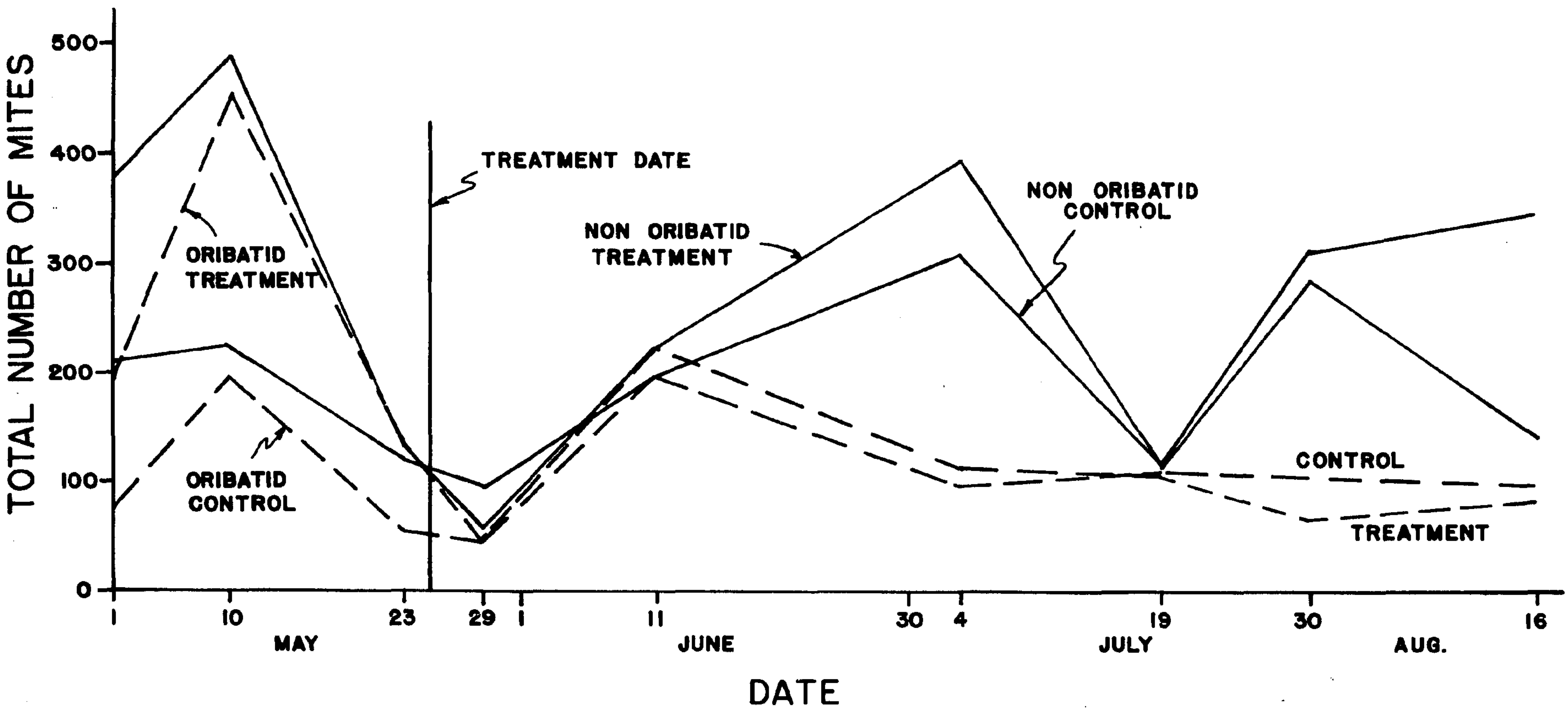

Fig. 16. Comparison of oribatid and non-oribatid mite populations on Dover, Ohio, watersheds in 1962 from nylon bags.

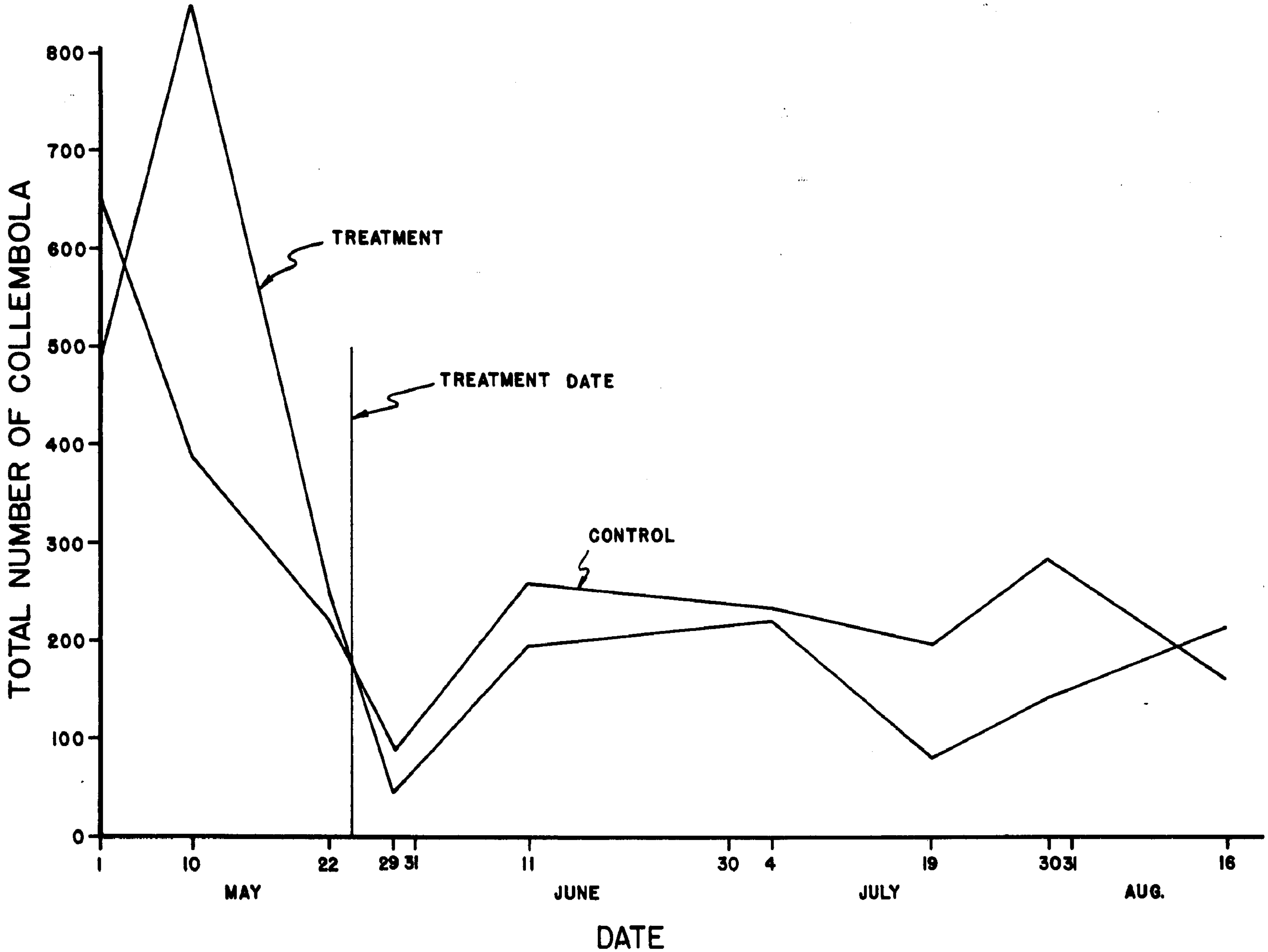

Fig. 17. Comparison of Collembolan populations on treated and untreated Dover, Ohio, watersheds in 1962 from nylon bags.

Table 15. - Correlation coefficients, r, for soil arthropods sampled from nylon bags with factors of the environment.

	Elevation	pH	Organic Matter	Phosphorus	Potassium	Distance from water	Cover Density Rating Tree	Cover Density Rating Shrub	Cover Density Rating Herb
Oribatid Mites	0.117	-0.079	0.265	-0.348	-0.009	0.197	0.344	-0.089	-0.397
Non-Oribatid Mites	0.165	-0.124	0.258	-0.350	0.168	0.239	-0.169	0.163	-0.228
Collembola	-0.422	0.332	-0.271	-0.008	-0.366	-0.340	-0.074	0.323	-0.008

calculations (Snedecor, 1956) of each arthropod group on nine environmental factors suggests that a predictive equation of populations based on environment is impossible and that except for the case of the strong negative relationship of phosphorus to non-oribatid mite populations (Table 16), the relationship of $b_1 = b_2 = b_3 \ldots b_9$ probably does exist.

A study was conducted to verify the time required for population recharge of the nylon bags and to aid in further work with this sampling device. Bags at the 10 sites in watershed no. 1 were used for the tests. Bags were left in the field for progressively longer periods after each typical extraction. The study was conducted between 30 July and 15 August 1962. The results are shown in Fig. 18. Since mites and Collembola compose 75 to 96% of the soil arthropods (Crossley and Bohnsack,1960:636) only these forms were counted.

The variability of the samples even under near-standard conditions is at once apparent. The low populations occurring after the bags were in place 12 hr cannot be explained. It is interesting to note, however, that this was the only time bags were picked up at night. The 12 hr period was from 9:30 am to 9:30 pm. There may be a behavioral factor here, not yet reported. Normalcy of the population is in question. Populations are not correlated with the weight of substrate. Correlation coefficients of litter weight with oribatid mites was 0.01, with non-oribatid mites -0.02, and with Collembola 0.06. This is in accord with the results of Crossley and Bohnsack (1960:629). Although the period of this study was without rain or other major climatic change, the humidity of the evenings and amount

Table 16. - Partial regression coefficients for multiple regressions of mites and collembola as dependent variables on environmental factors as independent variables, Dover, Ohio, watersheds 1962.

Arthropod	Sum	Mean	Standard Deviation	b_o	Elevation b_1	pH b_2	Organic Matter b_3	Phosphorus b_4	Potassium b_5	Distance from water b_6	Cover Density Rating: Tree b_7	Shrub b_8	Herb b_9	F
Oribatid Mites	2361	124.3	46.1	192.7	-0.518	-0.724	11.166[3]	-0.373[4]	-0.092	0.246	7.750	4.464	-20.059[2]	1.517
Non-Oribatid Mites	4119	216.8	60.6	77.843	0.316	2.940	8.742	-0.982[1]	0.223	0.087	-10.652	24.797[4]	-18.443[3]	1.782
Collembola	4891	257.4	124.9	1065.0[3]	-3.746[3]	3.618	-24.383	1.296[3]	-0.526	0.989[3]	4.260	30.051	-18.027	0.901

[1]at 18 degrees of freedom, a test indicates observation significant at the 5% level of confidnece; [2]significant at the 10% level; [3]significant at the 20% level; [4]significant at the 30% level.

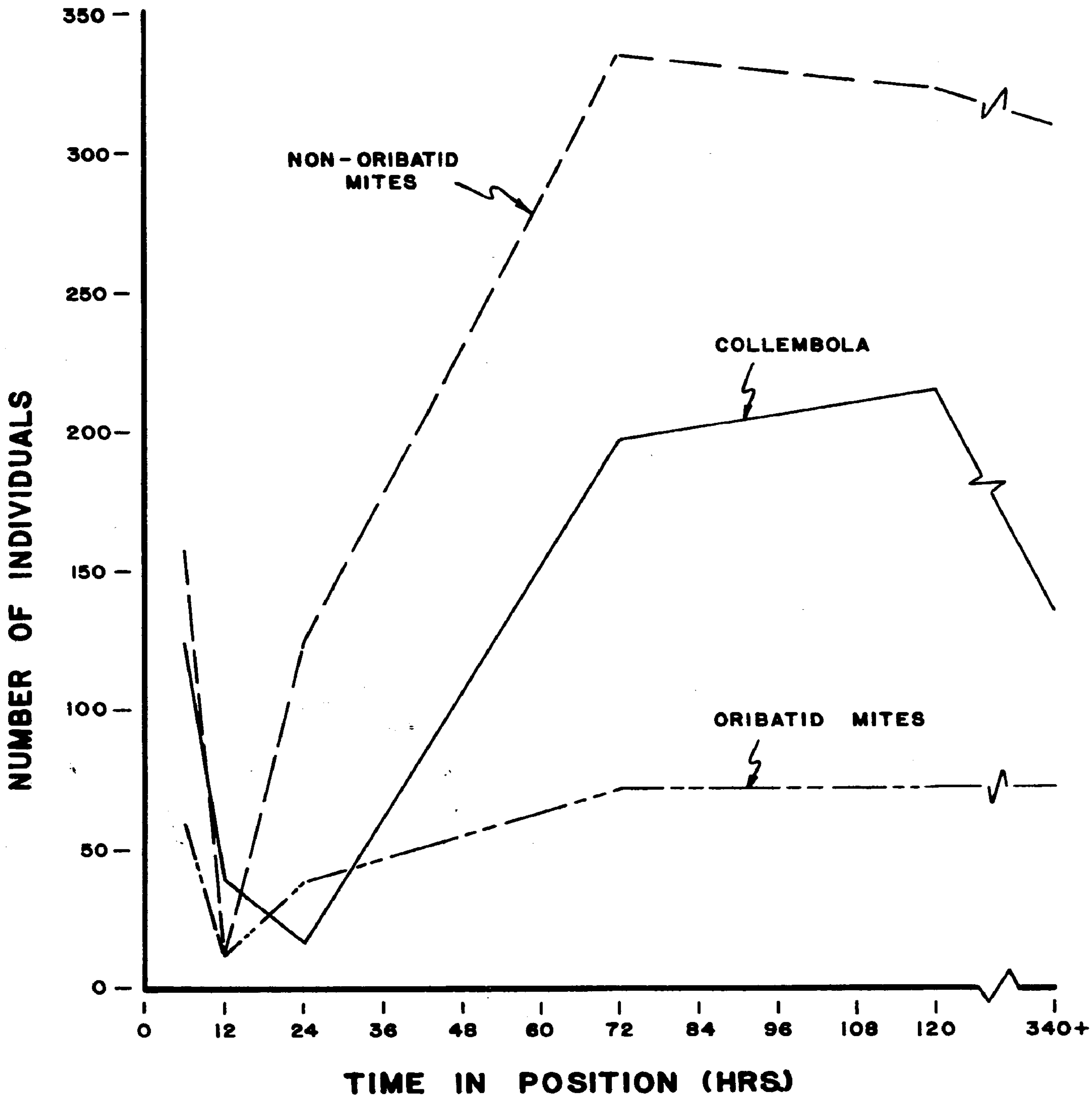

Fig. 18. A comparison of the recovery rates of microarthropod populations in 10 nylon bags containing leaf litter and soil placed throughout Dover, Ohio, watershed no. 1, August 1962.

of overcast during the afternoon are believed significant in influencing the desirability of the nylon bag habitat during its time in place and its condition at the time of removal for extraction. Weather is thought to restrict the recovery of the population to a norm, but once this has been established it appears fairly stable. Fig. 18 indicates that nylon bags should be left in position at least 72 hr to allow populations to reach a norm, a carrying capacity for the fauna of the nylon bag micro-habitat.

On 31 July 1962 a study was conducted of the soil arthropods around the plane crash. Based on radioactivity measured by the portable counter as presented later in Fig. 64 three core samples of leaf litter and the A soil horizon, 10.5 cm in diameter (as described for nylon bags), were taken in areas of 0.5 mr/hr and over, 0.4 to 0.5 mr/hr, and less than 0.4 mr/hr. These samples were placed in plastic bags and returned to the laboratory where they were extracted in Berlese funnels for about 10 hr. The litter samples were replaced in plastic bags and later sub-sampled for radioactive determination by the precipitation method. The results are shown in Table 17.

This table shows, again, wide variability between and among samples. There is a tendency toward reduced total populations of soil arthropods close to the crashed plane. The high insecticide concentrations here are so far removed from normal applications that the results have little value other than simply to recognize that in high concentrations, the formulation does reduce soil faunal populations for over 60 days, and perhaps longer. Because of the large amounts of insecticide involved, the breakdown may not have been complete and

Table 17. - Soil arthropods present in the upper 2 in. of the forest floor at the Dover, Ohio, area plane crash site 77 days after the crash on 15 May 1962.

Coordinate Locations of core samples in plane crash grid	Weight of core sample in grams	CPM/g of processed sample	Thysanura	Oribatid Mites	Other Mites	Psocids	Beetle larva	Total organisms/ g of litter
0.5 + mr/hr								
4-5-1	18.2	7.6238			2			0.11
2	24.8	0.8634			4			0.16
3	23.7	16.8776	1		3	2		0.25
Total	76.7		1		9	2		0.18
Mean	19.2		1		3	2		
0.4 to 0.5 mr/hr								
4-4.5-1	12.3	1.3044	4		3			0.57
2	15.6	0.7824			3			0.19
3	12.9	0.3822			2			0.16
Total	40.8		4		8			0.29
Mean	13.6		4		3			
0.05 to 0.1 mr/hr								
4-2-1	6.7	0.5184	1		2			0.45
2	8.1	0.2614		1	5	2	1	1.11
3	15.6	0.4964	2		6			0.45
Total	30.4		3	1	13	2	1	0.66
Mean	10.1		3	1	4	2	1	

actual malathion may have still been acting on the soil fauna.

Cryptozoa Boards

Four seasoned oak boards 20 x 285 mm (5-5/8 x 11-1/4 in.), 400 cm^2 in area were placed (Fig. 21) 2.4 m (8 ft) from each 61 m (200 ft) grid stake. Boards were placed on the moist soil after the top loose litter had been scraped away. Cole (1946) used the technique to compare cryptozoans of different types of habitat. He spaced 369 boards 1 m apart. This study utilized 92 boards in each watershed or 184 boards. As described by Cole (1946:54) animals seen on the ground or on the under surface of the board were counted after quickly turning the board on edge. Insects quickly disappeared, so larger forms were counted first during cursory observations, smaller ones were counted as the surfaces were systematically examined. The surfaces were generally shaded from direct sunlight by the writer's body but light was needed in the dense forest to make observations. (Cole, 1946:54). The boards were replaced in their original position. During the study several boards became accidentally displaced but similar populations formed after replacement. Numbers of ants and termites were estimated; each board was examined approximately 1 min. It required two people, a recorder and an observer, to examine all 184 boards in 1 day (6 hr afield). The writer made all cryptozoan board observations. Several specimens were collected during observations and identified by the taxonomist. The limited number of forms and a single observer made observations very reliable. Observations are general in that only gross faunal categories were recorded, e.g. all centipedes of several types were simply recorded as "centipedes", snails and slugs were combined as "snails". Cryptozoans under all four boards (1600 cm^2) were totaled. The temporary disturbance of turning the boards was not considered to affect the counts. Species collected and identified under the boards are shown in Table 18.

Unlike Cole (1946:54) mammal disturbance was tabulated as a separate observation and used as another index of mammalian activity. These observations are reported under mammalian studies. Earthworms and salamanders, also counted, are reported under those titles.

Great difficulty was found counting ants, Collembola, and mites (Cole 1946:54). They could not be counted with any degree of confidence. Numbers of ants, usually in large colonies, were estimated. Cole (1946:54) found that the "presence or absence of groups of ants as large as 25 may, however, be used as a criterion of ant abundance for statistical purposes." Mites and Collembola occurred in abundance on the area as seen from litter bag studies. Under a board, however, rarely more than 3 to 5 were seen. Though the board method does not provide a reliable census, or basis for total population estimate, it does provide an index and was used entirely for this purpose. Environmental factors on any day which caused differences in observed animals and those actually present were assumed equal on treated and control areas.

Two very obvious relations were noted. Thysanura were almost always observed with breeding colonies of black ants though they were not observed under boards having similar non-breeding colonies. Cryptozoans were much less abundant under boards after periods of

Table 18. - Invertebrates collected from under cryptozoa boards on the Dover, Ohio, watersheds, 1961 and 1962, and identified in the laboratory.

Insecta

- Collembola
  - Entomobryidae
- Orthoptera
  - Gryllacrididae
    - *Centhophilus*
- Hemiptera
  - Lygaeidae
- Neuroptera
  - Chrysopidae
    - *Chrysopa*
- Coleoptera
  - Carabidae
    - *Pterostichus*
  - Tenebrionidae
  - Elateridae
- Diptera
  - larvae
- Lepidoptera
  - Pyralidae (larva)
- Hymenoptera
  - Formicidae

Chilopoda
- Lithobiidae

Diplopoda
- Polydesmidae

Mollusca
- Limacidae (slug)

Symphyla

Araneida
- Thomisidae
- Gnaphosidae

Isopoda
- Trichoniscidae
  - *Trichoniscus*

prolonged dryness. Cole (1946) did not consider the board a community for he detected little species interaction or "interior intraspecific integration" among the cryptozoa.

Arthropods and mollusks observed under 10 boards in each tracer plot are reported in Table 19. It appears that even though four populations decreased in the treated area, to only one of these, the Collembola, can any degree of confidence be attached. In only one case, the mollusks, did a decrease occur and this could easily be due to chance. The control area total populations increased 282%, while in the same period the treated area populations increased only 176%. The control area populations increased 1.6 times more than did the treated area populations.

Results of the 1961 and 1962 observations of the fauna under cryptozoa boards are summarized in Table 20. While there were 14 groups observed under boards, only Collembola, thysanura, and arachnids were present in numbers large enough to give an observer any confidence in interpreting changes observed with time. Other forms were so variable and so poorly represented in samples that they were not examined except in total population response.

Ants were recorded where counted as individuals or as colonies. By only comparing presence or absence at stakes in each watershed no change is apparent between treatment and control. Ants occurred at between 13 and 21 stakes in each watershed. Actual numbers (individuals plus estimated colony size) were valueless because of their high variances.

Collembola (Fig. 19) appear to be influenced by the treatment.

Table 19. - Sum of the cryptozoans observed under ten 400 cm^2 oak boards in each of four 0.1 acre plots near Dover, Ohio. Plots 1 and 3 were controls, 2 and 4 were treated with 2 lb/acre of malathion in 10 gal/acre of formulation on 29 August 1961.

	Dates			
	Pre-treatment 28 August		Post-treatment 5 September	
Taxa	Treatment	Control	Treatment	Control
Collembola	21	9	13	11
Centipedes	4	2	1	2
Spiders	15	11	19	13
Thysanura	9	9	58	64
Ants	4	6	4	20
Earthworms	0	1	1	2
Millipedes	1	1	2	5
Snails and Slugs	4	2	1	1
Crickets	0	1	1	2
Beetles	0	3	4	5
Isopods	1	0	0	2
Total Individuals	59	45	104	127

Table 20. - Cryptozoans under 92-400 cm^2 oak boards in each watershed. Four boards were located 15 ft from each of the 23 grid stakes in each of the Dover, Ohio, watersheds, 1961-62.

	Pre Spray								Post Spray					
	6-2-61 Watershed		7-17-61 Watershed		4-30-62 Watershed		5-11-62 Watershed		6-11-62 Watershed		7-20-62 Watershed		8-24-62 Watershed	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Species														
Ants	8		160	370	540	1276	2871	2612	1376	2440	2148	3012	3683	2216
Collembola	104		71	30	83	66	51	34	29	40	1	2	3	13
Thysanura	0		72	64	65	66	45	59	124	106	57	102	191	133
Mites	2		0	0	26	58	14	8	13	15	9	13	2	1
Beetles (Coleoptera)	4		15	9	11	6	7	9	7	6	29	12	13	7
Centipede	25		44	10	16	11	15	12	13	8	6	8	5	3
Millipede	2		4	5	7	6	4	4	1	3	3	4	1	0
Spiders (Arachnida)	24		32	33	90	45	29	28	50	52	48	39	37	46
Cricket	0		10	14	3	0	0	1	4	1	6	12	14	4
Unidentified Larvae	5		5	5	2	20	6	10	59	7	28	3	1	2
Earthworms	20		13	8	9	16	9	9	5	2	0	1	0	0
Mollusks (Slugs and Snails)	8		13	4	19	26	12	20	14	15	6	9	9	3
Total (excluding ants)	194		279	182	331	320	192	194	319	255	193	205	276	212

The similarity of the populations in the two watersheds before treatment and the reversal post-treatment supports this observation. Populations had not recovered within 3 months after spray. This could have been caused by the prolonged dry period of the summer.

Thysanura numbers in Fig. 19 are thought to have been slightly depressed after treatment. Populations are so variable this is difficult to support. Mite observations were so uncertain that they were ignored.

No significant abnormal changes were noted in beetles, centipedes, millipedes, spiders, crickets, or isopods. Millipedes and spiders (Fig. 19) were very nearly alike in between-watershed population comparisons. There were 30% more centipedes in watershed no. 1 than no. 2.

A comparison of the total number of individuals (exclusive of ants and termites) on each watershed is presented in Fig. 20, although such summations are not entirely satisfactory. Wide ranges in numbers occurred in 1961 and 1962.

In summary there appear to have been real effects on Collembola, minor effects on Thysanura, and other populations remained unchanged. There was no evidence of population imbalances; no forms unduly increased post-spray in the cryptozoan board habitat.

Earthworms

The importance of earthworms in aerating and cultivating soil, increasing porosity, improving soil texture and watershed properties, serving as prey, converting vegetable matter to reusable products for

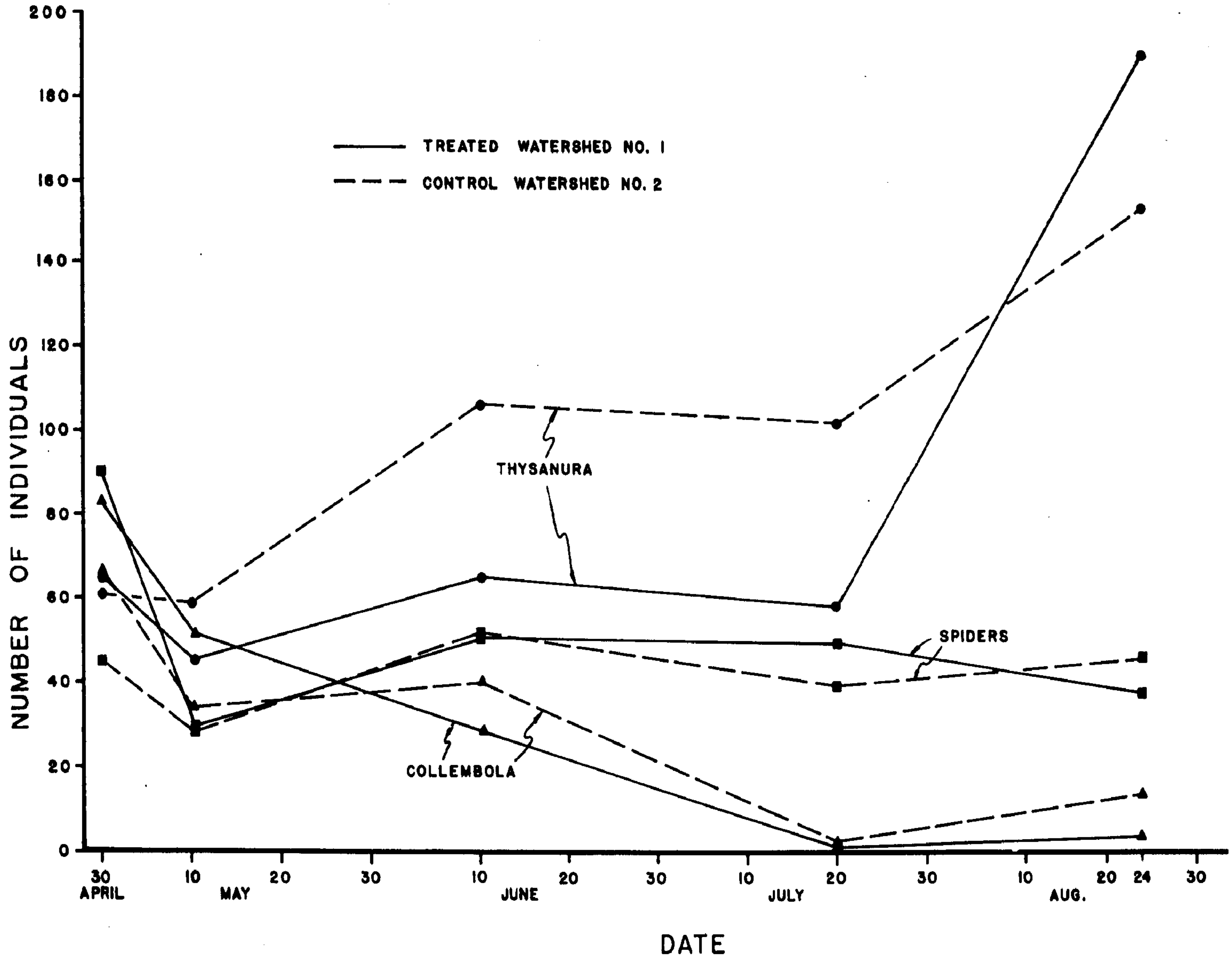

Fig. 19. A comparison of Thysanura, spider, and Collembola populations observed under cryptozoan boards on the Dover, Ohio, watersheds in 1962.

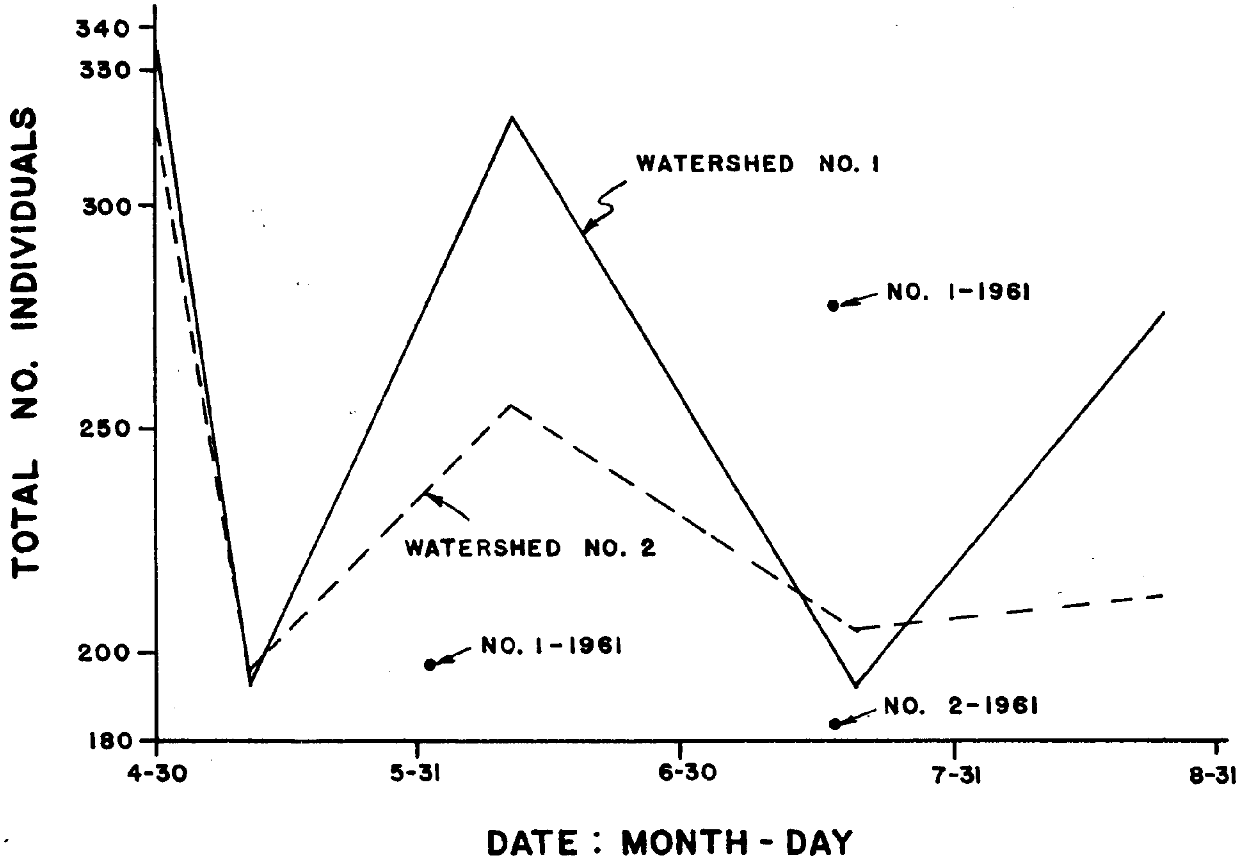

Fig. 20. Comparison of the total number of individual cryptozoans (exclusive of ants) under boards in watershed no. 1 and watershed no. 2 of the Dover, Ohio, study area, 1961 and 1962.

plants, and many other activities are essential in the forest soil community. Hopkins and Kirk (1957) commented that most insecticides are poisonous to earthworms if applied to the area of feeding. They found the 96 hr LD_{50} of earthworms to be 37.9 lb/acre of 4% malathion dust (1957:700).

Hyche (1956:410) reported that malathion was very effective as an acaride, and that it was highly toxic to earthworms at concentrations of 1 and 2.5% emulsions.

Cryptozoan Boards.

Earthworms observed under cryptozoan boards are reported in Table 21. Although a comparison of pre and post-treatment populations on the treated area with those on the control area indicates only a 68% survival, this observation is not real since an F test ($F = 1.4$) indicates no significant difference between the earthworm populations on the two watersheds before spray. There is no significant difference in those populations post spray. There are no effects of malathion on earthworms under cryptozoan boards.

The wide variations in numbers of worms observed both before and after treatment are attributed to moisture differences; the earthworms moved deeper into the soil as the surface dried. They were no longer seen under the boards. The correlated declines between the two watersheds indicate no insecticide effects.

Table 21. - Total number of individual earthworms observed under 92 400 cm^2 oak cryptozoa boards located throughout the Dover, Ohio, watersheds, 1961 and 1962.

Date Month - Day - Year		Watershed No. 1	Watershed No. 2
Pre Treatment			
6-2-61		20	-
7-17-61		13	8
4-30-62		9	16
5-11-62		9	9
	Total	51	33
	Mean	12.75	11.0
	s^2	26.9	19.0
Post Treatment			
6-11-62		5	2
7-20-62		0	1
8-24-62		0	0
	Total	5	3
	Mean	1.7	1

Tracer Plot Studies

Earthworms were purchased from a local fish bait dealer and 15 were placed in each of four 2.8 l (3 quart) galvanized cans. Each can was half full of moist top soil, decaying vegetation, and sphagnum in which the earthworms were purchased. One can was placed on each of the four tracer plots 1 day before the treatment. Many worms escaped or were taken by predators within 7 days post-spray. Results are shown in Table 22 and suggest insecticide effects.

Table 22. - Numbers of earthworms recovered on 4 September 61 (after 2 rains) from Dover, Ohio, tracer plot cans.

Plot Number	Dead	Alive	Total
T-1	5	8	13
T-2	0	0	0
T-3	9	5	14
T-4	10	0	10

Of 7 earthworm samples processed, 3 had significantly high radioactivity: 0.002, 0.005, and 0.009 cpm/mg. This represents activity accumulated by living worms within 6 days after spray.

Expulsion

Two central areas similar in soil moisture, vegetation, and apparent earthworm activity were selected, one in each watershed. A quadrat site was selected, the litter removed by hand down to the soil, and a m^2 string-outlined quadrat was sampled on each date.

Potassium permanganate, an earthworm expellant, about 30 g (1 oz) in 3.18 l (1 gal) of water (Evans and Guild, 1947) was applied evenly over the cleared quadrat in a water sprinkling can. Earthworms began emerging from the soil in 1 min. after application and continued for about 15 min. Those seen were removed from the plot, counted, and returned to the litter near the plot.

Svendsen (1955) in tests of the extraction method found that handpicking a large soil sample in the laboratory yielded more earthworms than the expelent method. The technique is believed to be suitable for the purposes intended, i.e. assaying effects of malathion on earthworm populations in similar soils, in similar areas, the same time each day.

Samples were taken first in watershed no. 2, usually during the afternoon of the date indicated. Each new quadrat on subsequent dates was simply moved several feet uphill from the last spot used. Results of extractions are shown in Table 23. An F test of heterogeneity between the populations of both watersheds indicates that natural real differences exist between the populations of the two watersheds. Calculations of chi-square are for a 2 x 2 contingency table (Table 24) of the population sums, yields 0.14. Therefore, there is no significant difference between the pre- and post-treatment populations and the insecticide had no effect on the earthworm.

On 29 May 1962, 4 days after treatment, samples were taken on both watersheds which yielded 4 earthworms on watershed no. 1; 8 on watershed no. 2. These differ greatly from established pre-spray pattern. On the next day, 3 quadrats were taken at each site yielding totals for areas 1 and 2 of respectively, 88 and 21. These data confirmed the expected negative earthworm effects and showed the variability of the earthworm samples within several feet of each other that can be caused by soil, geological strata, and moisture relations. These post spray samples showed inadequate previous sampling size of placement, but suggested that earthworm populations measured by properly stratified random samples will not have large variances. The correlation coefficient (r) between earthworm population under cryptozoan boards and that obtained from the expulsion method was 0.98.

Table 23. - Earthworms expelled from 1 m^2 quadrats on the Dover, Ohio, watersheds, 1962, by a potassium permanganate solution.

Date Month - Day		Number of earthworms Watershed no. 1	Watershed no. 2
5-7		41	16
5-10		23	14
5-14		26	11
5-22		32	11
	Total	122	52
	Mean	30.5	13.0
	s^2	63.0	6.0
5-25 - - - - - - - - - - Spray Date - - - - - - - - - - - - - - -			
5-29		4	8
6-1		33	0
		33	1
		22	20
6-12		5	4
7-14		26	11
7-25		3	16
	Total	126	60
	Mean	18.0	8.6
	s^2	186.7	57.3

Table 24. - A contingency table constructed from Table 23 for chi-square tests between pre- and post-treatment earthworm populations.

	Watershed 1	Watershed 2	Total
Pre-treatment	122	52	174
Post-treatment	126	60	186
Total	248	112	360

$\chi^2 = 0.14$ χ^2 at the 10% level of confidence for 1 d.f. is 2.7.

This is a very crude estimate of correlation, for dates were not the same and the two sampling methods, hardly comparable. The great positive correlation simply indicates substantiation of the expulsion method data by the cryptozoan boards.

After the treatment earthworms expelled were collected in 10% alcohol, returned to the laboratory and counted. On 29 May specimens from watershed no. 1 had activity of 0.061 cpm/mg, those on watershed no. 2 had 0.037 cpm/mg. No significant radioactivity was observed in specimens collected on 1 June 1962 and 12 June 1962. However, though not significant, earthworms from watershed no. 1 had a 1.0 net cpm while those from watershed no. 2 had no measurable activity. On 25 July 1962 levels of activity in worms from both areas were significant at the 10% level of confidence. Those from watershed no. 1 had 0.230 cpm/mg; those from watershed no. 2, 0.019 cpm/mg.

During the earthworm extractions, many white threadworms (nematode and Coleoptera larvae) were observed before and after the spray but no counts were made. Spiller (1961:312) reported some action of malathion on plant pest nematodes, also some antihelminthic action on hens fed excessive doses.

On 25 July 1962 "grunting" earthworms was attempted as a collection technique (Naggiar, 1962:17). Meter square plots were laid out as for $KMnO_4$ extraction in both areas. In the center of each an 18 in. long 0.75 x 0.75 in. hardwood stake was driven with a hatchet until only 4 to 5 in. of wood was exposed. The side of the hatchet head was rubbed across the top of the stake in long, even strokes for 10 min. producing a pig-like grunting noise and strong ground vibrations. No earthworms were collected in the watershed no. 1 plot, and only 2 in plot 2, though $KMnO_4$ used immediately after the grunting in both plots expelled 3 in no. 1; 16 in no. 2. The technique was ineffective.

Summary and Conclusion

Cryptozoan boards studies, crude estimates of earthworm populations, indicated no effects by malathion on earthworm population. This observation was substantiated by more accurate potassium permanganate expulsions in m^2 quadrats. "Grunting" is an ineffective technique under the conditions of this study. Quantities of radioactive sulfur in earthworm bodies increased with time.

Decomposition and Disappearance Studies

The effect malathion might have on retarding or preventing decomposition or insect attack on animal and plant matter was investigated. It was hypothesized that if scavenger and carnivorous insects were destroyed, their dead bird or mammal prey would remain on the area longer than on untreated areas. This retardation in energy flow could conceivably be a significant effect of an insecticide on an ecosystem.

Five, 4 in. cube cages of 0.25 in. hardware cloth were placed in a line up the center of each watershed. Each closed numbered cage contained a weighed (15 to 25 g) piece of moist lean beef and an inch cube (5 to 15 g) of apple. Reduction in the weight of the samples was expected to be less on the insecticide treated areas.

The study was unsuccessful and abandoned when after 1 week the samples had simply dried and showed no signs of insect attack. After

2 weeks, ants had begun to carry away small pieces of dried apple. Weight loss of a dead mouse in a test tube may be another possible approach to assessing insecticide effects on carrion-feeders.

Even the most skilled observers have had great difficulty in finding dead birds and animals placed afield to determine recovery-efficiency. Mice and shrews found dead in traps were placed on nearby plastic tiles. Without exception they had disappeared within 24 hr. These observations point out the impracticality of evaluating insecticide effects on wildlife on the basis of numbers of observed dead animals. Insecticide effects must be measured, conversely, by the disappearance of known live animals.

INSECTS AND OTHER ARTHROPODS

Malathion is highly toxic to many insects. It is one-fourth to one-third as insecticidal as parathion and is highly toxic to certain phytophagous mites, mammalian and avian lice, aphids, scale insects, flies and many other insects both sucking and chewing (Martin, 1953:152; Negherbon, 1959:451). Negherbon (1959) listed many insects upon which malathion has been tested. Spiller (1961:289) listed effects on Acarina and Insecta. Most crop pests will not be re-listed here in detail. LD_{50} in mg/kg varies from about 0.75 to 120 for a wide variety of insects. Effects are known for: house flies[1] (Lindquist and Fay, 1956); horse bot flies (Hoffman, R. A.,

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[1]"Common Names of Insects" (Laffoon, J. L., 1960) was used for this list.

1960); black flies and aquatic fly larvae, (Lea and Dalmat, 1954:139); Harlequin bug (Peterson, 1954); lawn insects such as fleas, leaf-hoppers, leaf bugs, and mites (Vance, 1956); mosquitoes (Gjullin and Peter, 1956:86); cockroaches (Hornstein et al., 1955:483); mange mites (Webb and Shepherd, 1959); plant mites (Ristich, 1956, and Hintz, 1953); garden centipedes (Howitt and Bullock, 1955:247) the garden millipede (Hennenberry and Taylor, 1961). It is impossible to generalize on the effects of malathion on the heterogenous population of a mixed hardwood forest. Insecticidal effectiveness varies not only between but within insect orders - even within species. Difference in susceptibility is due not only to inherent insect characteristics, but to behavioral patterns (Spiller, 1961:303).

With regard to beneficial insects, malathion has been shown highly toxic to bees (Anderson and Tuft, 1952; Atkins and Anderson, 1954:972; Johansen, 1954:716; Leiberman, et al., 1954). In comparison with other acaricides, insecticides, and fungicides, the organophosphates as a group present the greatest hazard to beneficial insects (Spiller, 1961:315; Harries and Valcarce, 1955:614).

The effect on insects of an aerial application of 2 lb/acre to a mixed hardwood forest cannot be predicted. Effect will range from none to near-complete extermination of species on the area. Variations of effect will occur not only between species but within species as application rates vary with environmental factors. Insecticide effects on certain populations are expected to be obscured by drastic predator-prey-host-parasite shifts caused by the insecticide. Natural population resistance and immediate recharge and stabilization of populations will be dynamic forces acting to obscure insecticidal effects. Egg and larval stages, unmeasured by sampling techniques, may be effected, the results of which will be postponed or will remain unrecognized. Aquatic populations may be effected with subsequent effects on insect larva, eggs, and later, adults. The result is a multi-dimensional web of action and interaction between and within species and their natural environment and an unnatural environmental hazard, malathion insecticide.

The Insect Fauna

The complexity of the insect fauna of the deciduous forest and its ecology can hardly be over exaggerated. One of the greatest problems of studying the effects of any unnatural environmental factors on the ecology of an area is simply to enumerate qualitatively and quantitatively the natural inhabitants of the area. Though working out interrelations is extremely difficult, the first step of finding out what organisms are present to interact is at present larger than can be taken. There are, for example, no techniques available for extracting all organisms (e.g. snails and rotifers) from a given sample of soil; some extractions are destructive for some forms, inefficient for others. Each insect group and life stage requires a different sampling technique. The sampling problems are enormous and vary for each study need. Shenefelt and Jones (1960) presented some of the problems of forest insect surveys.

Sampling methods and intensity for this study were balanced among the factors of available broad-spectrum sampling techniques, relative importance of the insects as related to other faunal segments, time available for sampling, rate at which the taxonomist could count and identify the specimens, and rate at which the radioactivity of identified specimens could be measured. The first year was spent in developing techniques, preparing a collection, and extablishing population comparisons between areas. The balance of effort for the 1962 study was obtained empirically. Morris (1960:254), reviewing studies of total insect populations over large areas, commented that of three major studies by statistically-oriented authors, the sophisticated stratification and optimum design with respect to area were disregarded for the arbitrary choice of the smallest sampling unit.

The purpose of the insect study was to discover what effects an aerial treatment of 2 lb/acre of malathion would have on insect populations of a deciduous forest. Since the insecticide has a short half-life, interest was not only in direct effects such as numbers of insects killed, but in predator-prey balance disruption as expressed in remaining insect populations and in speed of population recovery. The approach to the problem was much like that of Lagier (1949:50).

Unbiased rather than random samples were the objective, and most samples for between-area comparisons were taken in ecologically and geographically similar environments. Morris (1960:255) provided some justification for this approach:

> ...most of the objectives of insect sampling do not require strict randomization, at least in regard to area, and the additional expense that it imposes is therefore not always justified.

He commented that in biology there is still uncertainty as to whether a serious statistical violation results with the use of random theory with unbiased or systematic data. Roving versus permanent plots were considered for all sampling techniques but after trying roving sweep sample plots in 1961, the cost was prohibitive and the great variability even in similar areas made required sample sizes too great.

The sampling devices used for insect sampling are summarized in Table 7. Some random sampling was done to make as complete as possible a checklist of insects for the area. Hoffman and Merkel (1948) and Hoffman and Linduska (1949:105) in DDT effects-studies used tree-jarring onto sheets and cloth bottom traps under trees for arboreal species; light traps, fly traps, box-area traps, and adhesive boards for flying insects; traps baited with fish or molasses, and modified Berlese funnels for ground insects; and sweep net samples and general observation for other insects.

That samples of insects are not true functions of population size has become established fact. Light trap samples, for example, are incapable of sampling larva and pupal stages of insects and their effectiveness varies with temperature, barometric pressure, moisture on foliage, and vegetative stage (influencing the distance that light is visible). DeLong (1932) concluded that sweep samples were good only for qualitative sampling since sample sizes were influenced by light, temperature, shadow of the sampler, humidity, height of sweep above ground, vegetative differences, wind, and number and length of sweeps. Since then, however, sweep samples have been used extensively for population indices or estimates.

The practice of Shenefelt and Jones (1960:16) was followed in sampling habitat niches for populations rather than sampling for individual species. Over a long enough period and using as many techniques as possible, this approach was believed most efficient for the purposes of the study. It was early recognized that larval, pupal, beetle, and ground forms of life were inadequately sampled. Locations of insect sampling devices used are shown in Fig. 21.

Between-year variations in insect populations were expected and did occur though they were not radical (Shenefelt and Jones, 1960:6).

The entire insect picture should gradually change as the forest becomes more mature but within the 2 year study period, this change was unmeasurable.

No sub-sampling of insect catches was done as by Hoffman et al. (1949:6). All insects collected were identified, counted, recorded, and tabulated.

Sweep Net Samples

Though DeLong (1932) considered insect sweeping to be unsuitable for insect population estimates, later workers have used it extensively, primarily for lack of a better device. Crossley and Howden (1960:13) used 10 sweeps as a single sample for estimating insect biomass in a study on effects of gamma radiation on wild insect populations. They observed though (1960:14), that "the large standard errors attached to the estimates [of biomass] suggest that better sampling methods are needed if differences between years are to be demonstrated." They found that the 10-sweep method gave a

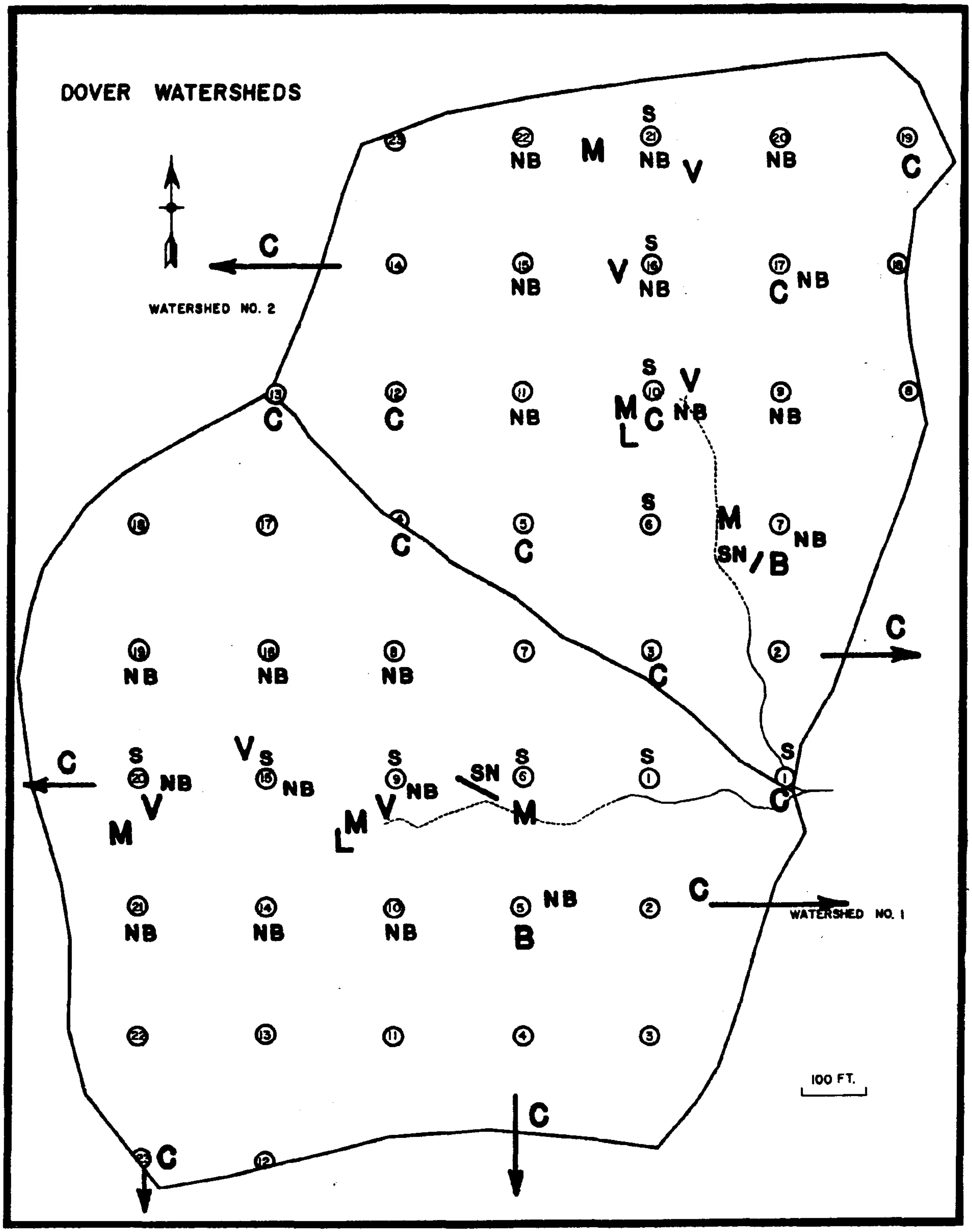

Fig. 21. Location of faunal sampling devices on the Dover, Ohio, watershed in 1962. Four cryptozoan boards were at every circled number; V-vertical sticky board trees; S-ground sticky boards; M-molasses traps; SN-sweep net line; C-catch cloths off watershed no. 1 and at all numbers in watershed no. 1; L-light trap; B-adhesive bands; and NB-nylon bags.

"reasonable approximation of the number of insects on one square meter of vegetation" (1960:11).

Tigner (1960:33) used 100 sweeps over insecticide treated fields to assess effects.

A commercial insect sweep net of marquisette netting, 30.5 cm (12 in.) diameter, 0.66 m (26 in.) long bag, and 0.91 m (36 in.) handle was used. Very small insects were observed to escape from the net. Sweeps of ground vegetation in units of 10 up to 50 were taken in 1961 but no satisfactory method was selected for obtaining insect counts suitable for between-area comparisons. Rain and differences in vegetation were the major variable producers. Even sweeping the "same" areas was impossible since the succulent vegetation did not completely recover from damage caused by walking through it, and many leaves were broken or removed by the sweeping net.

Trials in 1961 were unsatisfactory. Only one collection was made in 1961 that was considered worthy of reporting. One-hundred sweeps in vegetation were taken at random in groups of 10 to 15 sweeps at a time in each watershed. Table 25 summarizes the results.

Table 25. - Summary of results of 100 random sweeps with a net in each Dover, Ohio, watershed on 22 June 1961.

	Watershed 1	Watershed 2
Total number of individual arthropods	352	191
Total number of families or taxa	67	39
Families represented that were not found in the other watershed	48	19

In watershed no. 1 there were 172% more families, 184% higher density, and a greater variety of families. Of the 67 families in watershed no. 1, 71% were not collected in watershed no. 2. Watershed no. 2 had only 48% of its 39 families not represented in the large collection on watershed no. 1.

In 1962 the following method of sweep sampling was adopted. An area was selected near 1-6 in which the shrubs were low, dense, and varied but where they were open enough to allow sweeping the net across an 8 ft wide path. A 42 ft white cloth tape was tied in a straight line between two shrubs. Within this distance were taken 30 vigorous sweeps, approximately one sweep for every step. A habitat nearly like that in watershed no. 1 was selected near 2-7 and marked the same way. Sweeping shrubs was more satisfactory since the habitat was not destroyed by the sampling device and the vegetation was relatively constant. The number of sweeps or area was not planned; the method was adapted to fit the existing conditions. It allowed comparable samples to be taken from both areas with inherent differences becoming evident in pre-spray samples taken within the same year.

Arthropods collected by sweep nets in 1962 were noticeably reduced after treatment. Effects were persistent for over 7 days after treatment. Fig. 22 is based on total arthropods collected by sweep net sampling. Two unusual observations were omitted from the 24 May data: 101 Psocoptera and 152 Chironomidae. Again it can be seen from Table 26 that figures were so low and variable that accurate measures of effect are impossible. Sample radioactivity is shown in Table 27.

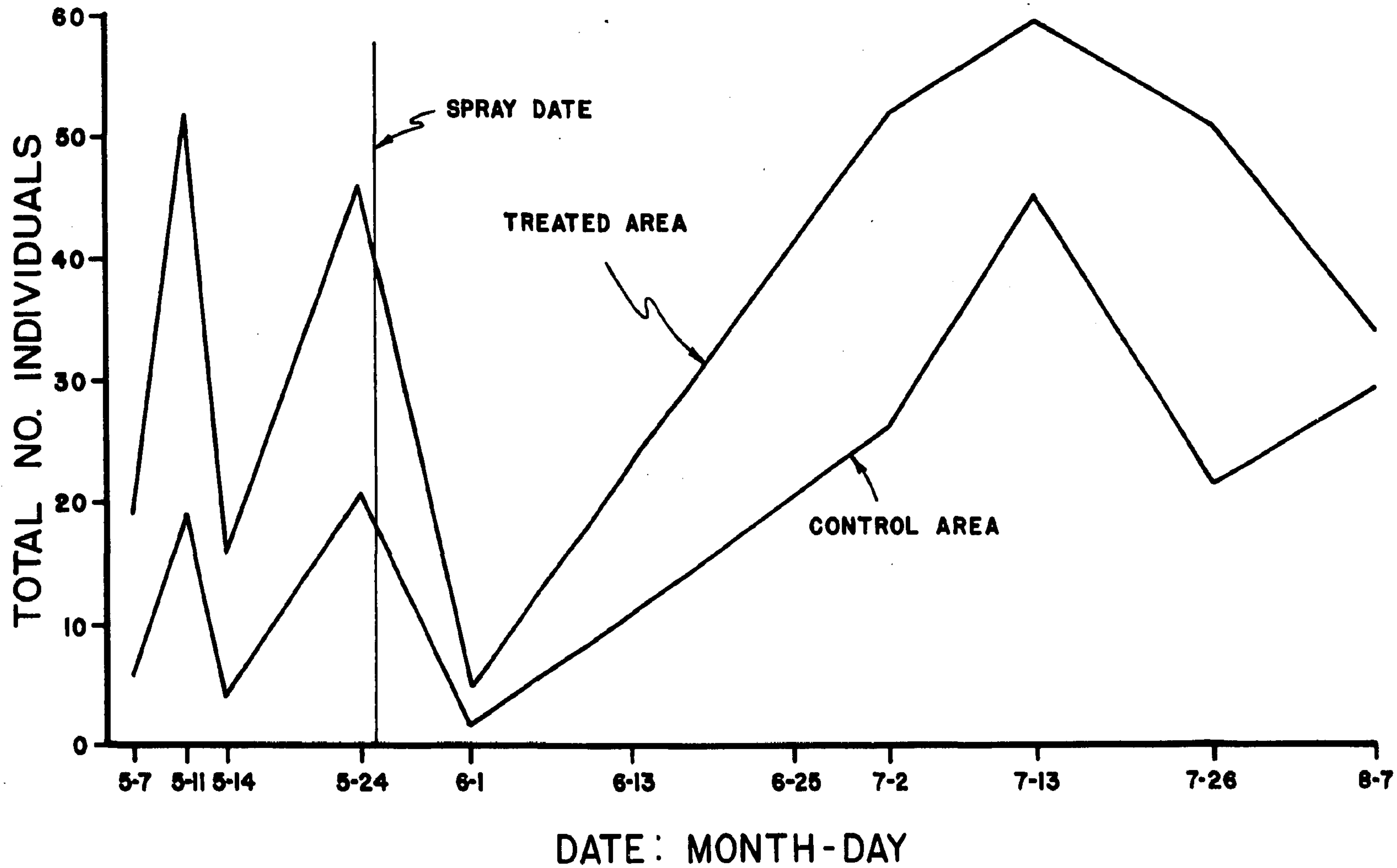

Fig. 22. Comparison of total number of individual arthropods collected in sweep net samples taken along a permanent transect in each Dover, Ohio, watershed, 1962.

Table 26. - Results of 1962 sweep net samples taken along two established transects on the Dover, Ohio, watersheds.

Orders and	May 7		May 11		May 14		May 24		June 1		June 13		July 2		July 13		July 26		Aug. 7	
families	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Orthoptera																				
Phasmatidae																				2
Psocoptera							101	2												
Hemiptera																				
Miridae																	3	5		2
Reduviidae							2													
Tingidae																	1			
Neididae											1									
Lygaeidae															2	2				
Homoptera																				
Cercopidae											2	1	3	4	8	3	5		3	1
Cicadellidae	1		24	4	4					1	3	5			12	5	20	5	11	3
Fulgoridae																1	1	2		
Psyllidae																2	1		2	
Aphididae	13																			
Neuroptera																				
Hemerobiidae			1												1	1				2
Chrysopidae															1	1	1			1
Coleoptera																				
Staphylinidae																	1			
Orthoperidae			12																	

Table 26. - Continued.

	May 7		May 11		May 14		May 24		June 1		June 13		July 2		July 13		July 26		Aug. 7	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Coleoptera (cont.)																				
Cantharidae			1						2			1	3	2	1	1	2	2		
Lycidae														1						
Elateridae			3											1						
Lathridiidae														1						
Mycetophagidae														1						2
Mordellidae															1		1			
Alleculidae														1						
Melandryidae													1	1						
Anobiidae																1				
Chrysomelidae			2		1												2		1	1
Curculionidae		1																		
Phalacridae					1															
Lepidoptera (Macro)															1					
(Micro)							1	1			2	3	1				1	3	1	4
Diptera																				
Tipulidae							2						1						1	
Chironomidae				1			152	10			2		2							
Simuliidae	1																			
Culicidae													1							
Bibionidae																	1	5		
Mycetophilidae													1		1	1				
Sciaridae	3	2	5	5	3	4	4				8		13	6	2	10				
Cecidomyiidae			2								2		5						4	
Stratiomyidae												1								
Empididae								2					2	1		1				

Table 26. - Continued.

	May 7		May 11		May 14		May 24		June 1		June 13		July 2		July 13		July 26		Aug. 7	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Diptera (cont.)																				
Phoridae			1	1	2								1		1					
Pipunculidae							1													
Acalypterates				2			20	2	1				2	4	5	2	4			
Calypterates									1	1										
Hymenoptera																				
Tenthredinidae		1	1		2															
Ichneumonidae															1					
Braconidae							2	1			2	3	3				1		2	3
Chalcididae					1										1					1
Cynipidae								1												
Chrysididae								1												
Formicidae		2		1			2		1				2	1	3	1			1	
Sphecidae														1			1		1	
Phalangida												2		1		1	1		1	
Araneida	1		1	5	2			1			1		6		15	12	1		6	7
Total	19	6	53	19	16	4	287	21	5	2	23	11	52	26	59	45	48	22	34	29

Table 27. - Radioactivity in cpm/mg of air dried sample of arthropods collected in 30 sweeps along permanent transect on the Dover, Ohio, watersheds. Counts were only made of family samples weighing over 0.2 mg.

Date	Watershed No.	Family or Group	Corrected Total Activity cpm/mg
11 May 1962	1	Elateridae	0.096
		Cicadellidae	0.307
	2	Araneida	0.134
24 May 1962	1	Chironomidae	0
		Cantharidae	0.289
		Acalypterates	0
		Bibionidae	0.365
		Araneida	0
		Psocoptera	0.039
		Reduviidae	0
28 May 1962	2	Tipulidae	6.42
		Cantharidae	0
		Bibionidae	0
1 June 1962	1	Cantharidae	0
13 June 1962	1	Cercopidae	0.321
	2	Calypterates	0.250
		Cicadellidae	0.115
		Microlepidoptera	0
		Phalangida	0.096
2 July 1962	1	Cantharidae	0
		Cercopidae	0
		Araneida	0
	2	Elateridae	0.089
		Cercopidae	0
		Alleculidae	0
		Lycidae	0
		Sphecidae	0
		Phalangida	0.320
13 July 1962	1	Cercopidae	0.422
		Cicadellidae	0
		Chrysopidae	0.326
		Formicidae	0.326
		Araneida	0
		Arctiidae	0.134
	2	Chrysopidae	0.192
		Phalangida	0
		Araneida	0.115
		Cercopidae	0
		Formicidae	0.307

Tracer Plots

The proximity of the tracer plots allowed much insect population interchange between treated and control plots. Results of studies on the tracer plots are shown in Table 28 and summarized in Table 29.

Samples were usually not large enough for radio-assay. Sweep samples taken on the tracer plots on 31 August yielded:

Plot 1	8.9 mg sample	- 0	cpm/mg
2	22.1 mg	- 0.4	cpm/mg
3	16.3 mg	- 1.08	cpm/mg
4	11.0 mg	- 0	cpm/mg

The activity in insects on plot no. 3 and lack of activity on no. 4 is probably accounted for by insect movement.

Light Traps

An ultraviolet light insect trap manufactured by Gardner Manufacturing Co. of Horicon, Wisconsin (Model IT-1B) was used for sampling large quantities of insects on the two watersheds. The trap with a 15 watt General Electric Black Light fluorescent lamp was operated first by two 6 v Ray-o-vac batteries in series, later by a 12 v storage battery to which was attached with alligator clamps a DC to AC power converter, Terado Model 50167 with 110 v AC output, 60 CT., 42 w continuous, 52 w intermittent current (Terado Co., 1068 Raymond Avenue, St. Paul 8, Minn.).

The light trap unit shown in Fig. 23 was moved about the area in 1961 in a crate with two 8 in. rubber-rim wheels. It was alternated between permanent spots in watershed no. 1 and no. 2 every week so that samples were taken from each watershed every other week. The trap was operated for 1 hr between 8:30 and 12 pm EST. The results of

Table 28. - Arthropods collected by sweep net, 20 sweeps in the shrub layer per plot, from the treated and control tracer plots, Dover, Ohio, 8-29, 8-31, and 9-4, 1961. The samples were taken just prior to application and 2 to 6 days after the treatment. Family groups are numbered for Table 29.

		Dates: Month-Day								
		8-29	8-31				9-4			
	Order and Family	Plots 2 and 3 Combined	1	2	3	4	1	2	3	4
1.	PSOCOPTERA		1							
2.	EPHEMEROPTERA							1		
	COLEOPTERA									
3.	Carabidae		1							
4.	Phalacridae							1		
5.	Chrysomelidae		1							
6.	Anthribidae						1			
	LEPIDOPTERA									
7.	Microlepidoptera	1	2							
	HOMOPTERA									
8.	Cicadellidae			3	1	3	5		7	2
9.	Fulgoridae	3			2				2	
	HEMIPTERA									
10.	Reduviidae								1	1
11.	Tingidae	1			1					
12.	Nabidae									1
13.	Miridae	4								1
14.	Pentatomidae	1								
	DIPTERA									
15.	Mycetophilidae	5	1	2	1	1	1		1	6
16.	Sciaridae	2	3	7	2	3	2	3	3	2
17.	Phoridae		1			3			1	3
18.	Dolichopodidae		1			1			1	
19.	Culicidae	1								
20.	Lauxaniidae		2	1	2	3			1	
21.	Acalypterates	3	1	5	1					3
22.	Drosophilidae			1		2	1		1	
23.	Cecidomyiidae					2				
24.	Chironomidae	1					3	1		4
25.	Sphaeroceridae							1	1	
26.	Empididae	1								1
27.	Tipulidae	5								
28.	Psychodidae	2								

Table 28. - Continued.

	Order and Family	Dates: Month-Day 8-29 Plots 2 and 3 Combined	8-31 1	2	3	4	9-4 1	2	3	4
	HYMENOPTERA									
29.	Ichneumonidae	3			1					
30.	Chalcididae		1	1	1	1				
31.	Braconidae								1	
32.	Diapriidae				1					1
33.	Formicidae	3					1	1		3
34.	Cynipidae									1
35.	Platygasteridae									1
36.	Vespidae	1								
37.	ARANEIDA	10	2	2	1	1	2	1	1	4
			17	22	14	20	16	9	24	34

Total individuals sampled,	Treatment plots	42	43
	Control plots	31	40
Total Orders sampled,	Treatment plots	4	7
	Control plots	8	6
Total Families sampled,	Treatment plots	11	18
	Control plots	17	15

Table 29. - Summary of arthropods collected by 40 net sweeps in Dover, Ohio, control (C) and treated (T) plots in 3 days in 1961. See Table 28.

Family Group Number From Table 28	Date and Plot 8-29 C	8-31 T	8-31 C	9-4 T	9-4 C
1			1		
2				1	
3			1		
4				1	
5			1		
6					1
7	1		2		
8		6	1	2	12
9	3		2		2
10				1	1
11	1		1		
12				1	
13	4			1	
14	1				
15	5	3	2	6	2
16	2	10	5	5	5
17		3	1	3	1
18		1	1		1
19	1				
20		4	4		1
21	3	5	2	3	
22		3			2
23		2		5	
24	1			1	3
25				1	1
26	1				
27	5				
28	2				
29	3		1		
30		2	2		
31					1
32			1	1	
33	3			4	1
34				1	
35		12	16	1	14
36	1			17	
37	10	3	3	5	3
Total	47	42	31	43	37

Fig. 23. Insect blacklight trap used throughout the study. The cart was used to move the trap between areas.

the 1961 and 1962 sampling of Lepidoptera, a representative order, are presented in Table 30.

In 1962, two of the above traps, one per area, were used. The trap was started in watershed no. 2 and a carbon tetrachloride ($C Cl_4$) saturated rag added to the collecting can. No. 1 was then turned on and poison added. At the end of the hour, the writer would spray the insects on the green baffles with about 2 cc of $C Cl_4$ and would spray about 1 cc into the collecting container, turn off the light, wait until no large moths could be heard fluttering, remove the collecting can and empty it into a cyanide jar on a piece of paper. The traps were operated at the same time except that no. 2 was started and stopped 5 to 10 min. sooner than no. 1. Variations between samples caused by climate were believed eliminated by this method. The collections were brought to the laboratory in numbered cyanide jars, left over night, then packaged and sent to the taxonomist.

Table 30. - Results of light trap collections of lepidoptera insects in Dover, Ohio, watershed no. 1 and 2 in 1961 and 1962. Trapping period was 1 hour on the night of the collection.

Date		Watershed Number	Families										
			Sphingidae	Saturniidae	Citheronidae	Arctiidae	Phalaenidae	Notodontidae	Lasiocampidae	Geometridae	Microlepidoptera	Pyralidae	Undetermined
1961	May 3	1					13			8	82		7
	May 25	1					8	1		11	46	12	18
	June 20	1				2	2			11	6	1	3
	July 6	1		1		1	3		1	1	56	4	
	August 4	1	1			28	3			10	71	1	
	May 18	2								13	30		
	June 7	2		1			13	1		27		3	4
	July 1	2		1		21	2		26	32	59	2	11
	July 28	2		2		34	2			9	112	6	3
	August 12	2				1	11			11	262	2	
1962	May 3	1					3			12	18		
		2					5			11	7		
	11	1				1	3			1	1		
		2				1	7			4	1		
	13	1					4			12	18		
		2				1	3	1		9	18		
	14	1					9			13	32		
		2		1		1	8			19	18		
	23	1				5	17			35	26		
		2				4	32			69	70		
	24	1			1		6			45	40		
		2				3	11			41	39		
	25	1			1	1	6			25	8		
		2				4	12			31	45		
	27	1	2		1	4				8	8		
		2				6	22			8	19		
	29	1				2				24	32		
		1				7	42			33	68		
	29	2					71			62	93		
		2				5	23			22	35		
	June 6	1				7	10			23	21		
		2				6	6			13	11		

Table 30. - Continued.

Date		Watershed Number	Families: Sphingidae	Saturnidae	Citheroniidae	Arctiidae	Phalaeniidae	Notodontidae	Lasiocampidae	Geometridae	Microlepidoptera	Pyralidae	Undetermined
1962	June 14	1					3	1		3	1		
		2		1		2	2			2	1		
	22	1				14	2		2	8	73		
		2				22	18		5	22	187		
	July 12	1	1		1	4					48		
		2				26	4			12	126		
	27	2		1	1	12				9	67		

Morris (1960:247) pointed out that an index of insect abundance was of little value due to the relation of factors other than density. The result of light trapping, for example, is a function of the population available to trapping, the activity of that population, and meteorological factors. King and Hind (1960:524) concluded that light trap catch depends on the activity of the population at the time of sampling and, secondarily, the size of the population. He stated the catch, C, was equal to AP, where A was activity, and P was the part of the catch which is the result of the population level. A, the activity, is the proportion of total activity not supressed by the aggregate effects of weather components, since flight response of the available population is almost entirely the result of current weather. Hollingsworth et al. (1961:308), for example, found wind reduced the catch in light traps. Barr et al. (1963) discussed other variables.

Since all these variables are nearly impossible to analyze, an accurate between-area or before-and-after comparison by light trapping is possible by working a fixed area at identical times. It is therefore possible to disregard population changes and also activity variation since this is obliterated by working with many variable species. Trap catch is a valid unit for comparing differences between insect population composition and density as they are effected by treatment. If another component H, the character of the habitat (primarily vegetation), is added to the equation, then C = APH and the differences in trap catches due to different locations and at different times can be negated and comparisons made more valid. In this study these inherent differences remain proportionately constant, since the same trap sites were always used and sampling was during near-identical times.

Tracer Plots

The light trap was operated in the travel lane between tracer plots 2 and 3 in 1961 before and after the treatment. The results in total numbers of arthropods collected on each date (month-day) were: 8-20, 37; 8-28,350; 9-1, 214; 9-4. 93; and 9-7, 89. The fluctuation could be due to changes in population, temperature, activity or other causes. The sampling radius of the trap was too great to make it of service in this part of the study.

1962 Data

The light trap data of 1962 best show the insecticidal effects of malathion. The Geometridae in Fig. 24 show the drop and rapid recovery of population. It is this rapid recovery that is masked by other sampling devices such as the molasses traps and sticky boards that are left in place several days. Of the 112 family groups sampled some of those showing notable reductions after treatment were Cantharidae, Phalaeniidae, Microlepidoptera, Tipulidae, and Cecidomyiidae.

Radioactivity of these groups is shown in Table 31. Such radioactivity indicates sub-lethal exposure to malathion, and acquisition of S^{35} contamination from the habitat or from feeding. It is important at this point to consider the findings of Schmidt and Weidhaas (1961) with radioactive organophosphates (none were malathion). The concentration of insecticide required to kill

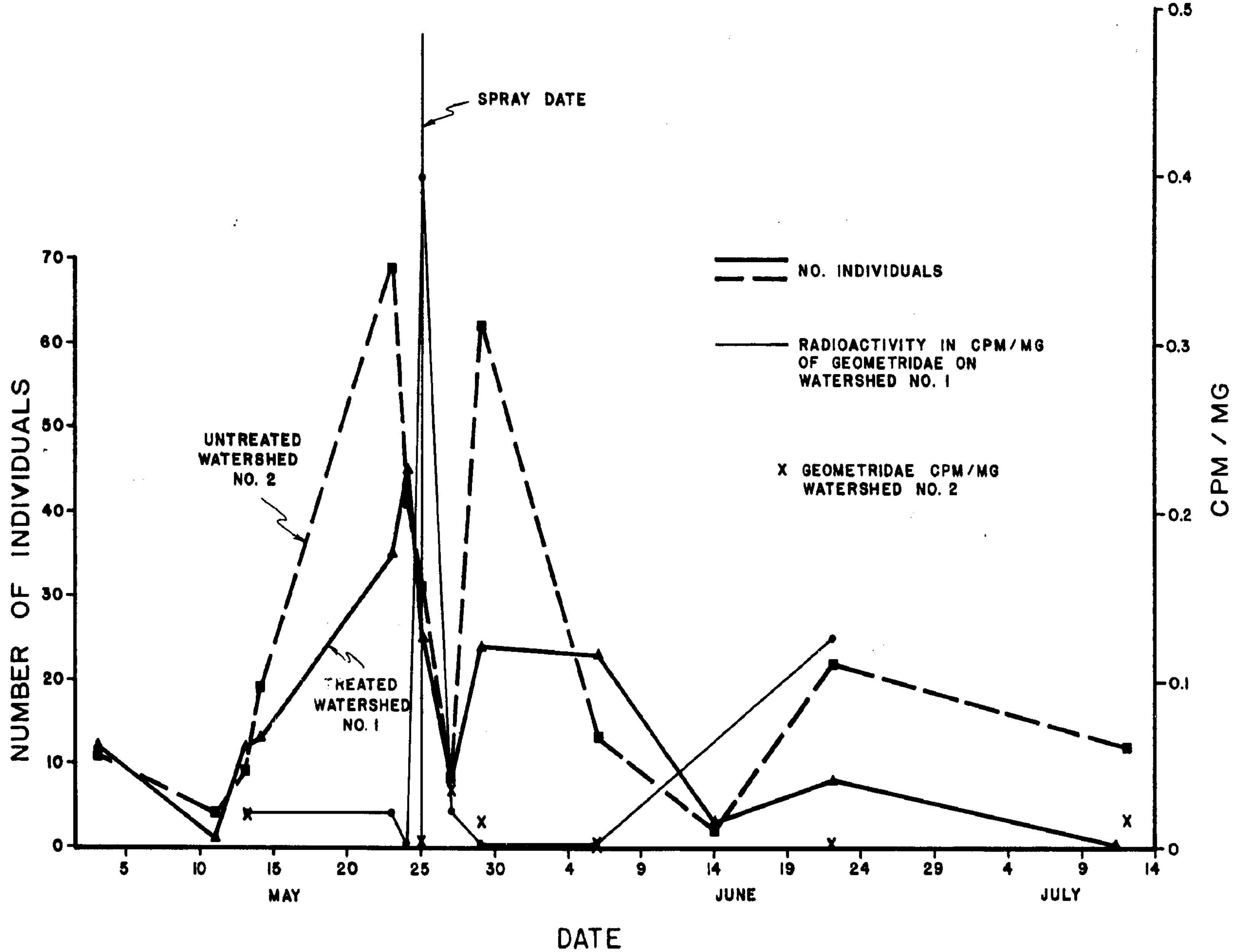

Fig. 24. Comparison of the light trap populations of Geometridae moths on Dover, Ohio, watershed no. 1 and 2, and the radioactivity of these collections.

Table 31. - Radioactivity of insects collected in the light traps, Dover, Ohio, watersheds, 1962, pre and post-spray application. A dash represents no observation; 0, no significant activity above background.

Order Family	Date	Radioactivity in cpm/mg Watershed 1	Watershed 2
Ephemeroptera	5-23	-	0
Orthoptera			
Blattidae	5-29	-	0
Psocoptera	5-23	3.500	0
	5-24	0	-
Hemiptera			
Corixidae	5-23	-	0
	5-29	0	0
Pentatomidae	5-3	-	0
	5-24	0	0
Reduviidae	6-22	0.0959	-
Homoptera			
Membracidae	7-12	0.134	-
Cercopidae	5-23	0	-
	6-22	0	-
Cicadellidae	5-24	0	-
	5-27	0	-
	5-29	0	-
	7-12	-	0
Fulgoridae	5-23	-	0
	5-24	0	-
	5-25	1.582	-
Neuroptera			
Corydalidae	5-29	0	-
	6-22	0	0.015
Sialidae	5-25	-	0
	6-6	0.058	-
Chrysopidae	5-29	0	-
Coleoptera			
Cicindelidae	6-22	-	0
Carabidae	5-13	0	0
	5-14	0	0
	5-23	-	0
	5-24	0	-
	5-29	0	0
	5-29	-	0

Table 31. - Continued.

Order Family	Date	Radioactivity in cpm/mg Watershed 1	Watershed 2
Coleoptera (Continued)			
Carabidae	6-22	0	0
	7-12	0	0
Haliplidae	5-13	0.228	0
Dytiscidae	5-13	0	-
	7-12	-	0
Gyrinidae	5-13	0	0
	5-14	0	0
Hydrophilidae	6-22	-	0
Silphidae	5-13	0.018	-
	5-14	0	0.015
	5-23	-	0
	5-27	-	0.035
Cantharidae	5-13	0	0.018
	5-14	0	0
	5-23	0.070	0
	5-24	0	0
	5-25	-	0
	5-27	0	-
	5-29	0	0.103
	5-29	0	0.034
	6-22	0	0
	7-12	0.250	0
Lampyridae	5-13	-	0
	6-22	-	0
Elateridae	5-13	0	-
	5-23	0	0
	5-24	-	0
	5-25	-	0
	5-29	-	0
Heteroceridae	5-13	-	0.385
Erotylidae	5-14	-	0
Mycetophagidae	5-29	-	0
Pyrochroidae	5-25	-	0.088
	5-29	0	-
	6-22	0.061	0
	7-12	0.211	0
Tenebrionidae	5-13	0	0
	6-22	0	0.077
Alleculidae	5-24	1.015	-
Melandryidae	5-29	-	0
Bostrichidae	5-13	-	0
Trogidae	5-29	-	0
	5-13	0.263	0

Table 31. - Continued.

Order Family	Date	Radioactivity in cpm/mg Watershed 1	Watershed 2
Coleoptera (Continued)			
Scarabaeidae	5-11	0	0
	5-13	0	0
	5-14	0	0
	5-23	0.018	0
	5-24	0	0
	5-25	0.464	0
	5-27	-	0.018
	5-29	-	0.015
	5-29	-	0
	6-22	0.055	0.006
Cerambycidae	5-23	-	0
	5-24	-	0
	5-29	-	0
	7-12	-	0.004
Mecoptera			
Bittacidae	5-29	0	0
	6-22	-	0.019
Trichoptera			
	5-14	-	0
	5-23	0	0
	5-24	0	0.493
	5-25	-	0
	5-29	0	0
	6-14	0	-
	6-22	0.062	0
	7-12	0	0.019
Lepidoptera			
Lepidoptera (unidentified)	5-29	0	-
Microlepidoptera	5-3	0	0
	5-13	0.053	0
	5-14	0	0
	5-23	0.018	0
	5-24	0.018	0
	5-25	1.858	0
	5-27	0.070	0.018
	5-29	0	0
	5-29	0.017	0
	6-6	0.058	0
	6-22	0	0
	7-12	0.009	0

Table 31. - Continued.

Order Family	Date	Radioactivity in cpm/mg Watershed 1	Watershed 2
Lepidoptera (Continued)			
Sphingidae	5-27	0	-
	7-12	0.002	-
Citheroniidae	5-24	0	-
	5-25	0.017	-
	5-27	0	-
	7-12	0	-
Arctiidae	5-11	0	0
	5-13	-	0
	5-14	-	0.029
	5-23	0.005	0
	5-25	0.258	0
	5-27	0.088	0.018
	5-29	0	0
	5-29	0	-
	6-6	0.006	0
	6-14	-	0
	6-22	0.0056	0.004
	7-12	0.019	0.010
Noctuidae (Phalaenidae)	5-3	0	-
	5-11	0	0
	5-13	0	0
	5-14	0	0
	5-23	0	0
	5-24	0.007	0.044
	5-25	0.241	0.018
	5-27	0.035	0.018
	5-29	-	0.013
	6-6	0.010	0
	6-14	0	0
	6-22	0	0.019
	7-12	-	0.004
Notodontidae	5-13	-	0
	5-14	0	-
Lasiocampidae	6-22	0	0.010
Geometridae	5-3	0	2.0
	5-11	0	0
	5-13	0.018	0.018
	5-14	0.058	0
	5-23	0.018	0
	5-24	0	0
	5-25	0	0.403
	5-27	0.018	0.035
	5-29	0.017	0.014

Table 31. - Continued.

Order Family	Date	Radioactivity in cpm/mg Watershed 1	Watershed 2
Lepidoptera			
Geometridae (Continued)	6-6	0	0
	6-14	0	0
	6-22	0.1253	0
	7-12	-	0.019
Diptera			
Acalypterates	5-29	-	0.026
Calypterates	5-23	-	0
	5-24	-	0
	5-25	0	-
	5-27	-	0
Tipulidae	5-13	0	-
	5-14	0	0
	5-24	0.070	0
	5-25	-	0
	5-27	-	0
	5-29	0	0
	5-29	-	0
	6-6	0.115	-
	6-22	0.019	0
	7-12	-	0
Chironomidae	5-27	-	0
	5-29	0.187	0
Culicidae	5-13	0	-
	5-14	0	-
Bibionidae	5-25	1.256	0
	5-27	1.015	0
	5-29	0	0
Myceiophilidae	5-13	0	-
	5-14	0	0
	5-24	0	-
	5-25	1.634	0
	6-22	0	-
Sciaridae	5-13	0	-
	5-14	0	0
	6-22	0.055	-
Cecidomyiidae	5-24	0	-
	6-22	0	-

Table 31. - Continued.

Order Family	Date	Radioactivity in cpm/mg Watershed 1	Watershed 2
Rhagionidae	6-22	0	0
Empididae	5-13	0	0
	5-14	0	0
	5-23	-	0
	5-25	-	0.368
	5-27	0	-
	6-22	0	-
Syrphidae	5-27	0	-
Hymenoptera			
Ichneumonidae	5-11	-	0
	5-13	-	0
	5-23	0	0
	5-24	-	0
	5-25	-	0
	5-29	0	0
	5-29	0	-
	6-6	0	-
	6-22	-	0.019
	7-12	-	0
Braconidae	7-12	-	0
Formicidae	5-14	-	0
Hymenoptera (Bees)	5-25	-	0.123
Phalangida	6-6	0.077	-

mosquito larvae did not necessarily reflect the amount of insecticide that entered the insect. Presence of S^{35} cannot be equated to contact with insecticide. Radioactivity in insects may represent the original insecticide, its metabolites, or both, and at a specific time an equilibrium between the amount taken up and that excreted (Schmidt and Weidhaas, 1958:643).

The reason for the presence of radioactive sulfur in a sample taken on 13 May prior to any 1961 applications is unclear. It probably represents a contaminated sample.

In Fig. 24 (based on Table 32) radioactivity corresponds, as would be expected, negatively with population size. In this case watershed no. 2 seems to have a higher population of geometers. The normal difference was rapidly regained after the treatment.

Molasses Traps

Molasses traps were made of No. 10 (about 3 l) tin cans suspended by 16 ga. wire bails from several inches to 2 ft above the ground. Only two traps were used in 1961 to decide on their usefulness and to suggest sample size. In 6 days 770 arthropods were collected. In 1962 three traps were put along a line in the center of each watershed. In each trap was placed a solution of one part black strap molasses (about 0.3 l) and 10 parts of water. The trap was left exposed and insects attracted or falling in were drowned. Insects were removed by straining the solution into a jar of 10% alcohol and replacing the solution in the can. Contents of three traps were combined in each watershed. Small quantities of molasses were added three times during 1962 when the traps became

Table 32. - Light trap samples of abundant families collected from the Dover, Ohio, watersheds in 1962 compared with their radioactivity in cpm/mg of sample.

Date	Watershed 1 Number Individuals	cpm/mg	Watershed 2 Number Individuals	cpm/mg
Geometridae				
5-3	12		11	
5-11	1		4	
5-13	12	0.018	9	0.018
5-14	13		19	
5-23	35	0.018	69	
5-24	45	0	41	
5-25	25	0.403	31	0
5-27	8	0.018	8	0.035
5-29	24	0	62	0.014
6-6	23	0	13	0
6-14	3		2	
6-22	8	0.1253	22	0
7-12	0		12	0.019
Arctidae				
5-11	1		1	
5-13	0		1	0
5-14	0		1	
5-23	5	0.005	4	
5-24	0		3	
5-25	1	0	4	0.258
5-27	4	0.088	6	0.018
5-29	2	0	0	
	7		5	
6-6	7	0.006	6	0
6-14	0		2	
6-22	14	0.006	22	0.004
7-12	4	0.019	26	0.010
7-27			12	
Microlepidoptera				
5-3	18		7	
5-11	1		1	
5-13	18	0.053	18	0
5-14	32		18	
5-23	26	0.018	70	
5-24	44	0.018	39	
5-25	8	0	45	1.858
5-27	8	0.070	19	0.018
5-29	32	0.017	93	0
	68		35	

Table 32. - Continued.

Date	Watershed 1 Number Individuals	Watershed 1 cpm/mg	Watershed 2 Number Individuals	Watershed 2 cpm/mg
Microlepidoptera (Cont.)				
6-6	21	0.058	11	0
6-14	1		1	
6-22	73	0	187	0
7-12	48	0.009	126	0
7-27			67	

diluted with rain water or when the solution developed mucilaginous bacteria. Trap attractiveness fluctuated widely with stages of fermentation and life stages of insects. Like other traps used, they reflected populations characteristic of their surrounding vegetation. Such traps, unlike the periodic sampling of light traps, may present a significant decimating factor to such insects as bees. For this reason and due to large samples obtained, only six were used, with some misgivings about over sampling in the 1962 study. They are an effective sampling technique for a segment of the insect fauna.

Tracer Plot Traps

On each of the four tracer plots, a molasses trap was hung at stake no. 14. The proximity of the traps to each other was undesirable due to movement of insects between plots and interactions of insects between traps and plots. The location was selected to increase constancy of site influence and elevation on the trap collections and to study, primarily, less motile forms. The results of the collections from four trapping periods, each 3, 3, 4, and 10 days long respectively, are summarized in Table 33. Collections from plots 1 and 3 are grouped under C; plots 2 and 4 under T.

The plots were sprayed the morning of 29 August 1961. The molasses trap had been emptied in the late evening of 28 August. A small number of insects undoubtedly entered the trap between the time of emptying the trap and the time of spray to possibly mask the spray effects.

It has been reported that the control plots were also contaminated by drifting spray. In Fig. 25 this drift and the general mobility of insects in and through the treated areas is reflected in total population drops in both areas. The control plots recover more rapidly and exceed pre-treatment levels. Even if the two groups that showed such sharp increases, the Drosophilidae (215) and the Formicidae (110), were eliminated, the mean population would be 27.7, still above pre-treatment populations. This 3.2 to 4.2 fold increase on control plots over treatment is of interest but is unexplained.

Before treatment both groups of plots had six taxa not represented in the other. After spray the population began to change. There were fewer forms on the treated plots unrepresented in the controls. The treated plots had less diversity of populations than the controls and similarity was not regained within 17 days after spray when the molasses traps were no longer operated. The arthropod groups, mostly families, that were represented in each pair of plots, but not represented in the other pair, on the same date are as follows: 28 August, 6 on the treated plots, 6 on the untreated plots; 31 August, 5 and 7;

Table 33. - Insects and arthropods collected by molasses traps from the 4 Dover, Ohio, tracer plots, 1961, listed by date and treatment plots T, or control plots, C.

Order and Family	Dates and Plot Designation 28 August		31 August		4 September		14 September	
	T	C	T	C	T	C	T	C
COLLEMBOLA		1		1			1	4
PSOCOPTERA			1				1	
HOMOPTERA								
Fulgoridae			1					1
Aphididae	1							
HEMIPTERA								
Miridae		1					1	
Nymph					1			
Tingidae								1
ORTHOPTERA								
Gryllidae								2
COLEOPTERA								
Staphylinidae	2	3		2	2	11	20	30
Nitidulidae	2	3			1	3	17	13
Curculionidae	1							
Erotylidae				1			2	
Carabidae								3
Silphidae						4		
Scarabaeidae								1
Hydrophilidae								1
Ptiliidae								2
Chrysomelidae							1	
Leiodidae								1
MECOPTERA								
Panorpidae		1					2	1
LEPIDOPTERA								
Microlepidoptera			2	1	1		4	2
Caterpillar								1
DIPTERA								
Larvae	26							
Sciaridae	4	2	4	2	1	2	10	25
Phoridae	1			1			1	3
Drosophilidae		4			2	5	67	215
Acalypterates		1				3	2	15

Table 33. - Continued.

Order and Family	Dates and Plot Designation 28 August		31 August		4 September		14 September	
	T	C	T	C	T	C	T	C
DIPTERA (Continued)								
Calypterates		1				1		
Chironomidae			1		1			5
Mycetophilidae			1	2		5	4	18
Rhagionidae				2				
Cecidomyiidae						2	6	2
Rhyphidae							3	7
Lauxaniidae							7	6
Helomyzidae							17	9
Tipulidae								3
Ceratopogonidae								4
Psychodidae							1	
Sciomyzidae							1	
HYMENOPTERA								
Ichneumonidae	1		2					1
Vespidae								
Vespula arenaria Fab.	1	4			1	4	21	16
Formicidae	10	4		2	1	49	26	110
Braconidae			1					
Chalcidoidea				1				
Diapriidae						1		1
Cynipidae								1
Platygasteridae							2	4
Proctotrupidae							1	
PHALANGIDA	1	22	3	3	2	3		1
PSEUDOSCORPIONIDA								2
ARANEIDA	1		1	2				1
TOTAL	51	47	17	20	13	93	218	512
Mean No. Individuals Per Day	17.0	15.7	5.7	6.7	3.3	23.3	21.8	51.2

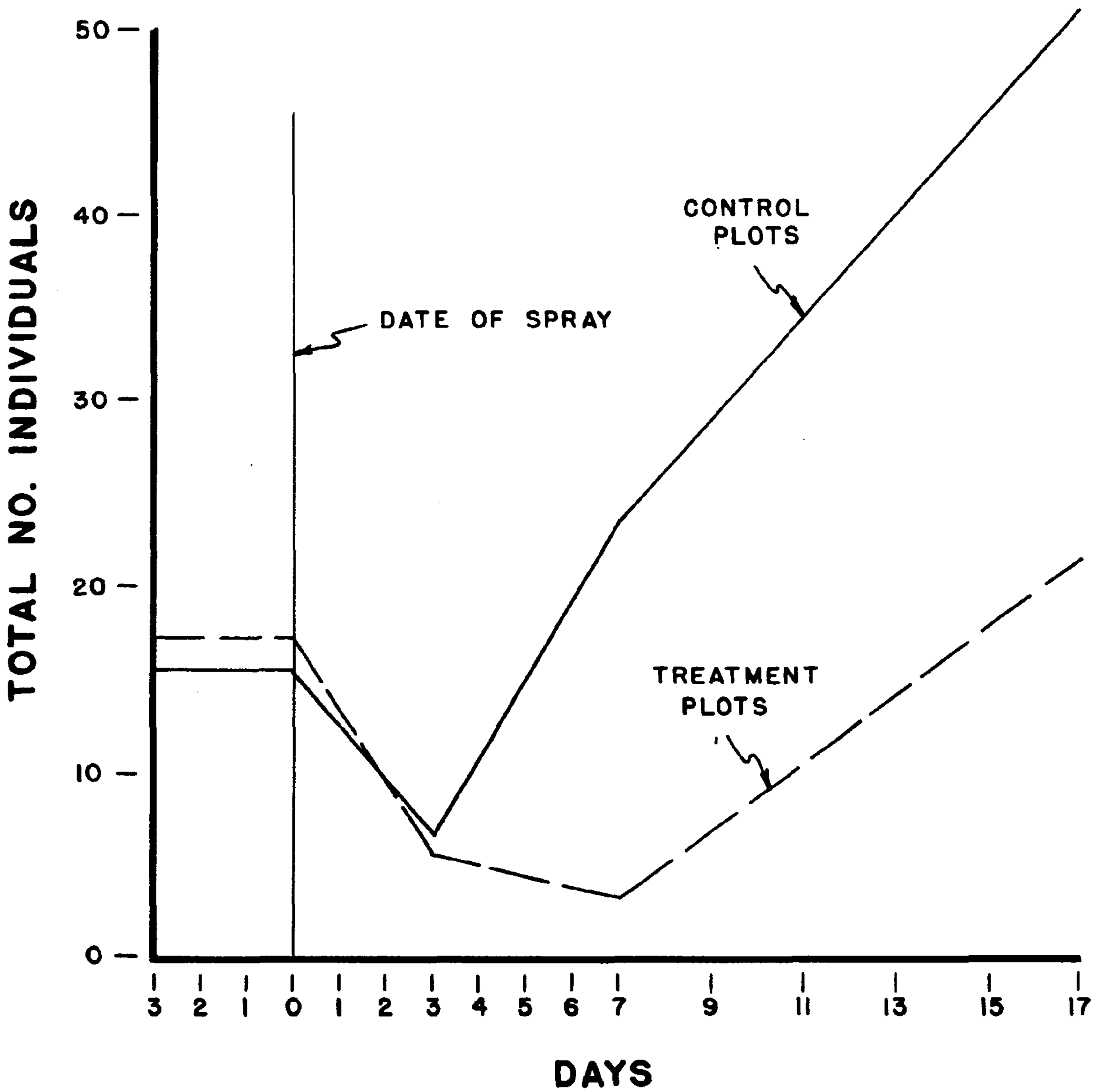

Fig. 25. Response of arthropod populations to malathion as measured by molasses traps on the Dover, Ohio, tracer plots, 1961.

4 September, 3 and 6; and 14 September, 7 and 18.

1962 Molasses Trap Collections

The results of molasses trap collections of Nitidulidae, sap-feeding beetles, are shown in Fig. 26 (Table 34). Table 35 presents the radioactivity of insect families recovered from the molasses collections. There was no correlation ($r = 0.49$, $t = 1.26$) between insects collected and the radioactivity of the trap solution of molasses and water. Thus, radioactivity is a characteristic of the population and not the results of diffusion between insects and sampling fluid.

Again, only one family is diagramed because others sampled are so few and variable that no conclusion can be reached on insecticide effects. After treatment none show major increases or decreases from pre-spray populations. There were 69 family groups represented in the data. The largest collection in any family was the 385 individuals made of Nitidulidae. Most observations from the tree traps in each area were in the range of 1 to 30 individuals in each watershed within the 12 collection dates.

Several observations can be made from Fig. 26. Radioactivity of insects on watershed no. 1, the treated area, was higher than of insects on watershed no. 2. Insects on watershed no. 2 did have radioactivity immediately after the spray but whether this was due to drift on the control area or interchange between areas is unknown. Lack of activity later indicates that there is probably little transfer of insects between areas. Whether radioactivity of populations in

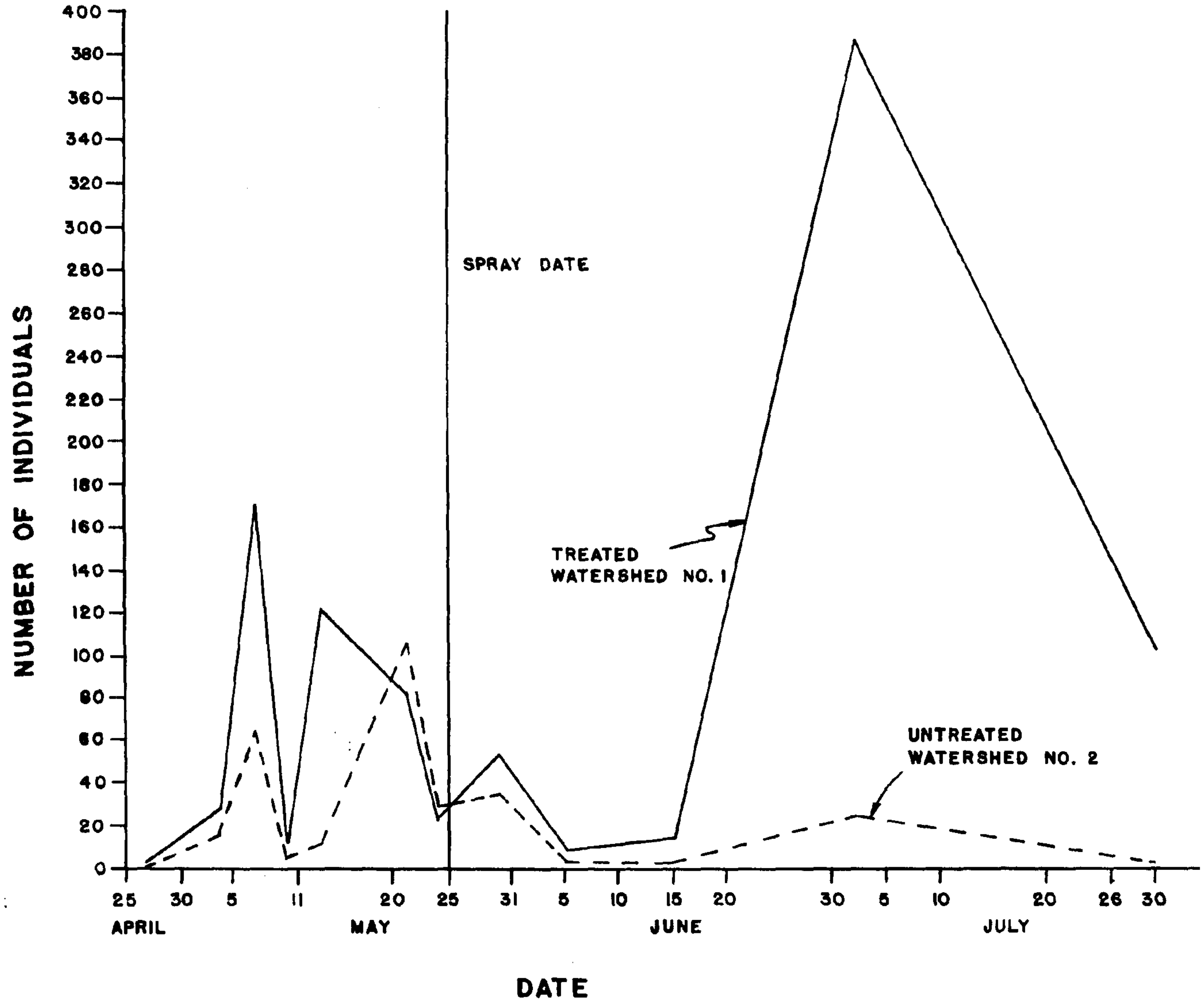

Fig. 26. Comparison of the molasses trap populations of Nitidulidae beetles on Dover, Ohio, watersheds no. 1 and 2, 1962.

Table 34. - Results of 1962 molasses trap collections of the insect family Nitidulidae and the radioactivity of each sample in corrected cpm/mg. See Fig. 26.

Date	Watershed 1 No. individuals	cpm/mg	Watershed 2 No. individuals	cpm/mg
4-27	1		0	
5-4	27		15	
5-7	171		63	
5-10	13		5	
5-13	121		11	
5-21	82	0.006	107	0
5-24	21	0.019	30	0.019
5-30	52	0.132	34	
6-5	8		4	
6-15	14	0.265	2	
7-2	385	0.019	23	0
7-30	103		1	

Table 35. - Radioactivity in cpm/mg of air dry sample of arthropods from molasses traps on the Dover, Ohio, watersheds, 1962.

Family	Date	Location Watershed 1	 Watershed 2
Pyrochroidae	7-2	0.038	
	6-5	0.395	
Araneida	7-2	0.113	
	6-15	0	
Silphidae	7-2	0.038	
	6-5		0.019
	6-15	0	
Nitidulidae	7-2	0.019	0
	6-14	0.265	
	5-30	0.132	
	5-21	0.006	0
	6-15	0	
	5-24	0.019	0.019
Scarabaeidae	7-2	0.002	0.076
	5-21	0.053	
Scolytidae	7-2	0	
Formicidae	7-2	0	
	6-14	0.057	
	6-5		0
	6-15	0.009	
	5-21	0.211	
Cerambycidae	7-2	0.019	
	6-14	0.057	
Ichneumonidae	7-2	0	
	5-30	0.075	
	6-5	0.414	0.038
	6-15	0	
Sphingidae	7-2		0.019
Staphylinidae	7-2		0.113
	5-21		0
	6-15	0.281	
Trogidae	7-2		0

Table 35. - Continued.

Family	Date	Location: Watershed 1	Location: Watershed 2
Elateridae	7-2		5.0
	5-30	0.470	
	5-24	0.250	
	6-15	0.056	
Tenebrionidae	7-2		0.095
Panorpidae	7-2		0.189
	6-14	0.586	
	6-5		0
	6-15	0.019	
Lampyridae	7-2		0.246
Vespidae	7-2		0.019
Blattidae	6-14	0.113	
	6-5	0.132	
Ptilodactylidae	6-14	3.308	
Geometridae	5-30	0.357	
	5-21		0
Bibionidae	5-30	0	
Gryllidae	6-5		0
	6-15	0	
Carabidae	6-5		0.038
Drosophilidae	5-21		0
Mycetophilidae	5-21	0.653	
Tipulidae	5-21		0.094
	6-15	0.489	
	6-5	0.056	
	5-24		0.230
Phalangida	5-21		0
Erotylidae	5-21		0

this study became less once it was acquired is not known. The large number of nitidulids caught up to 25 July (385) had relatively low radioactivity but this capture may have represented a hatch that had not yet had time to acquire the malathion breakdown products. Such animals eaten as prey would contribute to predators less activity per unit volume of food.

Data from collections of molasses traps and other sampling devices is available for interested scientists.

Sticky Boards and Bands

In 1961 and 1962 ten sticky or adhesive boards were hung on small wires at eye level in shrubs throughout the two study areas at grid points 1-1, 6, 9, 15, and 20 and 2-1, 6, 10, 16, and 21. They were made of 12.7 x 20.32 x 0.64 cm (5 x 8 x 1/4 in.) unpainted exterior plywood and covered on both sides (except for the 0.5 x 5 in. top edge) with a thin layer of tree tanglefoot (Tanglefoot Co., Grand Rapids, Michigan) applied with a putty knife. Later it was found that tanglefoot was inferior to "stickem" (Michel and Pelton, Oakland, California) (Kaloostian, 1961). The boards were not painted but were natural wood color. Each board had a total sampling area of 484 cm^2 (75 in^2.). They were carried afield and mailed to and from the taxonomist in wooden cases that kept them separated. The taxonomist, using a microscope, examined the boards. Under the microscope a board was supported on the points of four nails driven through a similar size but un-coated piece of plywood. The identification was difficult; no specimens could be saved.

insects were removed one by one as they were identified and tabulated. The complete boards were returned, tanglefoot was replenished or smoothed, and then they were replaced in the field. Two complete sets of boards allowed continuous sampling; as one was being counted and returned, the other was in position sampling insects. The boards and carrier were described by Sailer and Rozen (1958) and Rozen (pers. correspondence). They hung the boards in circular plots of 9.2 to 12.2 m (30 to 40 ft) diameter but recommended, based on their study, that a linear arrangement be made and more traps be used in similar sites (1958:12).

Sailer and Rozen (1958:9) found that malathion reduced the catch of the initial spray period significantly more than either DDT or methoxychlor. The population was depressed more by 2 lb/acre than by 1 lb. They found that within 6 weeks insect categories seemed to have made complete recovery though at 5 days after the treatment, leafhoppers, membracids, and mirids were virtually absent from all spray plots. Empid and dolichopodid flies, coccinellids, and cantharid beetles were greatly reduced but seldom eliminated from any plot.

> Parasite species such as the chalcidoids, braconids, and ichneumonids were less affected than the predators were as species developing in the soil and coprophagous forms such as sapromyzid flies were relatively undisturbed.

They observed that pupae occurring on the undersides of leaves and in galls were little effected. "...The ratio of parasites and predators to insects having other food habits was normal in August [the second post-spray sample] in the malathion 1 lb. plots..." Sailer and

Rozen (1958) removed some insect groups from calculation since they were evidently unaffected and tended to mask changes in less frequently occurring species.

Tracer Plots

Only one sticky board was hung in each plot at stake no. 17 and changed periodically. The results are shown in Tables 36 and 37 and Fig. 27. Since the area was sprayed on the morning of 29 August, the records on the first date are misleading. The majority of the families were sampled by the boards on 28 August; they were in position over 18 hrs. before the areas were sprayed. Assuming the pre-spray population capable of being sampled by this means was about 40 and the post-spray population about 9, then reduction was 78%. Since this device only samples highly mobile forms, the little difference between the closely spaced plots is easily understood.

In Table 38 are shown the structural changes that occurred in arthropod populations as they can be measured by this device. Not only was the loss in individuals great but almost half of the families disappeared from the samples. Following the spray, for the duration of the 15 day sampling period, the following forms did not reappear in the sample: Hemiptera - Tingidae; Homoptera - Cercopidae; Coleoptera - Erotylidae, Cucujidae, Phalacridae, Chrysomelidae, Scolytidae; Diptera - Psychodidae, Asilidae, Calypterates; and Acarina.

The Sciaridae, dark-winged fungus gnats; Cecidomyiidae, gall midges; and Phoridae, the hump-backed flies were all reduced to at

Table 36. - Total number of arthropods collected on sticky boards hung in tracer plots 28 to 30 August and 30 August to 4 September 1961 near Dover, Ohio.

	28 - 30 August				30 August - 4 September			
Order and Family	1	2	3	4	1	2	3	4
PSOCOPTERA	1	3	1		3		1	2
THYSANOPTERA	1	2	1	1	1			2
HEMIPTERA		1						
Miridae			2	1				
Tingidae		1	1					
HOMOPTERA								
Cercopidae	1	1	1	1				
Cicadellidae	2	2		3			1	
Psyllidae				1		1		1
Aleyrodidae		1	1	1	1			
COLEOPTERA								
Staphylinidae				1		1		
Orthoperidae		2	1		1			
Elateridae					1			
Erotylidae		1						
Cucujidae	1							
Phalacridae	1							
Chrysomelidae	1			1				
Scolytidae	1	1						
LEPIDOPTERA								
Microlepidoptera	1				1			
DIPTERA								
Psychodidae			1					
Chironomidae			1	3				
Mycetophilidae	1							1
Sciaridae	9	7	3	10	3	3		1
Cecidomyidae	14	10	2	9	2	3	4	2
Asilidae	1							
Phoridae	11	5	3	5	3		1	
Lauxaniidae		1	1					
Acalypterate		1		1				
Calypterate				1				
HYMENOPTERA								
Myrmaridae	11	7	8	6	1	1		5
Chalcidoidea	2	1	5	1	1	3	3	1

Table 36. - Continued.

Order and Family	28 - 30 August 1	2	3	4	30 August - 4 September 1	2	3	4
HYMENOPTERA (Continued)								
Formicidae							1	
ACARINA	1	1		1				
ARANEIDA		2		1				1
Totals	60	50	32	48	18	12	11	16

Table 37. - Total number of arthropods collected on sticky boards hung in Dover, Ohio, tracer plots 4 to 8 September and 8 to 14 September 1961.

	4 - 8 September				8 - 14 September			
Order and Family	1	2	3	4	1	2	3	4
COLLEMBOLA			1					
PSOCOPTERA		1	1			1		
THYSANOPTERA				1		1		
HEMIPTERA								
Miridae								1
HOMOPTERA								
Cicadellidae	2							
Aleyrodidae	1	2			1	1	1	
Phylloxeridae						6	2	1
COLEOPTERA								
Staphylinidae	3	3		2				
Orthoperidae		1				1	1	1
MECOPTERA								
Panorpidae								1
LEPIDOPTERA								
Microlepidoptera				1				1
DIPTERA								
Chironomidae		1			3	2		2
Mycetophilidae	1			1				
Sciaridae	3	5	4		1	1	1	1
Cecidomyiidae	6	5	7	7	3	2	4	6
Empididae	1							
Phoridae	5	7	3	2	5	3	4	3
Lauxaniidae		2	1		1			
Acalypterate				2	1			
HYMENOPTERA						1		2
Mymaridae	3			1		1	6	3
Chalcidoidea	3	3	2	2	1			
Formicidae						1		
ARANEIDA	1			1				
	29	30	19	20	16	21	19	22

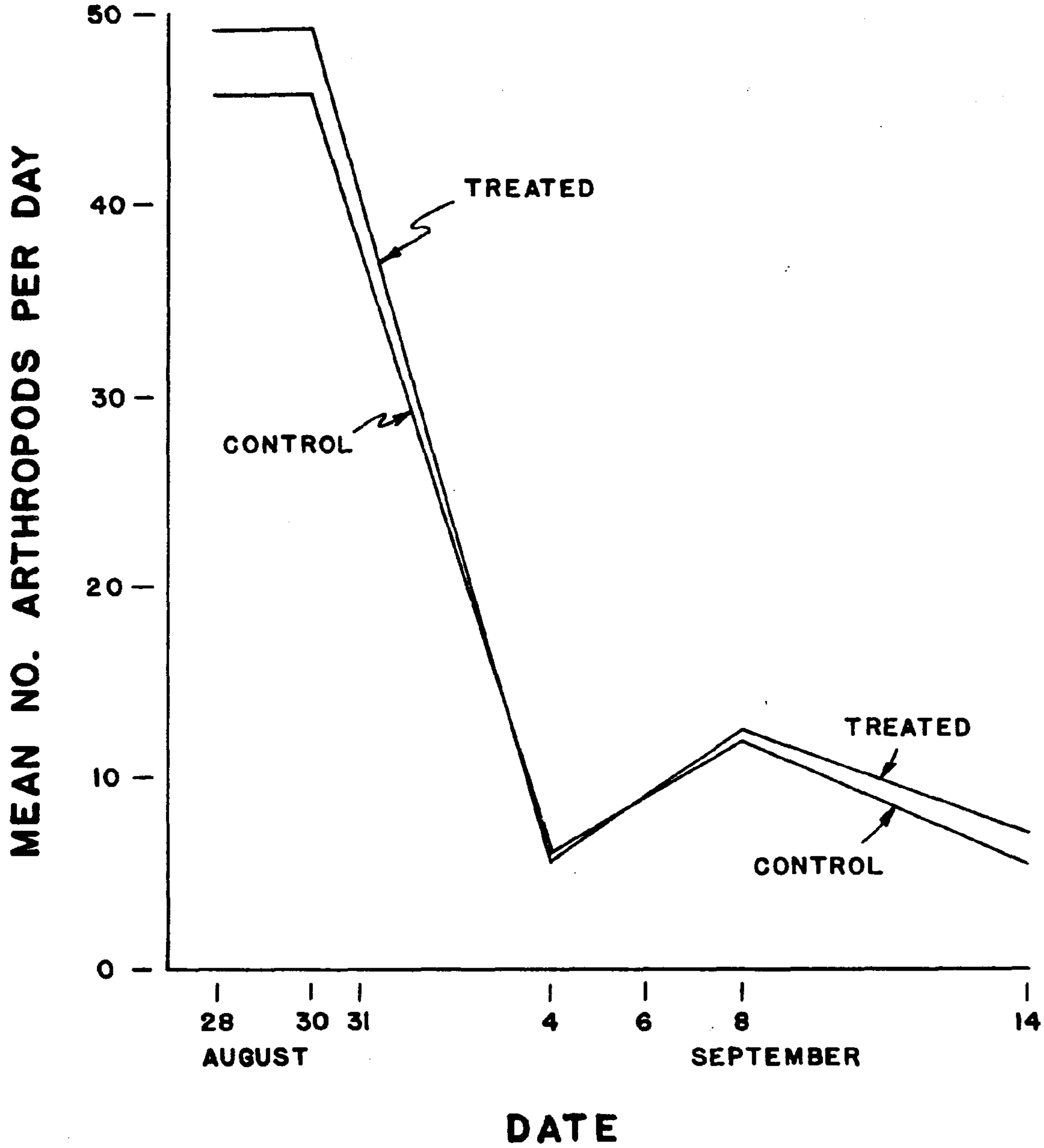

Fig. 27. Summary of influence of malathion application on the mean daily insects sampled by sticky boards on the Dover, Ohio, tracer plots, 1961.

Table 38. - A summary of the families of arthropods that were collected in one area but not collected in the other on the Dover, Ohio, tracer plots, 1961.

	Dates of Collection			
Area	28-30 Aug.	30 Aug.-4 Sept.	4-8 Sept.	8-14 Sept.
Treatment	7	4	4	7
Control	6	7	4	2
Total no. of families in both areas	31	17	19	18

least one-fourth their pre-spray populations.

On the other hand, four species appeared that were not collected before the treatment. Of these four, three were represented by only one specimen. Formicidae appeared on treated and control plots after the spray. Three unidentified Hymenoptera appeared on the treatment plots during the last sampling period. These increases are considered to be chance collections not representing population resurgance or invasion following application. Whether such a phenomenon occurs over a longer period will be examined in the 1962 data.

1961 - 1962 Data

Sticky boards were one of the most unsatisfactory of the devices used to study arthropods. Besides disadvantages of the field, laboratory, and taxonomic time spent with the difficult-to-handle boards, the results were nearly impossible to analyze. The largest sampling unit was 9.0 individuals per day. This was for 73

Chironomidae counted from five boards which had been in position for 8 days. The highest total capture was 94 Phoridae from five boards left in place 23 days. Most of the counts for 72 family groups were below 1 per day per watershed. Analysis was impossible. Boards left in place 10 days prior to the treatment had the lowest count of any 1962 sampling periods. Post treatment effects, therefore, could not be measured. The larger orders represented were Coleoptera and Diptera. In 1961 there were 709 insects counted on sticky boards from watershed no. 1; 561 from watershed no. 2. The faunal productivity or carrying capacity of the two watersheds differs by about 1.25, the same as measured by other sampling devices.

Vertical Boards

In 1961, no attempt was made to ascertain the insect population of the forest canopy except as they might be attracted by the sampling devices already in use.

In 1962 sticky boards were placed at 10 ft intervals from the ground into the canopies of three trees in the center of each area. Tree locations are shown in Fig. 21. Climbing irons were used to attach a pulley by wire to the highest point which could safely be reached. Twisted wires served as hooks for the boards which were held vertically 6 to 10 cm (3 to 5 in.) away from a hemp rope. The boards were raised and lowered in the trees as on flag-pole ropes. Maximum height attained was 60 ft, four trees had 50 ft ropes, one had a 40 ft rope. Davidson (1930) used a rope ladder and sweep net to show the dissimilar insect strata of the forest.

Results from sticky boards suspended in trees were as unsatisfactory as results from ground boards. Large population increases after spray were encountered at all levels. All numbers for individual family groups were so low and variable that no change could be concluded with confidence. No 1961 data was available for vertical sticky boards. Populations were generally equally distributed except at 60 ft where smaller numbers were evident.

Tree Bands

As a one-attempt sampling technique, tree bands of ordinary fly paper (latex and mineral oil adhesive) were put out on three trees 4 ft from the ground on each watershed 24 May 1962 and left until 29 May 1962, 5 days (see Fig. 21). On watershed no. 1 a sassafras, an ash, and a red oak were banded, numbers 1 to 3 respectively; on no. 2, a maple, an ash, and a red oak, numbers 3 to 6 respectively, were banded. The specimens collected were in worse shape than those on the sticky boards due to awkward handling provisions. Results are shown in Table 39.

Because of lack of knowledge of pre and post-spray populations sampled by this device, little can be said about the results. If the differences in insect abundance between the two areas hold as measured by tree bands, the total insect population was reduced by over 40%. Notable differences appear in the dipteran Phoridae, Empididae, and Sciaridae.

Catch Cloths

Catch cloths were torn in 0.65 m (26 in.) squares, 0.435 m^2

Table 39. - Arthropods sampled by tree fly paper bands left in place 1 day pre-spray and 4 days post-spray, Dover, Ohio, 1962.

	Location and Tree							
	Watershed 1				Watershed 2			
Order and Family	1	2	3	Total	4	5	6	Total
PSOCOPTERA			2	2	1			1
HOMOPTERA								
Cicadellidae	2	1	6	9	4	3	2	9
Fulgoridae	1		1	2		1		1
COLEOPTERA								
Staphylinidae			1	1				
Cisidae	1			1				
LEPIDOPTERA								
Microlepidoptera		1	1	2	2	1	1	4
DIPTERA								
Tipulidae	5	2	3	10	6	3	5	14
Chironomidae	10	7	18	35	11	10	22	43
Ceratopogonidae		1	1	2				
Rhyphidae					2	2	3	7
Bibionidae	1		1	2				
Sciaridae	11	21	51	83	67	51	80	198
Cecidomyiidae	2	2	7	11		1	2	3
Stratiomyidae					1			1
Empididae	1	1	2	4	4	5	2	11
Dolichopodidae	1			1				
Phoridae	26	6	9	41	23	9	27	59
Acalypterates	1	1		2	1		1	2
Calypterates			1	1		1		1
HYMENOPTERA								
Braconidae		1		1				
Chalcididae	2		3	5		2		2
Formicidae	1			1				
PHALANGIDA		3		3	7	3		10
Total				219				366

(4.69 ft^2) from unbleached muslin or white quilting cloth. Two cloths were laid horizontally within 4.5 m (15 ft) of each sampling spot and all corners were pinned with heavy nails. The combined effective sampling area of the cloths was 0.75 m^2 (8 ft^2). The location of the cloths are shown in Fig. 21. The spacing was designed to sample intensively the insects killed on the treated area, to determine "biologically" the extent of the spray effect off the treated area, and to correlate measured spray distribution with spray effect.

After the spray, the cloths were cleared of insects by four people, two doing the majority. Two methods were used, both of which are believed to produce near-equal results. The most-used method was to kneel before the cloth and with a pair of needle-point forceps pick each insect from the tray. This was most effective when there were only a few insects or when tree debris and dirt on the cloth was slight. When debris was great and field time limited, i.e. when a given number of cloths must be picked, the corners were raised in diagonal pairs and the debris collected near the center, then scooped into the square pill box in which most catch cloth collections were made. These boxes were later emptied into a white enamel tray in the laboratory and insects sorted before being sent to the taxonomist.

An indelible black ink cross on the cloth aided in examining and picking the cloths. Ants and spiders were active on many cloths and their influence on the counts, though unknown, is believed to be slight when insect kill was high, but in the latter days of insecticide effectiveness, their removal of killed insects could readily account for the low or even absence of kill-evidence.

In this study the sampling intensity was too great, for the manpower available could not collect insects from all cloths in the time available. Two people working together for companionship and safety on the 2-cloth sampling spots required about 20 min. to clear each sample spot. Some samples required additional laboratory time. Naturally, as insect kill decreased, so did picking time, but a set walking time and 5 min. minimal observation time for each cloth put severe limits on sampling intensity. Another limitation of the technique is the lack of knowledge of insects and the questionable conscientiousness of untrained labor. This menial task required such laborers while many other critical observations were being made.

During the first day, a 2-man team of trained and untrained personnel picked cloths, with occasional checks of each other's work. On the average, three insects (2 to 4% error) were overlooked by both observers. On one occasion a tray was picked, checked by the second observer, then its contents of oak flowers, dirt, etc. were dumped into a box and examined later by microscope. Four minute Hymenoptera had been overlooked. There is some bias in the catch cloth results but it is slight and assumed equal between compared samples. Later, after checks were removed, there is no knowledge of efficiency of clearing trays though it is assumed to be similar to checked work. The operation is tiring; tedious; and after several hours, boring. It cannot be done until cloths are dry from rain or dew.

A better approach to ascertaining actual kill would have been to reduce the frequency of samples and increase the size of the area of each sample. Large plastic sheets, if checked often to avoid rain or wind damage, would be very satisfactory. By raising the corners slightly, a concave, tray-like sampling area could be provided. Insects could be easily removed from these and counted in the laboratory. Twelve 0.61 x 1.2 m (2 x 4 ft) wooden edge, cloth bottom catch trays were used on the tracer plot but were not used later.

1962 Studies

Table 40 presents data on background counts of insects from catch trays prior to application of spray and shows post-spray results. Seventy family groups were collected from catch cloths from the 25 May application. The mean number of insects collected from all cloths in watershed no. 1 on 26 May was 71.9 ($s^2 = 2120.6$), on 28 May (a 2 day accumulation) 13.7 ($s^2 = 44.5$), and on 30 May (a 2 day accumulation) 21.7 ($s^2 = 301.5$).

Only two family groups were abundant enough in the catch trap sample for any meaningful population analysis (See Table 41 and Fig. 28). There was no correlation between maximum cpm on glass discs from the 25 May flight or 27 May "rainout" and the abundance of the dipteran Chironomidae, midges or the hymenopteran Chalcidoidea or chalcid flies. This precludes a bioassay of spray distribution. Catch cloth parameters are more a function of population size than the quantity of insecticide falling within the area. Even a general expression of malathion effectiveness on certain insect groups cannot be made based on catch cloth data because pre-treatment populations

Table 40. - Radioactivity in cpm/mg of air dry sample of arthropods collected from catch cloths on the Dover, Ohio, watersheds, 1962.

Date	Location	Family	cpm/mg
20 May 1962	1-1	Chironomidae	0.468
		Araneida	0
		Fulgoridae	0.516
		Phalangida	1.139
	1-7	Chironomidae	0.748
		Calypterates	0.392
		Nitidulidae	0
26 May 1962	1-1	Membracidae	0
		Stratiomyidae	1.353
		Carabidae	0.214
		Calypterates	0.623
		Acalypterates	2.100
		Plecoptera	0
	1-4	Formicidae	0
		Ichneumonidae	4.592
		Curculionidae	0.409
		Acalypterates	0
		Psylliidae	0
		Araneida	0
		Stratiomyidae	0
		Tipulidae	0.854
		Chrysomelidae	0.837
		Microlepidoptera	0.943
		Elateridae	0.107
	1-5	Buprestidae	1.460
		Trichoptera	0
		Tenthredinidae	3.791
		Calypterates	0.584
		Hymenoptera (bees)	0.107
		Scarabaeidae	0.160
	1-6	Formicidae	1.193
		Stratiomyidae	0
		Chrysomelidae	0
		Calypterates	1.602
		Carabidae	0.214
		Hymenoptera (bees)	3.364
		Chironomidae	1.032
	1-7	Elateridae	0.534
		Araneida	1.139

Table 40. - Continued.

Date	Location	Family	cpm/mg
26 May 1962 (Continued)	1-7	Formicidae	1.353
		Membracidae	0.427
	1-8	Ichneumonidae	0
		Formicidae	0
		Lygaeidae	1.086
		Syrphidae	1.068
		Elateridae	0
	1-9	Calypterates	0
		Araneida	0.498
		Chironomidae	2.261
		Formicidae	0.570
	1-10	Syrphidae	4.539
		Oedemeridae	0.605
		Chrysomelidae	0.534
		Acalypterates	1.727
		Chironomidae	1.121
		Formicidae	0.623
		Stratiomyidae	0.587
		Tachinidae	0.231
		Tricoptera	0.570
	1-11	Psylliidae	0.623
		Chironomidae	1.068
		Hymenoptera (bees)	5.732
	1-14	Chironomidae	0.659
		Empididae	0.285
		Calypterates	0
		Formicidae	0
		Cocinellidae	0.676
		Stratiomyidae	1.068
		Reduviidae	0.712
		Elateridae	0.107
		Tabanidae	0.142
		Acalypterates	0.516
		Calypterates	0.463
		Stratiomyidae	0.819
		Syrphidae	0.552
		Elateridae	0.783
		Formicidae	1.032
	1-15	Tingidae	0.890
		Panorpidae	0

Table 40. - Continued.

Date	Location	Family	cpm/mg
26 May 1962 (Continued)	1-15	Bittacidae	1.157
		Bibionidae	0
		Tipulidae	1.193
		Stratiomidae	0.926
		Calypterates	0
		Tingidae	0.570
		Ichneumonidae	0
	1-16	Lygaeidae	0
		Stratiomyidae	1.317
		Cantharidae	2.261
		Araneida	0.089
		Bibionidae	2.225
		Calypterates	0.249
		Acalypterates	2.528
		Microlepidoptera	0
	1-20	Tipulidae	2.545
		Syrphidae	0.445
	1-21	Psocoptera	0
		Formicidae	0
		Stratiomyidae	0
		Cerambycidae	0
		Tipulidae	0.285
		Chironomidae	2.278
	X-1-2-1*	Fulgoridae	1.638
		Formicidae	1.406
		Empididae	1.531
		Lygaeidae	0.338
		Calypterates	4.379
		Acalypterates	12.585
		Chironomidae	0
		Scarabaeidae	0.356
		Syrphidae	0.880
		Cantharidae	0.267
		Tingidae	3.311
		Chrysomelidae	1.193
		Chrysopidae	4.450
		Tachinidae	0.125
	X-1-2-2	Formicidae	0
		Membracidae	0
		Tipulidae	0

Table 40. - Continued.

Date	Location	Family	cpm/mg
26 May 1962 (Continued)	X-1-2-3	Calypterate	0
		Sphecidae	0.445
		Buprestidae	0
		Chironomidae	0.338
		Phalangida	0
	X-1-2-4	Ichneumonidae	0
		Tipulidae	0
	X-1-4-1	Curculionidae	0
		Carabidae	0
		Membracidae	0
	X-1-4-2	Chrysomelidae	8.259
	X-1-20-1	Hymenoptera (bees)	0.125
	X-1-20-2	Microlepidoptera	1.976
		Membracidae	0
	X-1-20-3	Elateridae	0
		Formicidae	0
	X-1-23-3	Scarabaeidae	0.053
	2-1	Membracidae	0
		Scarabaeidae	0
		Tipulidae	0.356
		Chironomidae	0.587
	2-2	Tipulidae	0
		Membracidae	0
	2-6	Calypterates	0
		Stratiomyidae	0.765
		Panorpidae	0
	2-10	Chironomidae	0.125
	2-17	Stratiomyidae	0
		Chironomidae	0.160
	X-2-2-1	Chironomidae	0
		Chrysomelidae	1.139
		Ichneumonidae	0
		Araneida	0.641

Table 40. - Continued.

Date	Location	Family	cpm/mg
26 May 1962 (Continued)	X-2-2-2	Chironomidae	0
		Curculionidae	0
	X-2-2-3	Formicidae	0
		Microlepidoptera	1.513
		Chironomidae	0
30 May 1962	1-1	Curculionidae	0.187
	1-2	Chrysomelidae	1.989
		Bibionidae	2.839
		Plecoptera	5.661
		Stratiomyidae	2.023
		Tenthredinidae	0.901
		Membracidae	0
		Tipulidae	4.097
		Cantharidae	0.697
		Elateridae	0.153
		Empididae	4.454
		Chironomidae	0.510
	1-3	Cantharidae	1.377
	1-4	Bibionidae	0
		Tipulidae	0
		Chironomidae	0
	1-10	Braconidae	5.950
		Bibionidae	0
		Cantharidae	0
		Miridae	1.071
		Ichneumonidae	1.173
	1-11	Formicidae	0
	1-13	Empididae	0.595
		Cantharidae	2.193
		Chironomidae	0.595
	1-14	Braconidae	0
		Formicidae	0
		Cantharidae	6.800
		Chironomidae	8.007
	1-16	Bibionidae	0

Table 40. - Continued.

Date	Location	Family	cpm/mg
30 May 1962 (Continued)	1-19	Tenthredinidae	0
		Lygaeidae	1.179
		Cantharidae	2.975
		Tipulidae	11.050
	1-20	Bibionidae	0
		Stratiomyidae	1.836
	1-21	Chironomidae	0
	1-22	Formicidae	1.139
	1-23	Bibionidae	0
	X-1-2-1	Chironomidae	0.83
		Ichneumonidae	0.51
		Stratiomyidae	0
		Formicidae	0.56
		Ephemeroptera	0
		Carabidae	0
		Bibionidae	1.054
	X-1-2-2	Formicidae	0
		Chrysomelidae	0.442
		Bibionidae	1.326
		Tipulidae	0
	X-1-2-3	Chironomidae	0
	X-1-2-4	Bibionidae	0
	X-1-2-5	Formicidae	0

*X locations are cloths placed along lines perpendicular to watershed boundaries. Subsequent numbers are: watershed, line, and distance in 50 ft intervals from the boundary.

Table 41. - Effect of malathion on the dipteran family Chironomidae, the hymenopteran superfamily Chalcidoidea and all arthropods after the 25 May treatment as measured from total insects collected from catch trays, Dover, Ohio.

Date: Month-Day	Sampling stations common to all three sampling dates 1-1	1-5	1-10	1-14	1-15	Mean $\bar{X}$	Variance s^2
Chironomidae							
5-26	42	40	63	68	58	54.2	158.2
5-28	5	11	3	5	4	5.6	9.8
5-30	25	13	22	27	2	106.8	17.8
Chalcidoidea							
5-26	0	12	8	4	3	5.4	21.8
5-28	0	0	0	1	0	0.2	
5-30	0	1	0	0	0	0.2	
Total							
5-26	65	88	130	134	136	111.0	1039.8
5-28	20	13	10	5	7	11.0	34.5
5-30	28	18	31	33	5	24.0	134.5

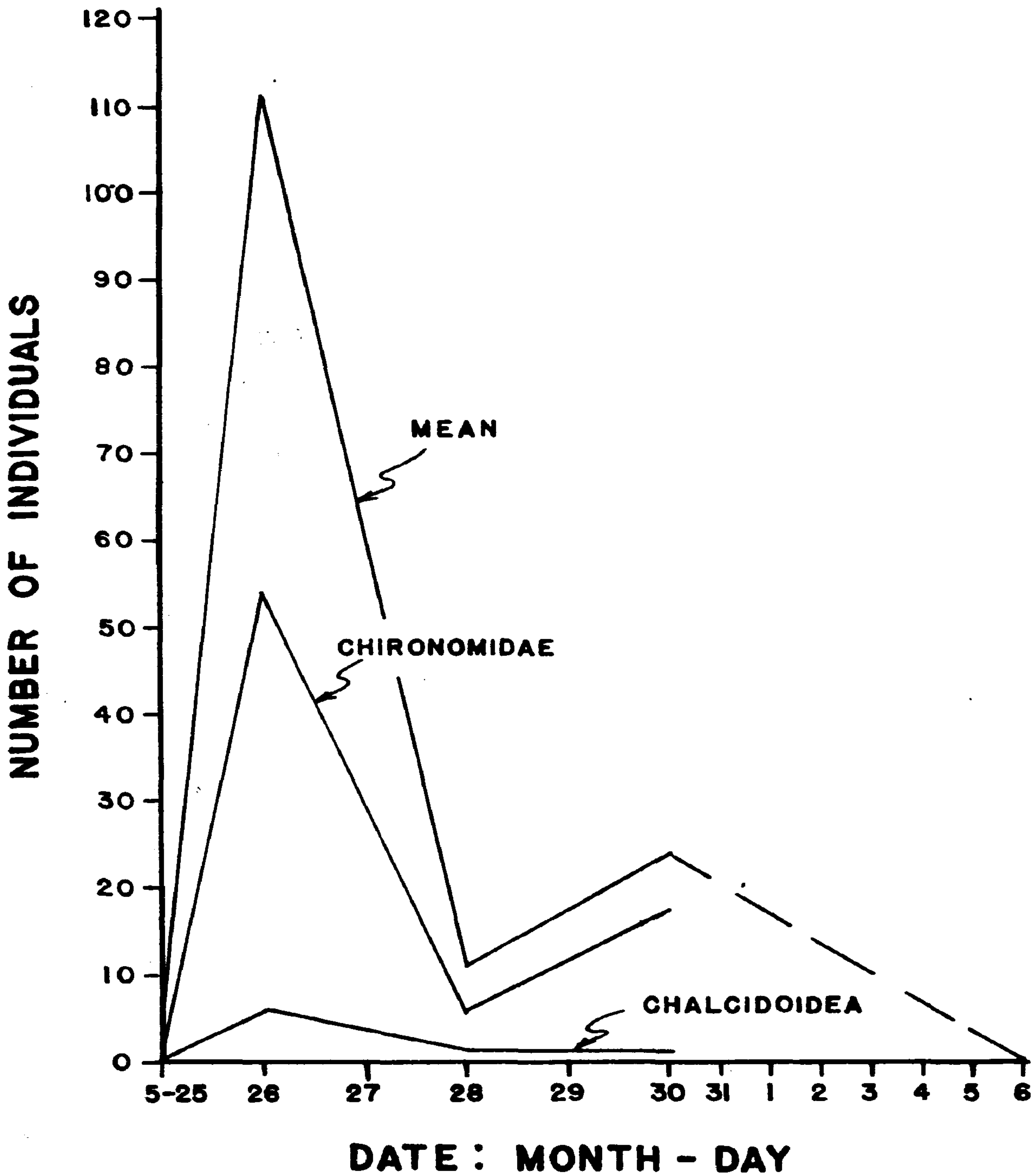

Fig. 28. Mean number of arthropods collected from all catch cloths on the Dover, Ohio, watershed no. 1 as compared with total number of midges (Chironomidae) and chalcid flies (Chalcidoidea) collected at five locations common to the three sample dates, 1962.

above a sampling area were unknown.

There was close similarity between total chalcids and the means of total insects killed but no indicator organisms were evident.

On 18 May 1962, 3 days after the spray, insects were collected from a few of the catch cloths. Numbers were much reduced. Only those found in quantity were processed for radioactivity. The elaterids increased from 0.232 cpm/mg (Table 42) to 0.307 cpm/mg, and the chironomids increased from 0.508 to 1.299 cpm/mg. Others, the calypterates and syrphids had no measurable activity above background. The chironomids collected at 1-1 and 1-9 showed no activity while those at 1-6 showed the relatively high activity of 1.299 cpm/mg. Dispersal maps indicate no measured insecticide applied at point 1-6. Activity of dead insects does not correlate with measured insecticide application. Measures of application are erroneous (as will be discussed later), or insects contacting sprays may move over 200 ft before the insecticide is lethal, or both.

The mean biomass of insects collected on the 4.7 ft^2 was 4 mg on the day after treatment of the forest. Expanded to the total acreage of the treatment area, the removal within 24 hours was about 2.5 lb.

Tracer Plot Studies

The results of catch tray studies on the tracer plots are shown in Fig. 29. Again most populations were so low that no significant change could be detected. The Sciaridae, the dark-winged fungus gnats, were most abundant in the population. The cross-contamination of the treatment and control plots (just as in the final spray

Table 42. - Radioactivity of insects collected on 17 May 1962 from catch cloths within the spray area after the crash-flight on 15 May 1962 on Dover, Ohio, watershed no. 1. Only those insect families were counted that had a total weight of over 0.2 mg/sample.

Family or Super Family	cpm/mg of air dry sample
Hymenoptera (bees)	2.480
Tenthredinidae	1.262
Cynipidae	0.783
Microlepidoptera	0.769
Chalcidoidea	0.696
Phoridae	0.682
Chironomidae	0.508
Calypterates	0.508
Acalypterates	0.406
Syrphidae	0.363
Ichneumonidae	0.348
Elateridae	0.232
Empididae	0.203
Carabidae	0.174
Formicidae	0.116
Plecoptera	No significant radiation
Tingidae	"
Vespidae	"
Braconidae	"
Coccinellidae	"
Histeridae	"
Sciaridae	"
Phalacridae	"
Hydrophilidae	"
Araneida	"

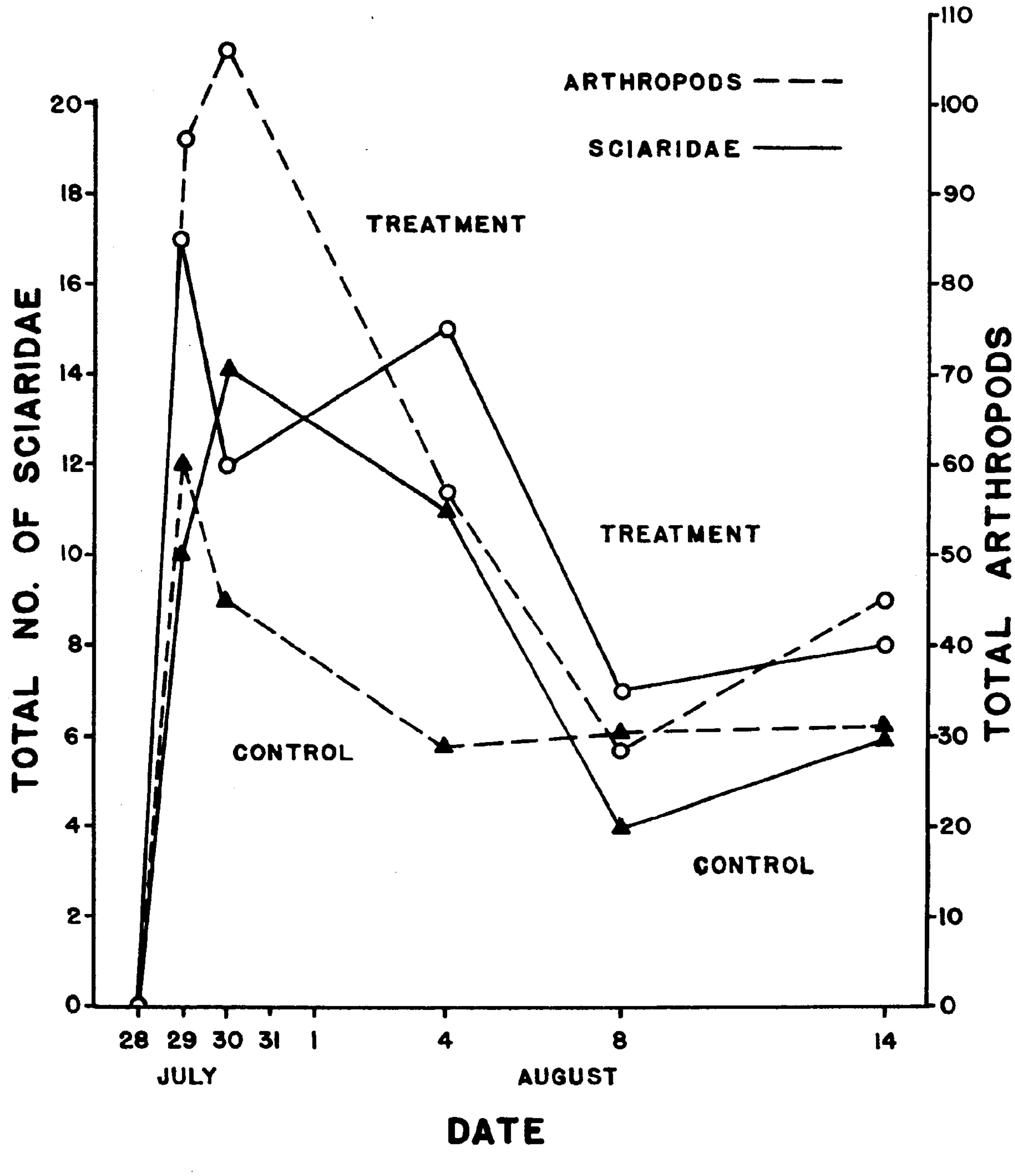

Fig. 29. Comparison of treated and control tracer plot Sciaridae and total arthropod populations as measured from the catch trays, Dover, Ohio, 1961.

application) does not permit conclusions with great confidence. Arthropods were still being killed by the insecticide 2 weeks after the spray but losses were not great. This period of kill was much longer than for the 1962 studies and reflects higher quantities of insecticide in a smaller area.

Spider Web Counts

The spiny-bellied spiders, *Micrathena gracilis*, of the subfamily Gasteracanthinae were the most striking in appearance and their webs conspicuously abundant in summer after July on both areas. Web counts over the normal 2 mile trapping route (Fig. 42) made after spray indicated identical populations and activity of this spider in the treated and control area. Pre-treatment (1961) populations on both watersheds were judged similar. Since large numbers of live spiders were observed immediately post spray on insect catch cloths, further support was added to the observation that malathion had little or no effects on spider populations.

Sunken-Can Traps

Tin cans (about 3 l) were dug into the ground so that the surface was level with their tops. Though primarily intended for shrew studies, these were effective for capturing ground arthropods. They were used late in the study, so no quantitative data is available for forms collected. Table 43 presents the variable radioactivity of the different families. The wide differences can only be explained in the general terms of variable behavior patterns including leaf-surface contacts and food habits. It is unlikely that

Table 44. - Radioactivity in cpm/mg of air dry sample of insects collected in sunken cans on plot no. 1, Dover, Ohio, watershed no. 1, 1 August 1962.

Family	cpm/mg
Tenebrionidae	0.0819
Blattidae	0.0503
Araneida	0.0438
Gryllidae	0.0395
Diplopoda	0.0352
Phalangida	0.0350
Geotropidae	0.0162
Gryllacrididae	0.0078
Carabidae	0.0073
Formicidae	0
Elateridae	0
Cerambycidae	0

this collection, made more than 2 months after the spray, involved arthropods that had received sub-lethal doses of malathion, but arthropods that had become radioactive through the food chain or by contact.

Miscellaneous Collections

Throughout the study individual insects were collected when it was convenient or when an unusual specimen was found. Several times random sweeps were made, and insects were collected and tabulated as "miscellaneous" from improperly functioning sampling devices such as the light trap for periods when no quantitative data could be obtained. The purpose of these collections was to add to the check-list of the area and to try to detect families of insects present that were not sampled by the various devices used. The list is presented in Table 44. The 1961 data is more precise and individuals, where

Table 44. - Arthropods collected from the Dover, Ohio, watersheds at random and by miscellaneous collections in 1961 and 1962.

Scientific Name
Odonata
Aeshnidae
Orthoptera
Gryllidae
Phasmatidae
Tetigonidae
Gryllacrididae
Hemiptera
Blattidae
Belastomatidae
Pentatomidae
Corixidae
Benacus griseus Say
Reduviidae
Triatoma
Lygaeidae
Coreidae
Leptoglossus
Homoptera
Fulgoridae
Cicadellidae
Coleoptera
Cicindelidae
Cicindela sexguttata Fab.
Carabidae
Carabus
Sphaeroderus
Pterostichus
Chlaenius
undetermined
Histeridae
Hydrophilidae
Cymbiodyta
Leiodidae
Silphidae
Colon
Staphylinidae
Ontholestes
undetermined
Pselaphidae
Coleoptera (Continued)
Cantharidae
Podabrus
Lampyridae
Ellychnia
undetermined
Lycidae
Ptateros
Endomychidae
Aphorista vitata Fab.
Dytiscidae
Ptilodactylidae
Heteroceridae
Geotropidae
Elateridae
Erotylidae
Curculionidae
Ptiliidae
Nitidulidae
Coccinellidae
Anatis quidecimpunctata Oliv.
Meloidae
Meloe angusticallis Say
Scarabeidae
Ateuchus
Hoplia
Cerambycidae
Anoplodera
Anthophylax
Larva, undetermined
Lepidoptera
Papilionidae
Papilia glaucus Linn.
Nymphalidae
Vanessa atalanta Linn.
Agaristidae
Alypia
Thyatiridae
Euthyatira
Geometridae
Dyopteris
Gonodontis
Larvae - undetermined
Lycaenidae

Table 44. - Continued.

Scientific Name	Scientific Name
Lepidoptera (Continued)	Hymenoptera
Saturnidae	Siricidae
Arctidae	Formicidae
Noctuidae	Ichneumonidae
Microlepidoptera	Halictidae
	Vespidae
Diptera	Apidae
Bombyliidae	
Bombylius	Plecoptera
Tachinidae	
Syrphidae	Tricoptera
Calypterates	
Tipulidae	Class - Arachnida
Chironomidae	Phalangida
Mycetophyllidae	Acarina
Cecidomyiidae	Araneida
Phoridae	
Larvae	Crustacea
	Isopoda
Siphonaptera	
Dalichopsyllidae	Diplopoda
	Chilopoda
	Symphala

possible, were identified to genus and species. Although 1962 comparisons are between families only, these total results are presented to give some picture of the family composition. The list will undoubtedly be increased by Ohio State University investigator James Galford who studied the insects of the same area in 1963.

Study of the Appendix table listing families and methods of capture indicates that there were fully 12 families not sampled by systematic methods. It is interesting to note also that there were two families collected from catch trays not collected by any other method and three families collected only on catch trays and at random. These observations indicate the relative inability of ecologists to describe their universe, much less to understand and explain its interactions.

SNAILS

Terrestrial snails were abundant on the area and were varied in species. Their importance in litter breakdown, nutrient recycling, feeding on fungi, scavenging on dead animals, and serving as prey gives them a significant role in the ecosystem. Terrestrial snails are relatevely resistant to many insecticides. If the effects of malathion had been great, i.e. had killed a large number of snails, the significance of this loss would probably not be too great due to rapid regeneration of populations. Ingram (1944) wrote of long egg laying periods and subterranean egg sites. The subterranean nature of most forms provides ample protected breeding stock with high reproductive capacities to rapidly replace lost numbers.

Since there are few known helminth parasites of birds and mammals with terrestrial snails as intermediate hosts, an insecticide would not be expected to alter significantly the incidence of parasitism by killing snails as potential intermediate hosts.

On 7 August 1962, 77 days post-spray, the litter from two 1 m^2 quadrats of forest litter, one near 1-5, the other near 2-3, was examined in white enamel pans. On watershed no. 1 three snails and three slugs were found: on watershed no. 2 five snails and three slugs were found.

Mollusks present on the study areas were identified by Mrs. C. E. Taft, Columbus, Ohio. They were:

Polygridae
- Triodopsis albolabris (Say, 1816)
- Triodopsis fraudulenta (Pilsbry, 1894)
- Triodopsis tridentata (Say, 1816)
- Stenotrema stenotrema (Pfeiffer, 1842)

Haplotrematidae
- Haplotrema concavum (Say, 1821)

Zonitidae
- Ventridens ligera (Say, 1821) or intertextus (Binney, 1841)
- Mesomphix sp.

Cionellidae
- Cionella lumbrica (Muller, 1774)

Sampling devices for snails, except by tediously examining the soil quadrats, were not discovered from a literature search, nor were any developed in this study. Strandine (1941) indicated that a 1 m^2 quadrat contained from 0 to 11.5 Succinea ovalis snails. With low densities, large variances, and inadequate knowledge for stratification of the area to reduce sampling intensity, extensive studies on the mollusks were not conducted.

One live snail was recovered from a 3 l (3 qt.) can on the tracer plot study 5 days after spray. It had not been placed there but was in the soil with earthworms. It represents slight evidence of survival in a container known to have been sprayed with malathion. One live Triodopsis snail collected near 1-9 had an activity of 0.36 cpm/mg.

One aquatic snail, probably Physa sp., from a bucket on a treated plot had no above-background radiation.

FISH AND AQUATICS

A Review

Malathion is recognized as an insecticide hazardous to aquatic life, but Henderson and Pickering (1958) and Henderson et al. (1960:84) commented that it is less hazardous than most chlorinated hydrocarbons, except BHC.

Parkhurst and Johnson (1955:115) found that aging as much as 144 hr "did not have any very important effect upon the toxicity of dilute solutions of Malathion 500." There is little or no oxygen demand by malathion (Henderson et al., 1960:78). "With most of the organic phosphorus compounds there are only minor increases in fish mortality between the 24 and 96 hr periods. This is to be expected with those materials which hydrolize very rapidly to less toxic materials" (Tarzwell, 1959:136).

Table 45 reveals that there are wide variances in the dosages required to kill fish. Tarzwell (1959:137) discussed the impossibility of judging the toxicity of these materials to all fishes on the basis

Table 45. - Summary of some studies of malathion toxicity to fish.

Author	Fish species	Dosage	Effect
Bodenstein, W. G. (pers. correspondence, unpub. data) Ent. Research Div., Agri. Res. Ser., U.S.D.A.	Mummichog Killifish, <u>Fundulus heteroclitus macrolepidatus</u>		More toxic than 1 ppm DDT at 21-26° C Toxic symptoms-4 to 6 hr, death 6 to 12 hr
Cope, (1960:18)	Sculpins, small cyprinids and suckers	1 lb/acre 0.20 ppm	Toxic 30 min. after spray
Darsie and Corriden (1959:696)	Rainbow trout fingerling, bluegills, sunfish	5 ppm	Toxic in less than 8 hr
			Toxic in less than 1 hr
	Goldfish	5 ppm	Toxic in less than 24 hr
	Carp	3 ppm	60% mortality
	Sea lamphrey larvae	5 ppm	No effect
	Fathead minnow, <u>Pimephales promelas</u> Raf.	25 ppm	LD-50 24 hr
		23 ppm	LD-50 48 hr
		22 ppm	LD-50 98 hr
	Ocellated killifish, <u>Fundulus ocellaris</u> Jordan and Gilbert	0.5 lb/acre	

Table 45. - Continued.

Author	Fish species	Dosage	Effect
Henderson *et al.* (1960:79)	Fathead minnows	12.5 ppm (57% active ingredients)	TLM-96 hr partially due to xylene solvent
		17 ppm (hard water)	TLM - 96 hr
		26 ppm (soft water)	TLM - 96 hr
Hilsenhoff (1959)	Larvae of *Tendipes plumosus*	0.1 lb/acre	Considerable toxicity.
		0.75 lb/acre	97% mortality within 7 days
	Fathead minnow	20 lb/acre	No toxicity
Hilsenhoff (1962)	Wall-eyed pike fingerlings	5 lb actual/acre	Mortality in aquaria tests
		2 lb/acre	No mortality in aquaria tests
Katz (1961)	Chinook salmon (*Oncorhynchus kisutch* and *tshawytscha*)	57% malathion	
		24.5 ppb	TLM - 24 hr
		23.9 ppb	TLM - 48 hr
		23.6 ppb	TLM - 72 hr
		23.0 ppb	TLM - 96 hr
	Fathead minnow	12,500 ppb	TLM - 96 hr
	Blue gill (*Lepomis macrochirus*)	95.0 ppb	TLM - 96 hr

Table 45. - Continued.

Author	Fish species	Dosage	Effect
Katz (1961)(Continued)	Three-spine Stickleback (*Gasterosteus aculeatus*)	5 ppt salinity 94.0 ppb 25 ppt salinity 76.9 ppb	 TLM - 96 hr TLM - 96 hr
Lewallen (1959:1-2)	Surface feeding minnow, *Gambusia affinis*	0.05 ppm (equivalent to approximate MLD for field control of *Culex* mosquitoes)	24 hour % mortality 30, 50, 40; average mortality 40%
McDuffie (1960, pers. correspondence)	*Cyprinodon variegatus* (indentification uncertain)	0.2 lb/acre aerial granules 0.25 lb/acre ground emulsion	Toxic
Mulla (1961)	*Gambusia affinis*	high and low rates	appreciable mortality within 48 hr of exposure. "At the larvae dosages DDT and parathion are somewhat safer than malathion."
Parkhurst and Johnson (1955:113; also see Johnson, 1955)	Fall chinook salmon 1.5 in. fingerlings	Malathion 500 in formulation Test 1 - 0.1 ppm	Killed some in 24 hr, nearly all in 6 days at 44-48° F. Critical concentration range 0.032 to 0.32 ppm.

Table 45. - Continued.

Author	Fish species	Dosage	Effect
Parkhurst and Johnson (Continued)	Fall chinook salmon (Continued)	Test 2 - 0.087 ppm and 0.1	Withstood for 6 days
		0.17 ppm 0.15 ppm 0.12 ppm	TLM - 24 hr TLM - 48 hr TLM - 96 hr
		Higher than 0.1 0.240 and 0.32 ppm (highest tested)	Toxic Killed nearly all fish within 24 hr
Springer (1956:177)	Killifish	0.2 lb/acre	Complete kill
Steiner, L. F. (pers. correspondence) unpub. data, Agri. Res. Ser., Ent. Res. Div., U.S.D.A.	Black mollies, yellow wags, rosy barbs, blue moons, flag fish, helleries, red moons, marble mollies, and black moons	0.15 to 0.80 ppm (concrete ponds)	No mortality
		1.60 ppm	No mortality
	Liberty mollies	1.20 and 2.0 ppm	Some mortality
	Rosy barbs and yellow crescents	1.20 ppm	Survived
	Dwarf gouramis and helleries	2.0 ppm	Survived
Tarzwell (1959:137)	All fish tested	0.78 lb/0.3 acre-ft	50% mortality
	Blue-gill sunfish	0.095	TLM - 96 hr

of tests with one species. Henderson et al. (1960:79) said, "The 96-hour TL_m value for an acetone solution of technical malathion to bluegills in soft water was 0.095 ppm as compared to 26 ppm for fathead minnows, a difference of over 270 times." Resistance and sensitivity vary widely not only between species but with factors of the environment, the formulation, and the physiological state of the fish. Haddock (1962:39) stated that conditions existing at different locations affected the results of his tests on midge fly larvae. Darsie and Corriden (1959:696) found no correlation between fish sizes and mortality. It is generally agreed that forage fish are more resistant to poisoning than game fish.

The pharmacology of the toxicity of malathion to fish has not been reported. General symptoms are: darkening skin, periods of high excitability with body tremors, complete loss of equilibrium, and extreme forward position of the pectoral and pelvic fins (Henderson et al., 1960:78). Darsie and Corriden (1959:698) report that the caudal peduncal of killifish was bent laterally and held that way.

Weiss (1959) studied the effects of malathion of brain acetylcholinesterase (AChE) of large mouth bass, *Micropterus salmoides*; goldfish, *Crassius auratus*; golden shiners, *Notemigonus crysoleucas*; and blue-gill sunfish, *Lepomis macrochirus*. His exposure of fish was analogous to a stream environ with a "slug" of poison. Both initial inhibitory and recovery effects were very rapid (1959:591). Recovery periods depend on the degree of exposure, the insecticide used, and the fish species (Weiss, 1959:591). Bass recovered in 20 days from malathion poisoning but AChE activity after exposure to parathion was only 60% of normal in the same time. Four to 6 hr of exposure to malathion were required to produce detectable enzyme inhibition in goldfish. Shiners exposed to 0.1 mg/l of malathion had reduced AChE activity below the lower limit of the normal range after 1 to 2 hr. It did not persist there until after 4 hr of exposure. After 12 days, fish had recovered to the lower limit of the normal range. Blue-gills reached the normal range at the end of 2 days. Weiss' studies (1959:592) yield a technique, the responses of fish-brain AChE _in vivo_, that permits assay of current or past low concentrations of organo-phosphorus insecticide pollution.

The results of laboratory tests on the effects of poisons on aquatic biota, Tarzwell (1959:138) explained, are 0.2 to 0.1 the values expected under field conditions of continuous exposure of an organism. He stated:

> Much more research is needed before safe concentration or application rates can be established to insure the protection of the aquatic biota. Full-scale field tests are needed in order to evaluate all the variables which may affect the toxicity of these materials to aquatic life in our lakes and streams.

Water is a unique faunal environment when this environment is treated with an insecticide. In air or on land, poisons settle out, assume strata or surfaces. In water the environment is totally polluted to the extent of the solubility and suspension of the compound; faunal contact with the poison is not specific but total.

Besides direct application of insecticides to water for mosquito and other insect control, much water is contaminated in the process of spraying adjoining lands. Further pollution occurs as insecticides are washed into waterways by runoff from treated lands. Vegetation and soil conservation practices retard this secondary contamination of water. Tarzwell (1959) stated some of the problems of water pollution from the drainage from insecticide-treated lands. Hoffman (1960:60) said, though, that "as yet no one has shown insecticide run-off from forests into streams in sufficient amounts to show a measurable increase in the mortality of bottom organisms above that caused directly by the spraying." Results reported herein of studies on dispersal within the soil confirm Hoffman's statement about forest run-off.

Many factors influence the runoff potential of insecticides on the forest watershed. They include the nature and depth of humus and organic debris and the molecular interaction of this with the insecticide molecule, the amount and rate of rainfall immediately after application and within the effective life of the insecticide, the depth and nature of impeding layers, and the hydrolic conditions of the forest soils. Whipkey (1962) characterized the hydrolic conditions of the forest soils as: those on slopes seldom reach saturation, static water tables are exceptional, very permeable in the root zone to water, hydrolic conductivity is difficult to define, elevation head is important, and turbulent flow may occur, at least for short distances, because of elevation head and numerous large biological channels.

Thornthwaite _et al._ (1960:1015) found that water surplus (the amount of water which falls over the watershed in excess of climatic and vegetation and after soils have reached water-holding capacity) is responsible for the redistribution of radioactive strontium in the soil. "On a longterm basis, the water surplus is the principal agent affecting the flux or the detention of strontium in the soil at a given place" (Thornthwaite et al., 1960:1018). Admittedly, it is dangerous to generalize from such observations to the field of insecticide-soil-water relations, but this is necessary due to the paucity of information on forest insecticide movement. The normal hydrolic condition of the sloping forest soils such as those of the study areas suggests that water surplus is rare. Water pollution by insecticides applied to old and mature forests appears to be from the following possible sources: net fall-through and channel interception, surface run-off from shingle-action on stream sides, and infiltration into sub-surface flow. The latter source, when present, would be most influential of the three major sources. Whipkey (1962) found that the lowest soil zone overlying water-impeding strata must be wetted before flow in the upper soil zones will occur. Solubility of the insecticide and soil minerals, molecular bonds with soil particles, soil porosity and the head of the rainfall and gravity flows - all would influence the insecticide burden of the water and its subsequent discharge into a channel. It is now believed that contamination of ground water by passage through treated soil does not appear probable.

Past attempts to measure insecticide quantities in streams have been largely unsuccessful due to the high dilution rates, separation or evaporation difficulties, and limited chemical and bioassay techniques for the small amounts of insecticides recovered.

Dover Studies

Samples

From a small dammed ground seep below the tracer plots and from the stream below the tracer plots, water samples were scooped into gallon size bottles. Taft Sanitary Engineering Center, Cincinnati, Ohio, evaporated the water in large pans over heat. Total radioactivity of these samples, both pre and post treatment, was very high, ranging around 1500 cpm. These values implied high levels of background radioactivity[1] in the water and stream sediments and eliminated possibilities for direct radioactive monitoring of S^{35} in the water.

Thus, it was necessary to isolate the sulfur from the water in order to determine the amount of malathion or its metabolites that were present. Evaporating the water was impractical because there were over 75 gal of water samples. Evaporation in a flat stainless steel pan was tried but besides the slowness of the procedure, even over heat, there was unknown volitalization of the sulfur, difficult-to-remove crustaceous deposits formed on the pan, the last evaporated sample was impossible to remove entirely, the clean-up of the pan for subsequent samples was difficult and the remaining S^{35} contamination was unknown. Two other concentration methods were tried, the flash evaporator and a three-ball Snyder column with heat and vacuum.

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[1]The observations were of timely interest since they were just following resumption of international nuclear explosion tests. Increasing fallout will abrogate opportunities for using isotopic tracers for field ecological studies.

Both were abandoned because they were too time consuming for the large volume of samples to be processed, were too difficult to clean, were impossible to empty entirely, and were producing inequalities in the final samples, especially when the water contained suspended solids.

A precipitation method was finally adopted. The entire gallon sample was filtered once through two, 15 cm, No. 1 Whatman filter papers in a 151 mm porcelain Buchner funnel using vacuum. This removed all sediments and while changing the picture of S^{35} in the water, it established one constant - the soluble, filterable portion of all water samples. (Water samples from the shallow stream frequently had large quantities of silt and algae present because of the disturbance caused by the sampling scoop.)

To the filtered sample was added 1 ml of Pirie's reagent which changed the pH from 6.8 to 1.5 and was believed adequate to encourage molecular breakdown of malathion and many of its metabolites. Use of Pirie's reagent resulted in larger precipitates than perchloric acid (60% C.P.) in tests. The acidified sample was left undisturbed for 24 hr in the same bottle in which it was collected. A 100 ml sample (0.026 gal) was poured from the vigorously shaken bottle, warmed, 5 ml 0.1 N sodium sulfate and 10 ml of 1.0 N barium chloride were added, and the sample filtered through the millipore filters described under the section on "Laboratory Procedures". The sample was dried, mounted on an aluminum planchet, and radioactivity of the $BaS^{35}O_4$ precipitate was determined.

To establish a standard, 500 Lambda (0.5 ml) of radioactive malathion formulation from load no. 2, 25 May 1962 flight, were added to 1 gal of distilled water. The mean corrected total activity of two samples prepared in the above manner was 5.2 cpm. There appeared to be no real difference in this standard and one prepared from water collected from stream no. 1. One cpm is equivalent to 1.3 ppm of malathion in water.

Six valid samples were obtained from preliminary attempts to evaporate the water sample to obtain a concentrated quantity of solution that could be processed by the previously described standard method. The results are shown in Table 46. The evaporation method and the mass precipitation methods correlate exactly (r = 0.99) but the actual recovery rate using the evaporation method is much lower since the activity was from 1 gal of water. Evaporation appears to detect presence or absence better, but quantitatively it is deficient by a factor of over 8.

Table 46. - Comparison of the evaporation and precipitation methods for determining radioactivity in Dover, Ohio, stream water samples, 1962.

Date: Month-day	Time	Location	Radioactivity of sample in cpm: Evaporation Method	Radioactivity of sample in cpm: Standard Method
5-25	7:25 am	In stream below dams	0	0
5-25	7:40 am		1.3	0
5-25	8:25 am		8.7	6.9
5-25	9:55 am		3.9	3.7
5-25	10:00 pm	Stream at dam no. 1	18.1	15.9
6-1	3:00 pm	Stream at dam no. 2	0	0

Activated Charcoal

Following the method of Van Valin and Kallman (1962) Nuchar (C-190 N Matheson, Coleman, and Bell, Norwood, Ohio) an activated charcoal was obtained and water samples of known quantities of radioactive malathion and sulfuric acid were assayed using this technique, modified slightly for isotopic rather than chromatographic techniques.

Five g of Nuchar were used but the large amount of filtrate could not be easily handled for counting or subsampling. Subsamples of the suspension within the vigorously shaken gallon bottles were taken and counted. Part of the records in the tests comparing evaporation, precipitation, and charcoal methods are missing. Charcoal appears to be 3 to 30 times more effective (i.e. results in a higher net cpm per unit sample of insecticide-contaminated water) than either of the previously used water analysis methods. It was not effective in absorbing S^{35} from $H_2S^{35}O_4$ added to water. The method needs further study but use of activated charcoal in similar future experimental water analysis seems justified.

Water analysis of the few samples collected following the 15 May flight show that at 8:25 am, 7 min. before the one spray swath, stream no. 1 had 4.1 cpm and stream no. 2 had 5.6 cpm. Very high concentrations were in the water by 2:05, 5.6 hr after application, stream no. 1 had 727.3 cpm, and stream 2 had 657.5 cpm. By next morning, 9:35 am, stream no. 1 had no detectable radioactivity.

Water samples were collected on 25 May 1962 through 21 June 1962, in clean gallon glass bottles. Just prior to and at half-hour intervals after the first plane pass over the area, water was collected at both dams and at a point 300 ft below the convergence of the two streams. The pond and upper reaches of stream no. 2 had been entirely covered on 24 May with a 15 x 40 ft piece of plastic to prevent contamination. That contamination observed on 27 May was after a 0.2 in. rain on the previous afternoon which undoubtedly washed the contamination under the plastic.

Disturbance of the discharge from stream no. 1 by insect sampling caused erratic changes in the water radioactivity. The mean cpm for water samples taken at 15 and later 30 min. intervals was 5.0 ± 3.5 (estimated standard error) or 2 to 11 ppm. This disturbance was also responsible for irregularities observed in samples taken 300 ft below the dams. The results of the water sample analysis are presented in Fig. 30.

Fig. 30 also indicates a positive correlation between occurrence of rain and peaks of radioactivity in water. Stream flow usually increases following rains unless the rainfall is slight and the ground very absorbent. It appears that to a point, the more rain there is, the greater will be the contamination of the water from the insecticide on the ground. However, this contamination is offset by greater dilution by the stream and also by increased discharge rate. This quantity of water would reduce both the amount and time of exposure of the fauna to secondary water contamination by insecticides. The majority of the secondary contamination is thought to be directly from the stream sides and from continued channel interception of "rain-out" from overhead contaminated leaves.

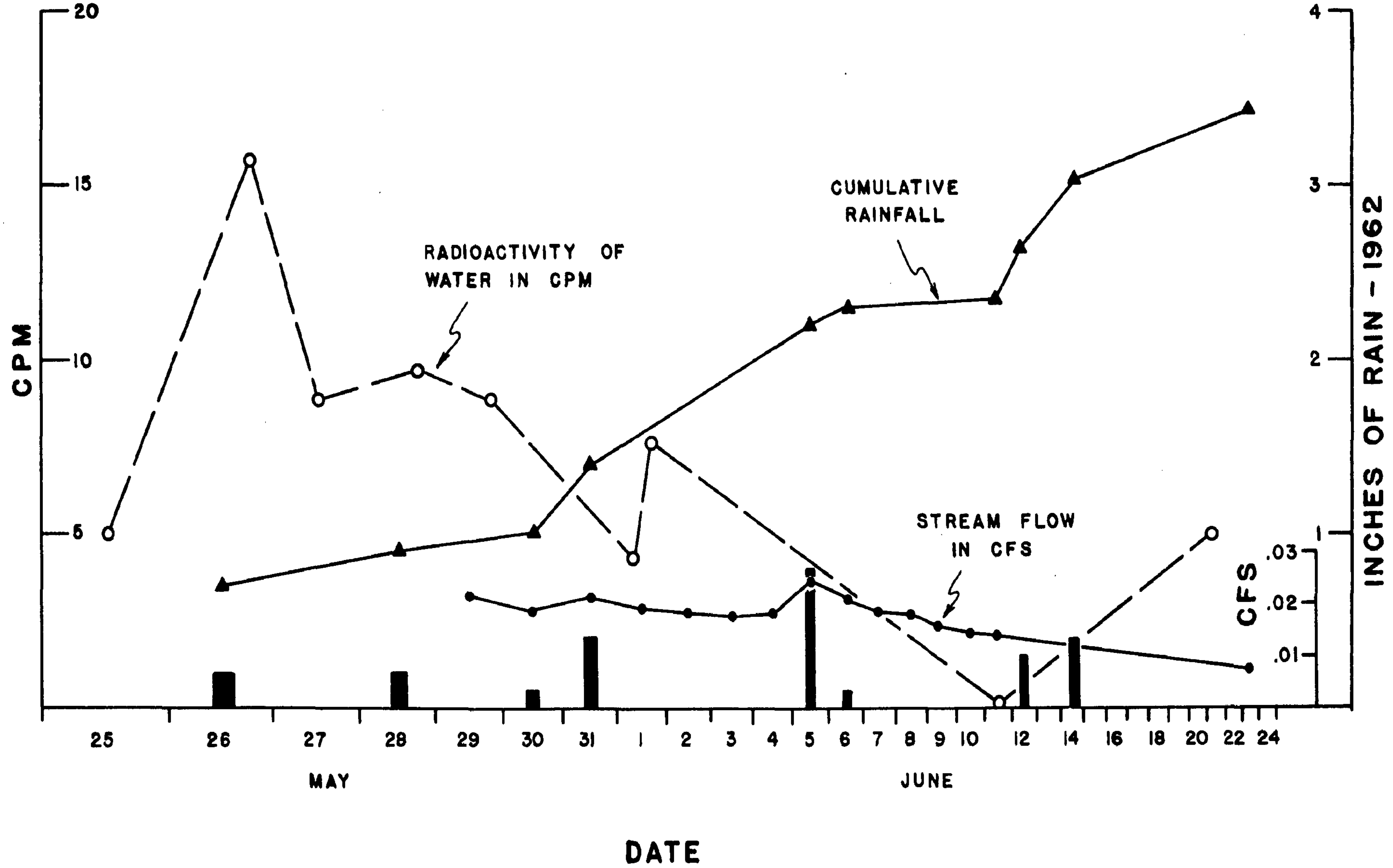

Fig. 30 Relationship of cumulative rainfall, dates and quantity of rainfall, stream flow, and radioactivity of water samples from stream no. 1, Dover, Ohio, study area, 1962. Dates are on logarithmic scale.

The Aquatic Environment and Methods of Study

The two small streams within the watersheds under study presented many complex problems of sampling and analysis. Few studies have been reported of investigations of the fauna of extreme headwaters known as the intermittent lotic or brook environment (e.g. Stehr and Branson, 1938). Most studies involve streams that at least for part of the year exceed several feet in width or, though more narrow, are unobstructed throughout their length. Populations are small in size and variety, for few animals can stand the stresses of an environment with annual fluctuation from near dryness to a foot of water, extreme crowding, and periodic swift currents. The stream no. 1 of watershed no. 1 reaches a maximum flow of 38.51 $ft^3/sec/mile^2$ (the 0.79 ft stage) and minimum flow of 0.005 $ft^3/sec/mile^2$ (0.02 ft stage) (Whipkey, 1961:2). Stream no. 2 is similar but ceases flow through the dam in mid-summer, although it maintains a 5 ft wide by 15 ft long pool. It is essentially dry throughout its bed to within 50 ft above the dam during the summer. Stream no. 1 retains a year-around flow with running water for over 75 ft above the dam. The streams unite 15 ft below the dams and meander one-half mile through a wooded bottom, an old rock road-culvert, and an overgrown pasture where it unites with another stream and flows 500 ft into the Tuscarawas River. Fish occur naturally 1000 ft upstream from the river but are blocked, except during flooding, from further progress by the culvert.

The streams and man-made pools serve as a food and water source for terrestrial forms. The water is a distinct environment and contains fauna totally different from the general forest, but yet is an integral part of it and it's character, a translocation vehicle for the insecticide, and a breeding area for terrestrial insects.

The literature suggested some possible effects on aquatics and consequently some approaches to their study. George (1959:253) stressed the need for insecticide treatment before infestations reach the upper drainages. This provides a faunal reservoir with constant discharge for rapid repopulation.

Burdick *et al*. (1960:17) reported that the effects of Sevin (a carbamate) became progressively greater downstream with reductions in weight of aquatic insects from 48.9 to 92.5%. These observations are the converse of those of Savage (1949:47) who concluded from a number of studies with DDT that:

> The effect of the poison rapidly diminishes as it is carried downstream from the sprayed area, particularly if there are stretches of quiet water, beaver dams, culverts, or weed beds.

Langford (1949:14) stated that minnows in quiet pools were killed but that kill of fish in rapid waters was negligible even though they fed on dying insects being washed downstream. Savage (1949:39) reported surface forms more affected than those totally submerged. Haddock (1962:53) stated that submerged aquatic vegetation tended to reduce the kill of midge fly larvae when low concentrations of insecticides were used but that it also seemed to prolong the effects of malathion. The list of complex and interacting variables influencing effects of insecticides on aquatics grows easily. Above direct toxicity, effects of starvation, predation, seasons and life stages of stream fauna, all the measurable characteristics of water, the stability of the watershed, the rate of siltation, and other factors (some conceivably unknown) confound the interpretation of effects observed.

Siltation, for example, in the pond of stream no. 1 was extensive. This was primarily a result of sluffing of the sides of the pond formed by the stream-gauging dam. Siltation in the spring of the year was greatest during periods of high flow which were brief, with increased turbidity usually lasting for only a day. If settling of the spray occurred or if it became bound to suspended solids or absorbed by algae, siltation could have made it unavailable to many aquatic organisms but concentrated it for such forms as the oligochaetes.

The quantities of silt and the narrowness of the stream presented many sampling problems out of proportion to the possible results of their analysis. No satisfactory methods were devised in 1961. Any sampling attempts changed the environment so drastically that they were considered impractical. Besides disturbing a relatively large area with sweep nets or bottom samplers, the entire pool became cloudy after the first activity. The extensive disturbance of wading was not acceptable; by sampling, small populations could be reduced beyond natural rapid recovery; silt filled even the large pores of a strainer; sorting of animals under a microscope was not feasible with the time and personnel available.

Slate Quadrats

In an attempt to count aquatics without disturbing their environment or removing a portion of them, two pieces of roofing slate were placed in each pond on the bottom with the intent of periodically counting crayfish and invertebrates visible on them. Numbers were too few, siltation too rapid (obscuring the underwater quadrat boundaries), and identification of certain forms too uncertain for the results to be meaningful.

Stream Quadrats

In 1962 four 0.5 x 1 m quadrats were outlined in stream no. 1 with stakes and string in selected good aquatic insect habitat 100 ft above the dam. Prior to 15 May the stones of two quadrats were overturned, stirred, and brushed with a vegetable brush into a 200 mesh/in. bolting cloth net much like a Surber square-foot sampler. Present in the samples were 2 Sialidae, 2 Ephemeroptera, 1 Staphylinidae, 4 Crustacea, 13 Plecoptera, 1 Tricoptera, and 4 larval salamanders. This list simply provides some idea of the forms that might have been subjected to the poison in the water. Carlson and Wenke (1962) are now studying effects of malathion on immature May flies and caddisflies. The samples were inadequate for reasons explained in the previous section. After the delay caused by the plane crash on 15 May it was impossible to take post-treatment (25 May) samples suitable for comparison with pre-treatment samples. Insect emergence and stream condition changes would have obscured insecticide effects. No post-treatment quadrat studies were made.

Drift Nets and Strainers

For the 15 May spray, drift nets of 200 mesh/in. grit gauze were placed across stream no. 1 in 6 places but were unsatisfactory. Finally, the flow of stream no. 1 was strained through a tea strainer for 3 hr after spray. The results are shown in Table 47. The mean deposition of insecticide as measured from glass discs around the pond was 48 cpm.

Within the first hour after spray a minimum of 1270 individuals passed through the dam's V. These were from the vegetation, air, water surface, and within the water. The second hour after spray 640 individuals were collected from the water, a reduction of 50%. By the end of the third hour the insects coming over the dam had dropped sharply; 598 were collected. A total of 2509 insects were collected in 3 hr. This decrease is linear.

The Chironomidae, Ceratopogonidae, Sciaridae, and Empididae forms constituted a major portion of the collection and their numbers-decrease follows the pattern for the entire insect fauna collected. No "indicator organism" i.e., one that was conspicuous, relatively small in number, and whose numbers paralleled those of the entire collection was apparent.

The decreasing catch represented the rate at which the surface strata of the pond and it's insect burden was discharged. Table 48 represents the collection of an immediate surface, sub-surface, and aerial kill as well as dead or dying insects from the vegetation.

Twenty-five feet above the dam a 6 in. high drift net trapped insects carried down toward the pond by the stream. The 3 hr collection thus described, therefore, represents the collection from the pond-and-above community only. Within the collection shown in

Table 47. - Insects drifting from the pond of Dover, Ohio, stream no. 1 and collected in a food strainer, 25 May 1962. The major kill occurred within the first 3 hours.

Order and Family or Super Family	Time 8:15-9:15	9:15-10:15	10:15-11:15
Collembola			5
Ephemeroptera			
Nymphs	12	6	5
Adults	1		
Plecoptera			
Adults			1
Psocoptera	1		
Hemiptera			
Corixidae	1	1	2
Miridae	1		
Lygaeidae	1		2
Homoptera			
Membracidae	2		1
Cicadellidae	1		1
Fulgoridae			3
Coleoptera			
Staphylinidae	4	1	6
Cantharidae	2	1	1
Lampyridae	1		
Buprestidae	2		
Phalacridae	1		
Mordellidae	1		
Chrysomelidae			1
Curculionidae	1	2	1
Scolytidae	1	1	
Trichoptera			
Adults	1		
Nymphs			1
Lepidoptera			
Agaristidae		1	
Microlepidoptera	12	5	3
Diptera			
Tipulidae	18	11	5
Psychodidae	1		
Chironomidae	502	218	233
Ceratopogonidae	20	11	10
Rhyphidae		1	
Bibionidae	4		
Mycetophilidae	15	8	10
Sciaridae	65	38	30
Cecidomyiidae	165	52	41

Table 47. - Continued.

Order and Family or Super Family	Time 8:15-9:15	9:15-10:15	10:15-11:15
Diptera (continued)			
Stratiomyidae	14	13	6
Tabanidae	3	1	
Empididae	155	75	58
Dolichopodiae	5	71	90
Phoridae	3		
Pipunculidae		5	2
Syrphidae	1	3	3
Acalypterates	89	39	16
Calypterates	18	22	18
Hymenoptera			
Tenthredinidae	2	4	2
Ichneumonidae	2	4	
Braconidae	13	3	4
Chalcidoidea	101	31	29
Cynipidae	28	10	5
Formicidae		2	3
Sphecidae		1	
Apidae			1
Number of Groups	39	30	32
Total	1270	641	598
Total	2509		

Table 48. - Arthropods collected on 26 May 1962 from a drift net located 25 ft above the dam across stream no. I, Dover, Ohio. Only eight individuals were collected from the net pre-spray.

Order and Family	Drift net 25 ft above dam
Collembola	3
Ephemeroptera	
Nymphs and adults	2
Hemiptera	
Miridae	1
Homoptera	1
Cicadellidae	
Membracidae	2
Fulgorida	3
Coleoptera	
Hydrophilidae	1
Staphylinidae	10
Cantharidae	2
Melyridae	1
Cleridae	1
Elateridae	1
Buprestidae	1
Mordellidae	3
Chrysomelidae	2
Lepidoptera	
Microlepidoptera	5
Diptera	
Tipulidae	3
Psychodidae	1
Chirionomidae	229
Mycetophilidae	10
Sciaridae	15
Cecidomyiidae	58
Stratiomyidae	2
Empididae	43
Dolichopodiae	73
Syrphidae	2
Sciomyzidae	1
Acalypterates	40
Calypterates	23
Hymenoptera	1
Formicidae	
Tenthredinidae	13
Braconidae	7
Chalcidoidea	62
Cynipidae	15
Sphecidae	1
Araneida	2
Total	639
n	36

Table 48, 639 individuals in 36 arthropod groups, there occur seven groups not represented in the above collection. Fourteen groups in the 3 hr collection are not represented in this drift net sample, Although the drift net had been left in place 28 hr, a smaller number and variety of insects were caught. This was due to the obstructions in the streams and the collection difficulties at the improperly functioning net.

After an immediate and drastic kill, the large volumes of dead insects in streams reduced rapidly and was thereafter a factor of the remaining insects (conversely, the direct kill or knock-down potential of the insecticide) and the surface area of the stream. This leads to the conclusion that the more effective the insecticide in immediate insect kill or knock-down, the less harmful will be the effects on lotic fauna feeding on dead or dying insects. This observation has management implications, for fish, confronted with a raft of dead insects rapidly flushed past overhead, will have little time to feed, whereas a few poisoned insects drifted by over a long period would ultimately provide more toxin to the fish.

Tracer Plot Study

Some indications of the effects of malathion on members of the aquatic fauna were obtained from fish, crayfish, and snails captured in nets from the lower stream, and placed in buckets of stream water on each of the tracer plots. Twenty-four hours after spraying, all but one of the brook sticklebacks, *Eucalia inconstans*, were dead on the treated plots. Those on untreated plots and all blacknose dace

Rhinichthys atratulus and a mud minnow, Umbra limi, were alive and in apparent normal health. Upon processing the fish, three sticklebacks had a significant (10% level) corrected total activity of 0.02 cpm/mg of air dry tissue. No other samples examined (12) from the tracer plots had levels of activity above background.

Eight days after the spray application additional fish died as shown in Table 49. Whether this death was a result of insecticide within the water or the unnatural environment of the bucket is not known.

From each of two buckets in tracer plot no. 1, were recovered four dead crayfish. Only one showed radiation (0.03 cpm/mg corrected total activity) above background.

Table 49. - Effects of malathion-S^{35} on fish in buckets on the Dover, Ohio, tracer plots, 1961.

Plot No.	Species	8-30 dead	8-30 alive	9-6 dead	9-6 alive	Quantity of solution
T-1	Stickleback	0	9	0	9	17 qts.
	Blacknose Dace	0	4	2	2	
T-2	Stickleback	10	0	0	0	17 qts.
	Blacknose Dace	0	5	3	2*	
T-3	Stickleback	0	7	1	6	17 qts.
	Blacknose Dace	0	4	3	1	
	Mud Minnow	0	1	0	1	
T-4	Stickleback	10	1	1	0	16 qts.
	Blacknose Dace	0	6	6	0	

*One fish died at the first straining of water in the bucket.

Rosenthal (1961:191) reported that the rate of uptake of S^{35} sulfate is logarithmically related to the activity of the isotope in water and is similar to that of Ca^{45} and Sr^{90}.

Cages

Four rectangular boxes 1 x 1 x 1.5 ft of 0.25 in. hardware cloth and wire corner fasteners were made and two placed in each pond several days prior to spraying. Crayfish and fish were caught from the stream near the river and placed in each cage. Four southern red-bellied dace, Chrosomus erythrogaster Raf. 1920 lived in pond no. 1 for 4 days pre-spray and 7 days post-spray without apparent ill-effects. They were removed and counted for accumulated radioactivity. None was found. Blacknose dace escaped from the cages but lived in the ponds for 2 weeks after spray. Those removed for monitoring exhibited no above-background radiation. Pond no. 1 was drained on 11 June 1962 and the fish reentered the stream from which they had come. No fish sampled had levels of radioactivity above background. Crayfish, Cambarus sciotensis, escaped from cages in pond no. 2 but were seen daily pre and post-spray in the pond. Those in pond no. 1 were removed on two occasions to observe buildups of radioactive sulfur in their bodies. One showed above-background quantities of 0.01 cpm/mg. Another, 1 week post-spray, showed the high corrected total activity of 1.6 cpm/mg of dry tissue.

Conclusions

Malathion is toxic to fish though its toxicity varies widely with many factors. Toxicity to stream organisms appears to be entirely from direct fall. No insecticide run-off from forests has been shown. Insect kill was high but decreased rapidly within a period of 3 hr. Sampling of the fauna of headwater intermittent lotic environment was difficult and no satisfactory method was devised. Slate quadrats, 0.5 m^2 quadrats, drift nets and strainers, and cages were used with only minor success. In a small scale study on plots in 1961, brook sticklebacks were very sensitive to malathion while blacknose dace and mud minnow appeared unharmed. Crayfish were either extremely susceptible to the poison or did not tolerate the bucket environment; all died. They contained no radioactivity so death was believed the result of an unsatisfactory holding environment.

Fish and crayfish free and in cages in the pond were unaffected by the spray which varied in concentration from 1.5 to 8.5 ppm on 25 May 1962. A concentration of 6.5 ppm was observed on 21 June 1962 although a zero concentration was observed 10 days earlier.

Laboratory studies of the effects of malathion on aquatic fauna offer only slight evidence of the effects of the insecticide in the natural environment. The effects observed in laboratory tests are increased or ameliorated by such natural factors as amount and formulation of insecticide applied, amount reaching the forest floor and stream surface, species present and their behavior, life stages, ranges, reproductive potentials, and the vigor of individuals and populations, flow rate of water, volume of water in relation to surface area, subsurface recharge of stream flow, chemical and sediment including microscopic organism burden, temperature, shading, bottom characteristics, oxygenation, and other factors. The long list of factors, their multi-interactions, their obscure nature, and slight hope of immediate clarification suggests the need for for caution in applications of insecticides in and near water. The effects of an insecticide on aquatic fauna must be discovered and weighed; a most-suitable time and method objectively sought; and the expected results appraised before application for each individual environment treated. In the case of this study, the only conclusion that can be drawn is that there were no significant observable or lasting effects on the aquatic environment or its fauna.

REPTILES AND AMPHIBIANS

Because of their low population densities, discontinuous distribution, and secretiveness, the reptiles and amphibians, as affected by insecticides, have been poorly studied. Stickel (1951) reported on DDT effects on box turtles, Fashingbauer (1957) reported on DDT effects on wood frogs and Matschke (1961) made studies of spray effects on snakes and frogs in Alabama. Most reports on such effects are brief observations made in connection with study of other faunal segments.

Methods

Following the plan of Imler (1945) five snake traps with 20 ft long drift fences were constructed, three parallel to the ridges and two perpendicular to the streams. To these were added traps for

Eastern Box Turtles (Terrapene c. carolina).[1] See Fig. 31. These traps made no captures. Since the trap has proved effective elsewhere, its inefficiency on this study area is thought to be due to low reptile density, low individual sampling intensity (20 ft), low height of fences (1 ft), and clear visibility of the entire trap to reptiles (unlike the swamp area in which Imler used the device).

The ineffectiveness of the adaptation of the trap for box turtles is partially explained by the escape after 12 hr of a turtle placed inside the trap. Following this a 4-in. weld wire fence addition was made to prevent burrowing under the fence and the top edges were turned inward to prevent the turtles climbing over. Perhaps a better enclosure is needed since a turtle in a tracer plot cage was observed hanging by its foreclaws from the top of a hardware cloth cage 1 ft from any side and over 4 in. from the floor.

On 5 August 1961 an attempt was made to obtain an index of reptiles in watershed no. 1 by use of an electric blanket. Since snakes respond positively to heat it was believed they might be attracted to a large warm area at night. A single-bed, tan, 5 x 8 ft General Electric Co. electric blanket was plugged into a converter attached to a 12 v storage battery and operated from 10:50 pm to 7:30 am. No reptiles were attracted.

The technique was not tried again since the converter overheated after prolonged use. It was also observed that the blanket became

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[1]See Table 50, a checklist of reptiles and amphibians on the study area, for scientific names of reptiles and amphibians.

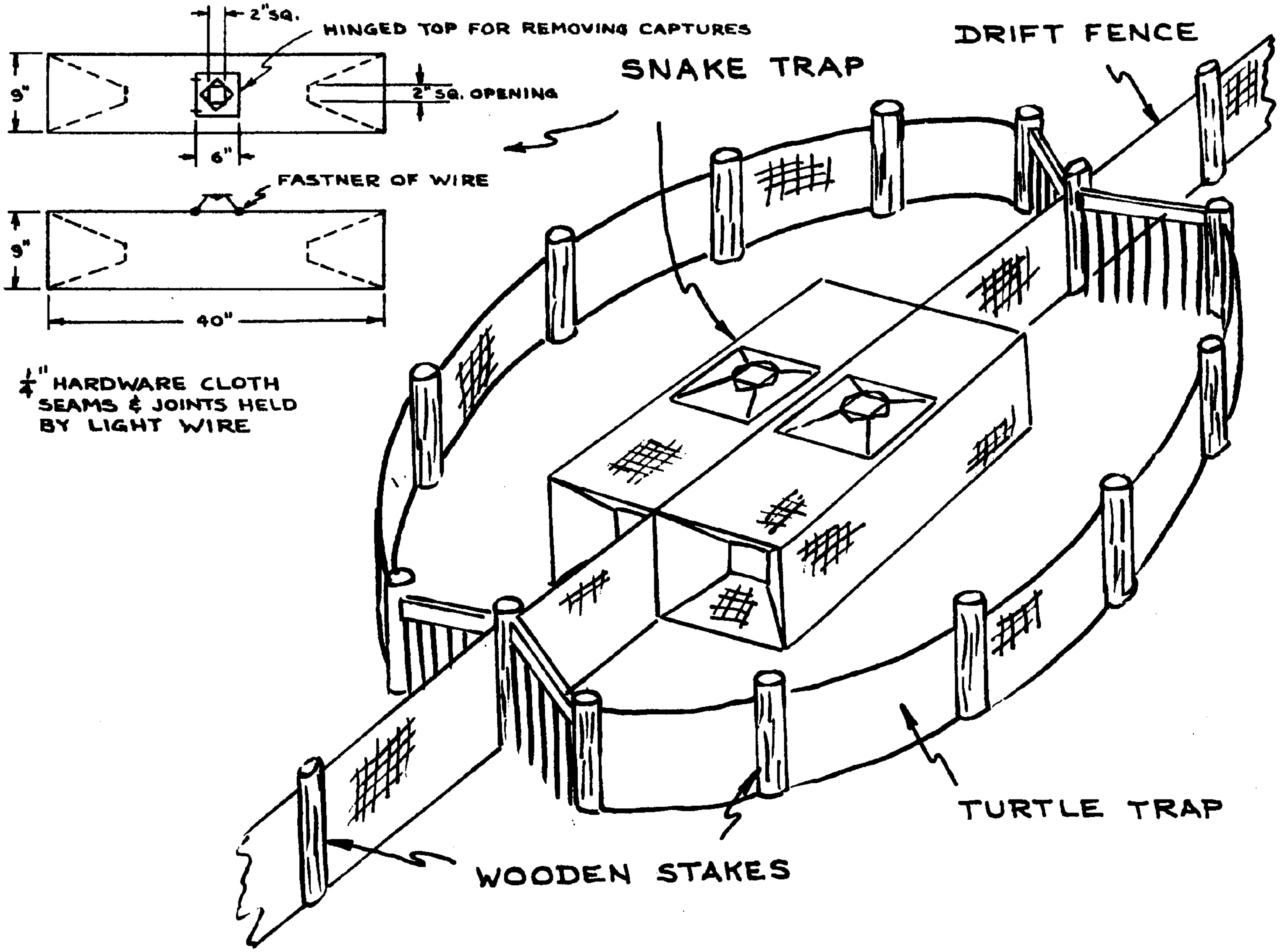


Fig. 31. Diagram of snake and box turtle trap used at five locations within the Dover, Ohio, study area, 1961 and 1962.

Table 50. - Checklist of reptiles and amphibians on the Dover, Ohio, watersheds 1961-62.

Snakes	Black Rat Snake, Elaphe o. obsoleta
	Eastern Garter Snake, Thamnophis s. sirtalis
	Northern Water Snake, Natrix s. sipedon
Turtles	Eastern Box Turtle, Terrapene c. carolina
Frogs	Wood Frog, Rana sylvatica
	Green Frog, Rana clamitans mellanota Raf.
Toads	American Toad, Bufo a. americanus
Salamanders	Red-backed Salamander, Plethodon c. cinereus
	Slimy Salamander, Plethodon g. glutinosus
	Northern Dusky Salamander, Desmognathus f. fuscus Raf.
	Red-spotted Newt, Diemictylus v. viridescens
	Red-spotted Salamander, Pseudotriton r. ruber Sonnini
	Northern Two-lined Salamander, Eurycea b. bislineata Green

moist and the temperature of the inter-coil space was equal to that of the soil, while the temperature along the coils was 75° F. The effective heating area was not considered adequate for the purpose. The hum of the converter located only 6 ft from the blanket may have had a repellent effect. It is believed that the technique has possibilities for obtaining indices of wild, undisturbed reptile populations over a short period of time, when a direct-line power source is available, and when a piece of roofing metal can be used to conduct the warmth of the blanket coils over a wider area, or when a similar large-surface area electrical warming device can be developed economically.

Twenty-four pieces of roofing felt, 35 x 36 in., (Viskalt No. 30 saturated felt, Flintkote Co.) were placed throughout the two watersheds near select grid sites (average distance apart of 400 ft). Their purpose was to provide a standard size shelter and attractive sunning spots for reptiles and amphibians. These creatures are often found under such paper around old buildings. After leaving the tar-paper squares in place for 1 week they were looked under at least twice a week for 5 weeks pre and post-spray. No reptiles or amphibians were discovered on or under any paper in more than 500 observations.

Salamanders

For the tracer-plot study, salamanders were collected from the stream and stream edges below the watersheds several days before spraying the tracer plots. Three were placed in a 3 l (3 quart) tin can on each of the four plots. Each can was one-fourth full of moist soil and rotting vegetation. Some salamanders escaped before an aluminum-foil retainer was pressed around the edges of the cans. In plot T-3 on 4 September 1961 there was one live salamander, *Desmognathus f. fuscus*. In T-1 a live *Pseudotriton r. ruber* was collected. These salamanders had a total activity in cpm/mg of 0.004 and 0.007 neither of which were significant at the 10% level. All salamanders escaped from treated T-2 but in T-4 one dead and two live salamanders were found on 4 September 1961 (9:30 am after two rains). Salamanders were covered by the vegetation and could have easily escaped the insecticide. In the case of salamanders, contact with insecticide or contaminated vegetation appears to be dependent upon the multiple functions of behavior, moisture conditions of the substrate, and absorptive properties of the ground cover.

After noticing on mammal plot 3 on watershed no. 2 that *Plethodon cinereus*, the Red-backed Salamander, was abundant under 6 in. by 12 in. pieces of roofing felt, these felts were moved to the stream edges and banks as standard environments under which to count salamanders and thus note insecticide effects.

Two small papers were placed every 25 ft beside or in the stream on moist leaves and two were placed on the drier soil bank several feet from the stream. There were 12 sites in each stream, each site having four papers. Observations were cursory in that most salamanders could not be captured without destroying the environment. Since no effects on salamanders had been observed on the tracer plots, none were expected in the final application. Effects were measured in total numbers of salamanders observed. Most observations were of

Desmognathus f. fuscus and are listed in Table 51.

Table 51. - Total number of salamanders observed under 48 pieces of 6 x 12 inch tar paper in and along beds of streams 1 and 2, Dover, Ohio, 1962.

Date: Month-day	Stream 1	Stream 2
5-11	4	3
5-14	5	7
5-22	2	3
6-12	0	0
7-13	3	2

Only 15 salamanders were observed under cryptozoan boards during the entire study. No changes could be detected.

Toads and Frogs

American toads were present in the forest but were not abundant. Along the 2 mile trap line, an average of three was seen each day. Toads were occasionally found in small mammal traps. No more than ten were caught in traps during the entire 2 years study except on 16 May 1961 when seven were caught in 1 trap-night. I think their entrance into traps is due more to chance than to "seeking" warmth or bait-feeding insects. The only index of toad abundance was the mean number seen per 2 miles of trap line. No changes were noticed post spray, nor were population shifts into or out of the area detected.

A toad was collected from each study area 5 days post spray. It was preserved in alcohol and 2 weeks later was homogenized in a

Waring blendor, dried, weighed and samples prepared for counting. The toad from watershed no. 1 had an activity of 0.013 cpm/mg while the one from area 2 had .002 cpm/mg. The activity of the toad was 7.2 times greater on the treated area. Toads seemed to behave normally.

Box Turtles

Box turtles discovered on or along the trails of the grid were numbered as shown in Fig. 32, and a record kept of their sex, general characteristics, and location ($\pm$ 10 ft). Turtle observations are presented in Table 52 and Fig. 33A. These populations are less than the 73 to 74 on a 30 acre tract reported by Stickle (1951:162). Turtles were discovered at a rate of 0.12 per field day.

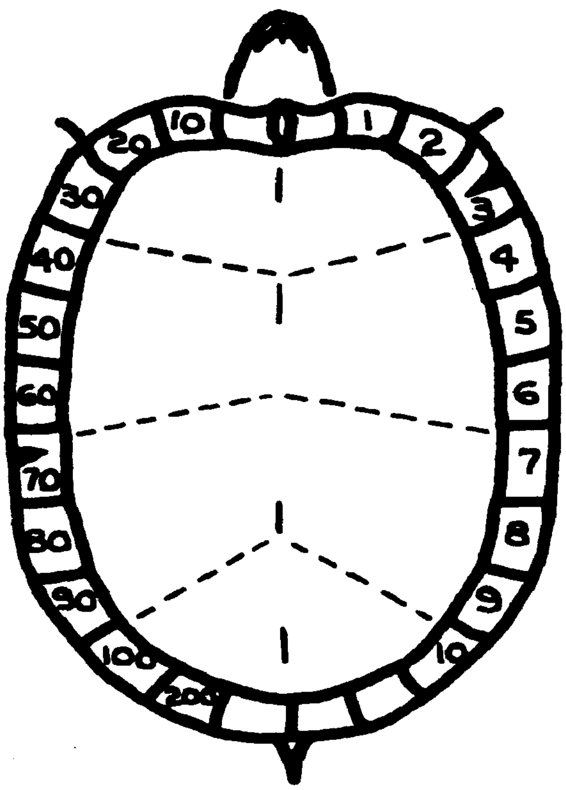


Fig. 32. - Box turtles were permanently numbered by notching one or two scutes with a knife. Turtle no. 73 is shown.

Table 52. - Summary of random collections of box turtles in 1961 and 1962 on the Dover, Ohio, study area.

Location	Date	Male	Recap-tures	Female	Recap-tures	Unknown	Recap-tures	Total
Watershed 1	1961	6	2	2		5		15
	1962	1		1		5		7
Watershed 2	1961	2		4	1	4		11
	1962		1		1	4	1	7
Vicinity of Study Area	1961		1	1		2		4
	1962			1	1	1		3
Total		9	4	9	3	21	1	47

Some box turtle movement data was obtained. Turtle no. 6 moved a minimum of 1310 ft between 2 June 1961 and 5 July 1962 (398 days); no. 13 moved 730 ft between 18 July 1961 and 13 July 1962 (360 days); no. 2 moved 710 ft between 15 May 1961 and 8 July 1962 (418 days); no. 7 moved 350 ft between 5 June 1961 and 2 July 1961 (27 days); no. 8 moved 325 ft between 6 June 1961 and 5 September 1961 (91 days); and no. 10A moved less than 100 ft between 6 June 1961 and 13 October 1961 (130 days). See Fig. 33A.

On the day after the second spray application (26 May 1962) turtle no. 48 was caught in a cat trap at 1-10. It was the only turtle trapped throughout the study. It's radioactivity was 0.06 mr/hr with higher carapace concentrations than on the plastron where the

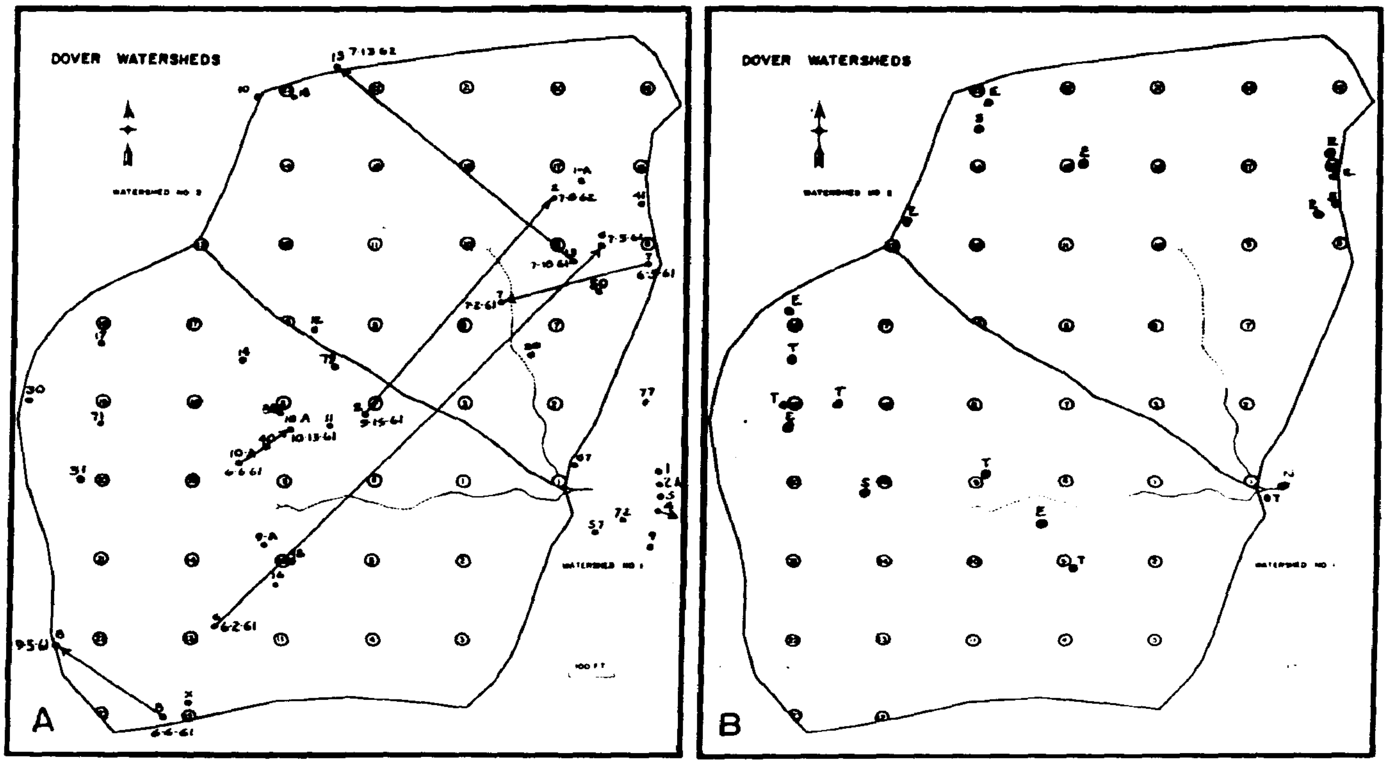


Fig. 33. A. Location of box turtles found on the Dover, Ohio, watersheds 15 May 1961 to 1 September 1962. B. Observations of snakes in 1961 and 1962. E represents Elaphe; T, Thamnophis; N, Natrix; and S, shed skins.

activity was background.

Box turtles were held in 3 x 3 x 1 ft wire cages on the tracer plots and fed garden vegetables for a week prior to treatment. They made no observable response to the poison. On 30 August 1961, turtles in T-1-7, T-2-7, and T-2-29 cages were active. On 6 September, 1961 all were active and in apparent good health. On 13 September, 4:00 pm, three turtles in the cages were killed, frozen, and later processed. Results are in Table 53.

Table 53. - Radioactivity of box turtles from Dover, Ohio, tracer plots after malathion-S^{35} treatment in 1961. *Designates activity significant at the 10% level.

Location	Description	cpm/mg
T-1-8 (below and right of 8)	Scapula	0
	Liver	0
21 days post-treatment	Plastron	0.011*
	Activity in area (cpm)	4.6*
T-2-8 (below and left of 8)	Scapula	0.006
	Liver	0.006*
21 days post-treatment	Plastron	0.013*
	Activity in area (cpm)	909.1*
T-4-8 (below and right of 8)	Scapula	0.002
	Liver	0.009*
21 days post-treatment	Plastron	0.024*
	Activity in area (cpm)	18.4*
T-2-26 (below 26)	Scapula	0.067*
	Liver	0.039*
27 days post-treatment	Kidney	0.033*
	Plastron	0.036*
	Activity in area (cpm)	5000.0*

Snakes

In 1961, four black rat snakes were seen on the watersheds, two in each. Two were marked by cutting with scissors a notch in a caudal scale. There were no recaptures.

In 1962 more snakes and a greater variety were observed. Four rat snakes, five eastern garter snakes, and one northern water snake were observed. Locations of snakes are shown in Fig. 33B.

Conclusions

There appears to be no literature on the effects of malathion on amphibians and reptiles. These animals are very difficult to sample adequately. In the Dover forest, traps, an electric blanket and tar paper quadrats were ineffective sampling devices for reptiles. Collecting and marking individuals was the only feasible study method. Indices based on time-distance-niches-inspected would have been of greater value and are feasible for a study in which reptiles are a major interest.

Reptiles appeared unaffected by the insecticide. Caged turtles built body loads of S^{35} proportional to the amount of spray delivered to their area. The correlation coefficient, r, between scapula cpm and cpm of glass discs in the vicinity was 0.99. More snakes were discovered in 1962 than 1961 within comparable periods on the same area. Snakes were observed active on the treated watershed within 1 week post-spray.

Amphibians appeared unaffected by the insecticide. Toads built high total body loads of S^{35} but whether due to dermal contamination and absorption or ingestion is unknown. No observable differences were noted in toad or frog populations or behavior. Salamanders, sampled with success under tar papers, were unaffected.

In the writer's opinion, the cryptofauna would be unaffected by even more toxic, short-toxicity insecticides applied at a rate of no more than 10 gal/acre of formulation. The great absorptive capacities of the forest soil make the insecticide unavailable to these forms. Since they normally remain hidden or inactive very early or late in the day when aerial spraying is most satisfactorily done, chances for direct contact are limited. Influence of an insecticide on their food supply is another factor, however. It may be concluded from the results that an insecticide with toxicity persisting less than 2 weeks will not deplete food supplies of the reptiles and amphibians sufficiently to cause noticeable decreases in their populations.

BIRDS

Literature Review

Results of research by DeWitt (1955) and others cover the chronic effects of other insecticides on birds and mammals. Hickey's report (1961:8-9) that the 1.5 million lb of insecticide used in Wisconsin's agricultural and forestry operations have slight over-all direct effect on bird life reduces some concern. Often, however, possibilities for poisoning are taken as probabilities. This apparently is not the case with malathion for it is generally rated as safe for use with birds. Darsie and Corriden (1959) cited six references attesting to the non-toxic nature of the substance to poultry.

Negherbon (1959) summarized the known effects on poultry. Table 54 is a summary of some reports on the effects on birds. Dahm (in Metcalf, 1957:110) suggested that malathion is poorly absorbed by the skin of birds and is unavailable for absorption from the feathers. Malathion is unlike the other organo-phosphates which, in this respect, are more toxic to warm blooded animals than chlorinated hydrocarbons.

Sherman and Ross (1960:431) studied the effects of manure from malathion-fed chickens on house fly larvae. They found that the manure of chicks fed on a diet of 1.102 ppm malathion in feed appeared to be more toxic to fly larvae than the manure from hens on the same diet. In relation to the other materials, malathion was relatively ineffective. In their tests malathion caused some mortality or subclinical toxicity which was expressed as poor growth or decreased egg production.

Malathion has been used for poultry louse and mite control. After one treatment of dust, control is evident for at least 150 days (Rodriguez and Riehl, 1961). Furman and Weinmann (1956:450) and Reid (1956) reported reinfestation of lice. Residual effectiveness of roost sprays as determined by artificial reinfestation was largely dissipated within 3 to 5 weeks.

Furman and Weinmann (1956:447) reported a 2% dip killed 25% of tested turkeys and a 4% dip killed all tested birds including a goose. Dead birds showed little overt pathology. "...Slight mottling of the liver and hyperemia of the brain were suggestive of toxic effects." Negherbon (1959:462) reported that malathion does not penetrate

Table 54. - Summary of some accounts of toxicity of malathion to birds

Author	Species	Dosage	Effect
DeWitt *et al*. (1960)	Bob-white	400 mg/kg	Approximate lethal dose to 50 per cent or more of the subjects (chronic poisoning).
	Ring-necked Pheasants	1600 mg/kg	
	Bob-white	50 mg/kg	Maximum concentration in diet permitting normal survival.
Dicke *et al*. (1955: 342)	chicken	Exposure to feet for 10 weeks. Contamination of feed and water for 6 weeks	No harm. On food, no harm and no repellent action.
Furman and Weinmann (1956:447)	laying hen	50 ppm in diet for 10 days	No reduction in egg production, or hatchability, nor was off-flavor detected.
	domestic turkey	2% solution on manure and wire	No toxicity in 1-month-old turkeys.
	turkey, chicken, goose	4% emulsion dip	LD_{100}-24 hours.
Harding and Quigley (1956:867)	chicken	4% dust at .5 and .25 lbs/ 20 sq. ft	No effect on hatchability of 500 eggs or no residue contamination on interior of 72 eggs.
Rodriguez and Riehl (1961)	chicken	1 lb. dust per 20 ft of pen. Dust added to dust baths	No effects. Good control for 150 days.

chicken egg shell and was not found in yolk or egg white. He reported (1959:454) radiotracer studies showed that 60% of the malathion consumed orally by hens was eliminated in the feces in 2 to 4 days; 75% in 5 to 6 days. Of the excreted radioactivity 97% was in the form of water soluble metabolites, degradation products. The maximum amount in tissues was less than 3% of the amount fed. The maximum amount of radioactivity from sprayed hens occurred in the feces 24 hr post treatment. Less than 12% of applied malathion was absorbed from the feathers.

Studies to date appear to be limited to the Galliformes. The questions are legion that must be answered for the wild birds of the forest, the majority of which are insectivorous with greatly different behavior and physiology. George and Mitchell (1947:789) pointed out that

> ...If a large forested area were treated with DDT [This will probably hold true for other insecticides, including malathion] at a rate sufficient to eradicate the insect population at the time when young are being fed in the nest that they, being fed poisoned insects just subsequent to spraying, and not receiving sufficient food later through a scarcity of insect life, would suffer or succumb from DDT toxicity itself or from a combination of weakness and toxicity.

Success of birds in conflict with insecticides is ameliorated by some naturally-occurring phenomena. Odum and Norris (1949:417) observed that birds tend to stay in their niches and seek live prey rather than be attracted to piles of DDT killed insects. Ants were observed to carry away dead insects and may be an important factor in reducing the hazard to birds. On the other hand, recent investigations of the Patuxent Wildlife Research Refuge, Laurel, Maryland, indicate that sub-lethal doses of insecticides to birds may make them more susceptible at a later date to what would ordinarily be considered a non-lethal dose.

Short residual effects of malathion would minimize dangers of the poison to transients or migratory birds. Although sub-lethal effects are not known, it may be generalized that at moderate rates of aerial application, malathion does not directly harm wild birds. No such generalization can be made about secondary effects of an application. The question remains: How and through what channels are wild bird populations affected by the removal of part of their food supply?

Bird Study Limitations and Considerations

Personal

In this study the birds were not as well surveyed as were mammals. In 1961 the writer was not familiar with the birds on the area. Apparent bird abundance increased throughout the year, not only with migration or production, but with the writer's increased knowledge, study, and familiarity with the area and bird habitats. The writer also discovered in 1962 that he was deaf for birds with very high frequency calls, adding further to the inadequacy of the 1961 data. Besides these personal shortcomings, the woodland, as in most studies, was difficult to survey for birds. The few birds of the shrub layer could be readily seen; birds of the tree canopy, however, some 60 ft or more above the observer in dense foliage, were very difficult to locate and identify. Much identification was by song alone and no record of a bird was made before voice and name were associated. Duplication of counts of moving, singing birds, is believed to be small, yet remains an unmeasured variable.

Mobility

Lay (1958) pointed out the difficulty of studying highly mobile bird populations on small areas treated with insecticide.

The interrelationships of mobility, time, area studied, space, territory, behavior, insecticide dispersal, and plant succession are exceedingly complex as they influence insecticide effects on bird populations.

Population Estimation

In 1961 several methods were tried over different routes. The results were qualitative rather than quantitative and are included only in the checklist of birds seen in the area. Based on 1961 studies, the following method was selected for use in 1962. Using the route shown in Fig. 34 a sample strip census (Pettingill, 1956: 290) was used to obtain a measure of the birds on the two study areas. The surveys usually started 0.5 hr after sunrise and were taken weekly. The line was marked with red plastic ribbons. Closer to the date of spray counts were taken more frequently. A slow, continuous rate was made as quietly as possible with pauses every 5 to 10 paces. Approximately 1 hr was required per watershed. All species seen and heard were recorded on maps of the area. Permanent 200 ft grid locations allowed great accuracy in recording locations of observed birds. Binoculars, 8 x 40 and 8 x 30, were used. In addition to the strip census, birds seen or heard at night when operating insect light traps were recorded.

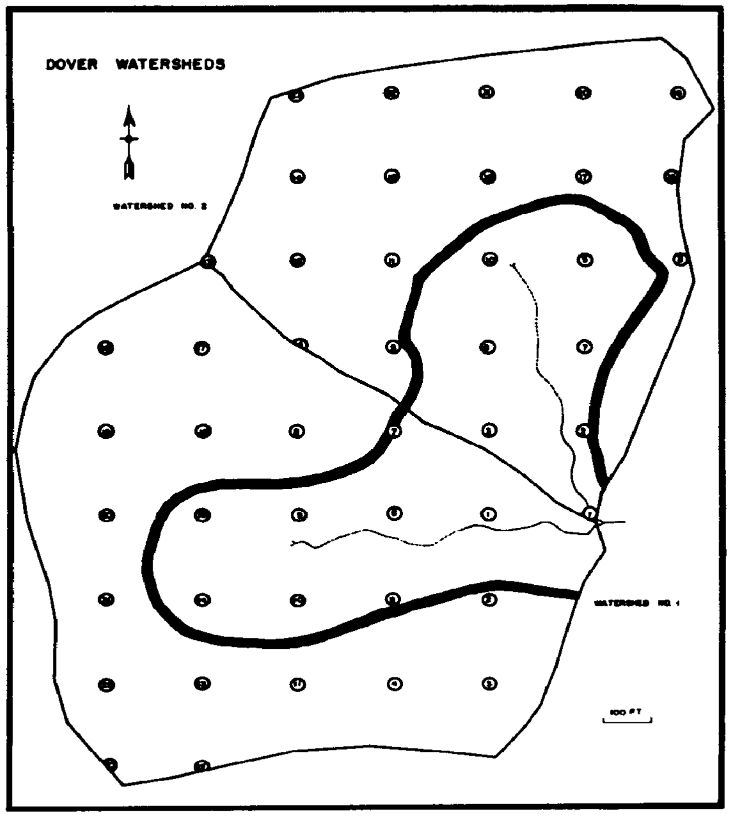


Fig. 34. Permanent transect used in 1962 for bird population studies on the Dover, Ohio, watersheds.

Two ground-level 4 x 15 ft mist nets were unsuccessful in capturing birds for radiological analysis. A 410 ga. shotgun was used to obtain samples for processing. Birds were shot off the area for pre-spray background counts on bird tissue. Only a few birds, those seen first and not selected by species, were shot. Numbers collected were limited to minimize territorial change phenomena.

Results

The results of studies are shown in Table 55 and 56 and Fig. 35. No effects were observed from the 15 May single swath spray. The afternoon following the second spray, 25 May, there was notable silence of birds on the treated and control area as compared to areas east of watershed no. 1. This silence was noticeable for 2 days

Table 55. - Birds known breeding on the Dover, Ohio, study area in 1961 and 1962 (AOU Checklist, 1957).

Ruffed Grouse	Blue-gray Gnatcatcher
Yellow-billed Cuckoo	Red-eyed Vireo
Ruby-throated Hummingbird	Cerulean Warbler
Red-bellied Woodpecker	Ovenbird
Downy Woodpecker	Kentucky Warbler
Acadian Flycatcher	Brown-headed Cowbird
Wood Pewee	Scarlet Tanager
Tufted Titmouse	Cardinal
White-breasted Nuthatch	Rufous-sided Towhee

during which time it became less noticeable. Bird calls on the control area were more frequent than on the treated area and became normal more rapidly. The differences were noted by three observers. By the fourth day there was no noticeable difference between areas. The reasons for this silence are not known. A few birds were seen alive on the treated area, but the total number ever seen during any 1 day, other than on bird-count days, was very small, rarely more than 10. The silence could have been due to emigration, to some behavioral responses associated with the loss of food, or to sub-lethal insecticide effects. Post-spray populations then became "normal" and no differences could be measured between years, between areas, between treatments or before and after spray.

Young birds were successfully reared in the contaminated area, eggs were hatched and re-nesting occurred (Kentucky Warblers, Acadian Flycatchers, Wood Thrushes, Yellow-billed Cuckoo, Red-eyed Vireo, and probably Titmice, Chickadees, and Woodpeckers). These observations correlate with those of Hoffman and Linduska (1949:108) who

Table 56. - Checklist of birds observed on the Dover, Ohio, study areas, 1961 and 1962 (American Ornithologist's Union, 1957). (F) indicates species seen only in flight over the area.

Great Blue Heron (F)	House Wren
Turkey Vulture (F)	Winter Wren
Cooper's Hawk	Robin
Red-tailed Hawk	Wood Thrush
Ruffed Grouse	Hermit Thrush
Bob White	Swainson's Thrush
Killdeer (F)	Gray-cheek Thrush
Woodcock	Eastern Bluebird
Mourning Dove	Blue-gray Gnatcatcher
Yellow-billed Cuckoo	Golden-crowned Kinglet
Black-billed Cuckoo	Ruby-crowned Kinglet
Screech Owl	Cedar Waxwing
Great Horned Owl	Yellow-throated Vireo
Whip-poor-will	Red-eyed Vireo
Chimney Swift (F)	Black and White Warbler
Ruby-throated Hummingbird	Magnolia Warbler
Flicker	Myrtle Warbler
Pileated Woodpecker	Black-throated Green Warbler
Red-bellied Woodpecker	Cerulean Warbler
Yellow-bellied Sapsucker	Chestnut-sided Warbler
Hairy Woodpecker	Black poll Warbler
Downy Woodpecker	Ovenbird
Great Crested Flycatcher	Lousiana Waterthrush
Eastern Pheobe	Kentucky Warbler
Acadian Flycatcher	Yellow-breasted Chat
Least Flycatcher	Wilson's Warbler
Eastern Wood Pewee	Canada Warbler
Rough-wing Swallow (F)	Red-winged Blackbird
Purple Martin (F)	Common Grackle
Blue Jay	Brown-headed Cowbird
Crow	Scarlet Tanager
Black-capped Chickadee	Cardinal
Carolina Chickadee	Rose-breasted Grosbeak
Tufted Titmouse	Indigo Bunting
White-breasted Nuthatch	Purple Finch
Brown Creeper	American Goldfinch
	Rufous-sided Towhee

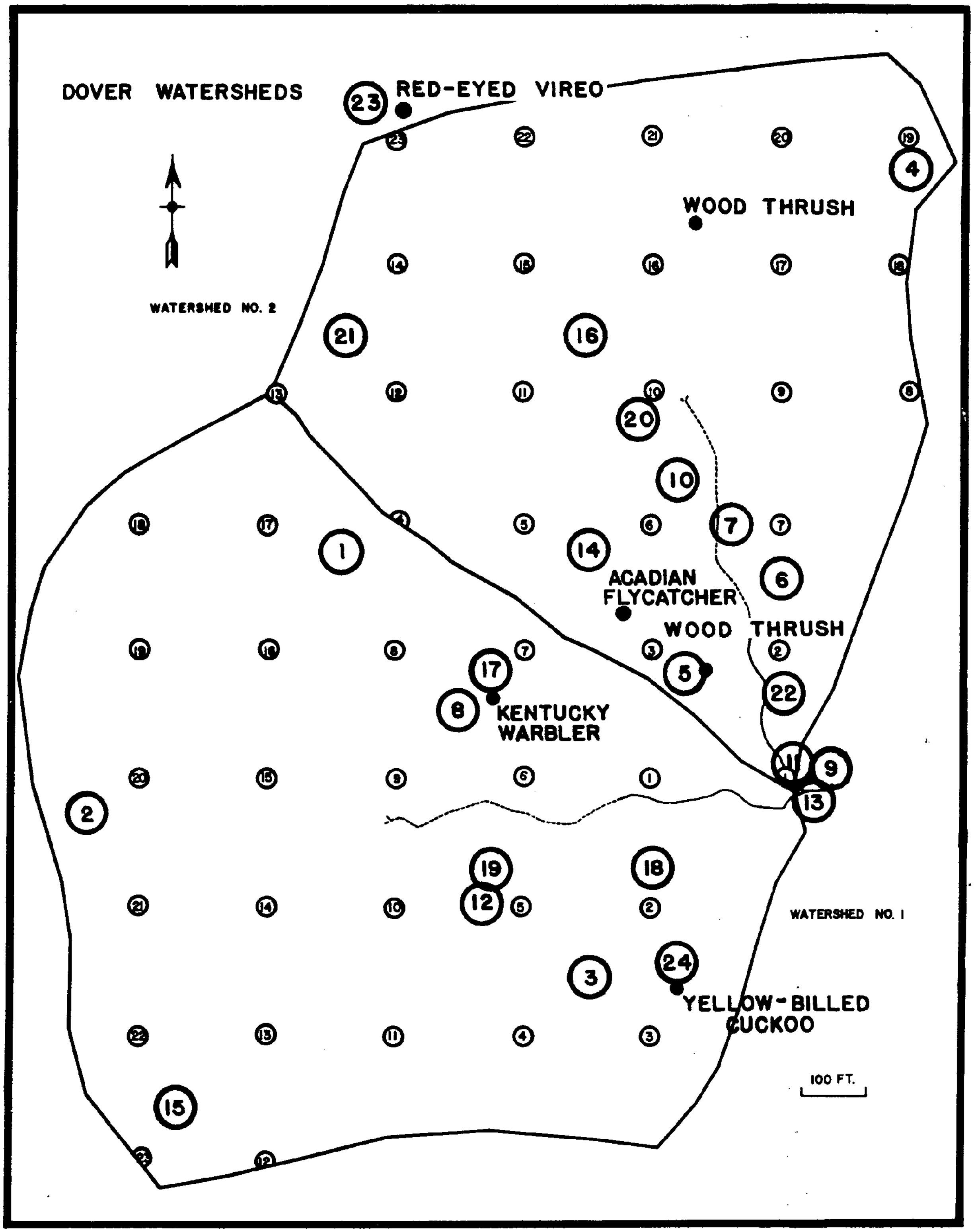


Fig. 35. Breeding territories and known nest sites of some birds of the Dover, Ohio, area, 1962. Territory centers are shown as: 1 and 2, Ruffed Grouse; 3, Cardinal; 4, Tufted Titmouse; 5, Wood Thrush; 6 and 7, Ovenbird; 8 and 9, Scarlet Tanager; 10, 11, and 12, Acadian Flycatcher; 13, 14, and 15, Kentucky Warbler; 16, Towhee; 17, Wood Pewee; 18 through 23, Red-eyed Vireo; 24, Yellow-billed Cuckoo. Nesting sites, black dots, are labeled.

that even though 1 lb/acre of DDT caused a sharp drop in insect populations, recovery was rapid and the temporary loss was of no real consequence to birds. Tigner (1960:33), studying the effects of an insecticide on pheasants, stated that

> A loss of insect life in a particular field should not seriously affect adult pheasants or their young, because they would move to a nearby field where insects are abundant. However, if large areas were sprayed with the more toxic insecticides, such as parathion, a severe food shortage would result with the subsequent starvation of young birds.

Ruffed Grouse

The Ruffed Grouse, *Bonasa umbellus*, was the only game bird breeding on the area. (Woodcock and Bob White were only recorded). Since it was seen frequently, is a ground feeder, has an important role in forest ecology, and was thought to serve as a good indicator organism, it was studied more intensively than other bird species. Dates, time, and location of flushes were recorded. These are plotted in Fig. 36. Time and location were correlated with activity of observers on the area, not bird behavior. Birds shifted locations between years but did not seem to shift after spray.

A newly hatched covey was seen on the morning of the spray, 25 May, on the ridge above 1-20. These young were in the contaminated area at the time of spray. On 10 August 1962 a covey of eight birds flushed from below 1-21 were believed to be the same family. No adverse insecticidal effects were evident. Watershed no. 1 had 2 known grouse nests, watershed no. 2, 2 nests. A nest 100 ft away from the plane crash was deserted immediately after the crash, undoubtedly due to the great disturbance in the area.

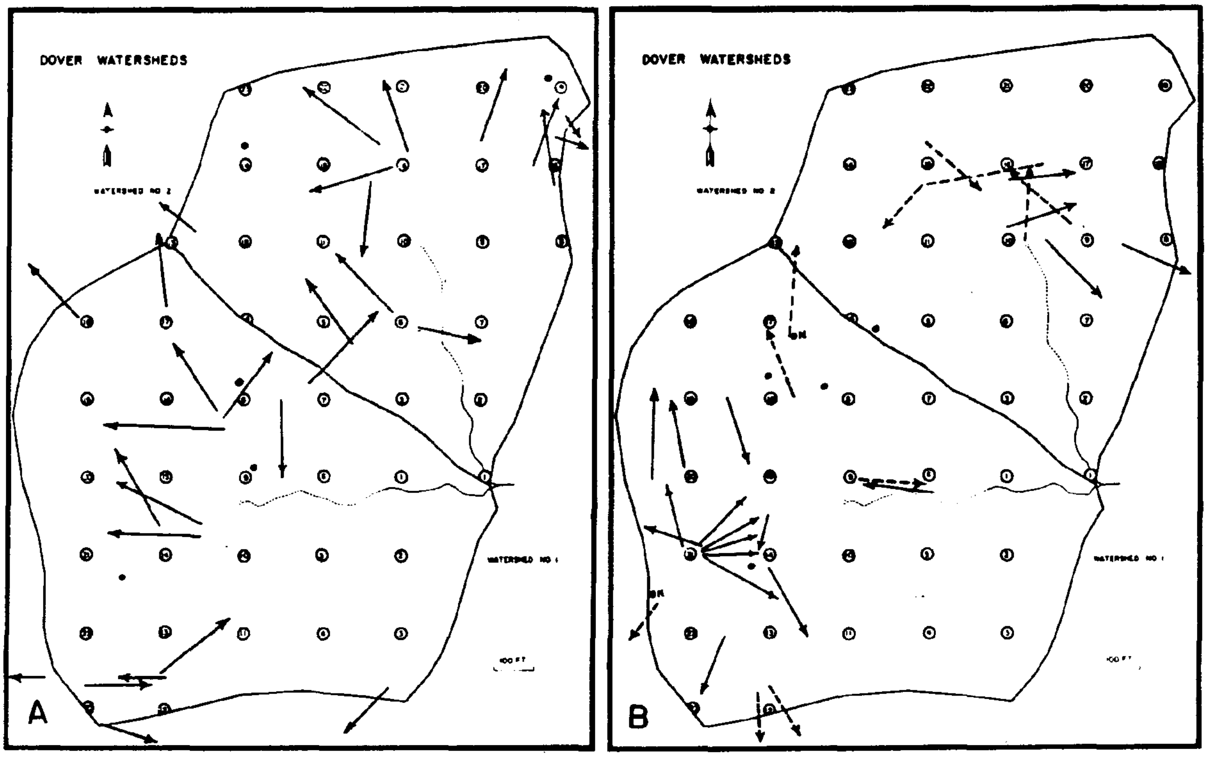


Fig. 36. Ruffed Grouse flushing locations and directions observed in 1961 (A) and 1962 (B) on the Dover, Ohio, watersheds. Dashed lines represent grouse observed pre-spray; "N" represents nest locations.

There were five grouse "centers" or areas from which birds were flushed in 1961 and 1962. Four were common to both years; there were two less in 1962. There is no evidence that grouse populations were effected by the spray either in terms of survival or altered behavior.

Radioactivity Levels

Since no dead or moribund birds were found or observed, the radioactivity levels of collected birds is believed to represent amounts of malathion, it's metabolites, or by-products encountered by birds in their normal activities on the areas (Table 57).

The anomalies in the data in Table 57 cannot be explained. Especially interesting, but unexplained, is the quantity of activity in the pre-spray Downy Woodpecker and Myrtle Warbler. The post spray gizzards and gizzard contents of the Red-eyed Vireo and Acadian Flycatcher are interesting in their mutual exclusiveness. The gizzard and it's contents have no apparent contamination exchange. External contamination appears highest of all organs or structures.

Willard (1960:148) studying gross beta activity of birds on the White Oak Lake bed of Oak Ridge, Tennessee, found activity on the feathers was very variable and though some activity could be washed off, it was not possible to remove all of it.

In summary, the malathion did affect bird behavior, but produced no detectable lasting adverse effects. The insecticide and it's metabolites were present in the body tissues of birds, but were so variable in quantity and location that no conclusion other than that

Table 57. - Radioactivity in cpm/mg of whole organs of birds collected pre-spray off the study areas and post-spray from watershed no. 1, Dover, Ohio, 1962.

	Pre-spray, 11 May			Post-spray, 11 June		Post-spray, 3 August		
	Myrtle Warbler	Downy Woodpecker	Kentucky Warbler	Acadian Flycatcher	Hairy Woodpecker	Red-eyed Vireo	Acadian Flycatcher	Wood Pewee
1. Feathers	0	0.0469	0	0.1134	0.0421	0	0.2112	0.1285
2. Skin	0	0.0775	0	0.0699	0	0.1542	0.0443	0.0983
3. Liver	0	0.0097	-	0.0226	0.0151	0.0127	0.0145	0
4. Gizzard	0.0110	0	0	0.0298	0.0108	0	0.0121	0.0054
5. Gizzard Contents	-	-	-	-	-	0.0512	0	-
6. Heart	0	0.0089	0	0.0389	0.0140	0	0	0.0229
7. Intestine	0.0076	0	-	0.0226	0.0162	0	0	-
8. Tibial Caps	0.0151	0.0065	-	-	-	0	0	0.0894
9. Lungs	0	0.0251	-	0	0.0762	0	0	0
10. Trachaea	0	0	0	-	0.0654	0	0	-
11. Costal Facet	0	0	-	-	-	0.0497	-	-
12. Brain	0	0	0	-	-	0.0432	0	-
13. Pectoralis major	0	0.0069	0	-	-	0	0	-
14. Testes	0	0	-	-	-	-	-	-

it entered and remained in the bodies of the birds could be reached.

MAMMALS

Mammals are the most abundant large animals of the forest and widely influence the plant-animal-abiotic interactions of the forest. They play varying positive and negative roles in the development and dissemination of forest reproduction; consume injurious insects and fungi; mix, aerate, and improve the water retention properties of the soil; serve as prey for larger forms; and enrich the soil by their excreta and dead bodies.

Studies of wild small mammal populations are becoming more and more critical as needs increase for knowledge of population dynamics and social stress, as primary consumer fauna become ecologically more important, and as the effects of potentially harmful agents such as insecticides, fire, and radioactivity receive more careful scrutiny.

Mammalian Effects of Malathion: A Review

Mammalian toxicity of malathion is less than that of DDT and falls in a category along with methoxychlor, TDE, BHC and chlorthion (George, 1957:23). It is 100 times less toxic than parathion and is reported to be the safest of the organo-phosphate insecticides.

Reported LD_{50}'s vary widely. Negherbon (1959) listed many observations. Martin (1953:152) set acute oral LD_{50} to rats and mice as 480-5800 mg/kg; Metcalf (1955:306) stated that for rats the figure is 1400 to 5834 mg/kg. Variations are due to differences in male and female reactions and the degree of purity of the test material.

Zavon (1958:12) emphasized the importance of the relationship of feeding habits and nutritional condition of mammals to toxicities of poisons. Mammal species variation is generally small (Metcalf, 1955: 306). Golz and Shaffer (1956:3) noted, that acute oral toxicity is inversely related to increasing grades of malathion purity.

Barnes (1953:64) observed that the approximate average single lethal oral dose to male rats is less than 1000 mg/kg.

> Malathion, 99%, was fed to rats for two years at rates as high as 5000 ppm without mortality or tissue damage, although growth was decreased in males but not in females. Growth rates were normal at 1000 ppm, and ChE cholinesterase levels of plasma and red cells were normal at 100 ppm, moderately depressed at 1000 ppm, and markedly depressed at 5000 ppm. Some female animals survived the feeding of 20,000 ppm (Metcalf, 1955:309).

Metcalf (1955:306) reported that dermal LD_{50} is greater than 4000 mg/kg for rabbits. Chronic toxicity and inhalation effects are reportedly low (Frear, 1955:82). Guinea pigs were not killed by skin application of up to 12,300 mg/kg of 95% technical malathion. They suffered no primary skin irritation or gross evidence of systemic toxicity. Malathion, 0.05 ml of 95% technical, in the rat eye produced slight symptoms which completely subsided in 24 hr. Eight-hour exposure of rats and mice to a near-saturated atmosphere resulted in excessive preening, depression, and labored breathing. One mouse died 30 min. after termination of exposure. All survivors appeared to have made full recovery (Golz and Shaffer, 1956:5). The same investigators (1956:3) reported that when symptoms less than a coma appeared, they were normally of short duration and unless death occurred in several hours, recovery was rapid and apparently complete.

These symptoms in small mammals are excessive salivation, depression, and tremors.

The USDA made studies on the fate of malathion applied to four cows. They were sprayed twice, 1 week apart, 2 with emulsions, 2 with suspensions each of 0.5 and 1.0% concentrations. Cows excreted malathion ranging from 0.08 to 0.36 ppm in all samples of milk taken 5 hr after spraying. Traces were found in samples taken after 1 day, but after 3 and 7 days, samples were free of contamination. The higher concentrations caused heavier residues than the lower concentrations and the suspensions caused heavier residues than the same concentrations applied as emulsions (Claborn, 1956:8). Goulding and Terriere (1959:341) reported that use of a 10% dust contaminated milk with malathion for 60 days. Their findings support the above statements of milk contamination. Golz and Shaffer (1956:8) found that an emulsion formulation at the rate of 0.2 mg/cm^2 per week apparently reduced red blood cell colinesterase activity in cows to 80% of the pre-treatment value. Spraying and dipping beef cattle reduced ChE activity during the period of application with practical to complete recovery after 3 months. No gross evidence of toxicity was observed in test animals. Later, however, Radeleff and Bushland (1960:141) found a minimum toxic dose of orally administered malathion for adult sheep and goats of 100 mg/kg. For 4 day old calves it was 20 mg/kg. They found a maximum non-toxic dose on calves to be 10 mg/kg and on sheep and cattle, 50 mg/kg. Varying sprays applied to Jersey cows to the point of runoff resulted in no significant ChE depression in 24 hr after spraying. Angora goats dipped in 95%

technical malathion in varying formulations evidenced no observed effect on ChE activity. March et al. (1956:682), using P^{32} as a radioactive tracer, discovered that malathion was rapidly absorbed after spraying cows and that elimination was rapid, the greatest amount being excreted in 24 hr. Residues were present up to 140 and 237 days. Only 2.7% of the spray remained on the animals after 2 weeks. Up to 2 ppm of residue was found in bone, liver, thyroid, thymus, and pancreas. Highest residues, 3 to 18 ppm, were found in the hide. Claborn (1956:9) found no storage of malathion in the fat of beef cattle. If this is true of other animals, it precludes the availability of stored poison for re-introduction into their systems following periods of stress.

Beck's studies (1953:571-72) on the effects of malathion on boar spermatozoa showed that it had a weak inhibitory effect on respiration and glycolysis and that motility was decreased.

Gjullin et al. (1955:508-09) found malathion-sprayed alfalfa fed to cows did not effect the flavor of milk. Objectionable odor in wheat treated with 8 ppm of malathion and stored 68 days was reported by Walker and Locke (1959). This presents a possibility of the poison making some plant foods objectionable to mammals.

B. S. McGinnes, Leader, Virginia Cooperative Wildlife Research Unit, (1960, pers. correspondence) suspected that malathion killed immature wild cottontail rabbits when dusted on their fur.

Metcalf (1955:306) elaborated on the symptoms of malathion poisoning in humans.

> The muscarinic effects in order of their appearance are anorexia and nausea, followed by vomiting, abdominal cramps, sweating, salivation, and in some cases by pupillary constriction....The nicotinic effects of twitching the eyelids and tongue are early symptoms and are followed by fasciculations in the muscles of the face and neck and in the extraocular muscles, and then by general fasciculations and weakness.

Single aerial applications of malathion at 0.46 lb/acre produce human exposure less than 1/120,000 of the LD_{50}'s of experimental animals (Caplan *et al.*, 1956:332).

Population Studies

Much emphasis was placed on the study of mammals, though malathion was not expected to be directly toxic to them in the quantity used. Even young, which may be more susceptible to direct poisoning, would probably be protected in nests and burrows and be unaffected. The interest in secondary effects of the poison promoted the intensive mammalian studies. Effects on mammals were hypothesized to be the result of food chain disruption, necessitated feeding-behavior changes, and inter and intra-specific conflict resulting from home range shifts. If these effects occurred, intensive studies would be needed to detect and measure them.

Mammalian response to an insecticide application is variable due not only to inherent tolerances of some species and individuals but to the amount and mode of insecticidal contact. Mammals may contact poison in varying amounts by: (1) having it settle directly on them at the time of application, (2) walking over or through contaminated ground and vegetation, (3) eating contaminated vegetation, (4) grooming after direct or indirect contamination of fur, (5) eating other poisoned animals or food, (6) drinking contaminated water, and (7) inhaling insecticide particles.

The results of these contacts may range from immediate death to no effects. The intermediates include: (1) weakness with consequent susceptibility to to predation, disease, and parasitism, (2) storage within tissues with effects gradually becoming evident or effects evident on discharge of the tissue load within the body as in the case of stress-induced fat consumption, (3) passage of the poison through the placenta or milk with affects on young, (4) alteration of reproductive success, and (5) disruption of food chains with resultant changes in inter and intra-specific competition and predation (Jackson, 1952:259). No attempt was made in this study to evaluate these effects. Population changes detected are an expression of their combined action.

Determining the population size is the first step in understanding the impact of the population or its loss in the community. Mammalian population estimates are difficult to secure as indicated by Davis (1963). Reliable population models are not available and many assumptions necessary in a trap-retrap approach to estimates are invalid. These assumptions will be discussed in the section on population estimation. Patric (1958:17-21, 28) discussed at length factors which may contribute to sampling error. Some factors are briefly reviewed here with comments on the conditions of this study to allow proper evaluation of the results presented.

1. Presence of the research worker. Regrettably no wildland study can excape this factor and it must color in many ways ecological

observations. Since mammals are largely nocturnal, little contact-influence was believed exerted on them. Field workers wore drab clothes, refrained from loud talk, and attempted to be unobtrusive. Paths were followed for much of the field work, no cutting or other influences were exerted except those required in moving and placing sampling devices and in operating them. This is to say that no greater and probably less influence was placed on the area and it's fauna than in other similar studies and that this influence should be considered in constant proportion to the sampling effort in all small mammal studies where reasonable steps are taken to keep influence minimal. This variable of investigator-influence can and should be objectively studied later.

2. Inter- and intra-specific variation in capture rate. The shrews, for example, are difficult to capture. Adults and males are usually more frequently caught than young or females. Trapability of males and females varies with seasons of mating, nest building, and litter tending. In this study no attempt was made to account for variable individual mammal trapability. Populations of mammals estimated or determined are reported as expressions of trappable animals only. Hereinafter, references to mammal population size will be only to that portion of the population that can be sampled, i.e. trapped, and will be minimal. Only that portion of the population vulnerable to capture at least once is available for study.

3. Variations in trapping methods. Trapping methods were varied in the first year of study to determine most effective methods to be used in the critical second-year. Varying mammal response to

different baits clearly points up the need for constant sampling methods for valid population indices.

4. Seasonal and weather variations. There seems to be some correlation between sampling results and weather. Patric (1958:21) stated, "It seems most likely that small mammals, with their limited size and high metabolic rate, must pursue their specific habits regardless of normal weather variation." Getz (1961:169), however, reported that small mammal trap response to weather conditions does vary.

5. Inability to distinguish between resident and non-resident mammals.

6. Statistically inadequate samples.

7. Effects of handling individual animals. Patric (1958:17) noted that trapping arrests animal activity, causes excitement, and occasional injury. Some mammals in this study injured their faces in trying to escape the metal traps. All animals were toe-clipped or ear tagged so some injury occurred to all. Golley (1961:333) speculated that the imposition of trap stress on an already stressed adreno-pituitary system (as from an insecticide) may influence reproductive activity and survival and produce changes in population density.

> At least, an investigator studying the ecology of mammals by intensive live-trapping should consider the possibility that repeated handling may induce changes in the natality and mortality of the sample from which he draws conclusions about the entire population (Golley, 1961:333).

Trap stress was assumed approximately equal in both treatment and control area populations, obviating the need for further investigation of this factor in the study.

8. Removal of the sampling unit, i.e. the trap, by malfunction, escapes, extraneous springing, or bait loss. Twigs, acorns, rain and wind sprung some traps. In this study millipedes caused greatest loss of trap-nights in May and June, camel crickets, Rhaphidophorinae, replaced the millipede in this function in July, August, and September, 1961 and 1962. Ants were abundant but presented no real bait-removal problem as they have in many other small mammal trapping studies.

The Role of Home Range in Trap Spacing Selection

Home range is the area to which an animal commonly restricts its activities and over which it normally travels (Hayne, 1949:1 and Patric, 1958:157). One of the most thorough treatments of the subject of the role of home range in trap spacing selection is that of Calhoun and Casby (1958). They recognize the merit of Hayne's (1949) "center of activity" concept and his index of the relative frequency with which an animal is found per unit area at different radii from this center.

Home range studies were conducted in order to ascertain what species and proportion of individuals live their entire lives on the study area and are totally dependent upon the ecological conditions there. It seemed likely that a portion of the species present might spend part of their lives and perhaps the most sensitive stage, in another habitat, thus being much less closely associated with the treatment. Home range studies were also essential to meaningful evaluation of the results of the relatively wide-spread grid trap pattern finally selected. Hayne (1950:38) showed that a significant relationship exists between the relative position of traps to each other and population estimates and that there is no evidence of a difference between sexes in this relationship.

Murray (1957:451), Patric (1958:27), and others have evidence that traps block or restrict normal activities and home range utilization, though Stickel (1954:11) found that in a woodlot, traps did not block the travels of transients on the area. Traps seem to influence small mammal distribution and add bias to home range determinations and consequently to population estimates.

Stickel (1954:12) warned against the temptation to use data from a small number of animals from traps of wide spacing to calculate home range. "The more animals that are dropped out as a result of wide spacing, the more serious is the error of basing average home range calculations on the data from the few remaining." Estimates are smaller at close spacing, greater at widely spaced traps.

Home Range Studies in the Dover Watersheds

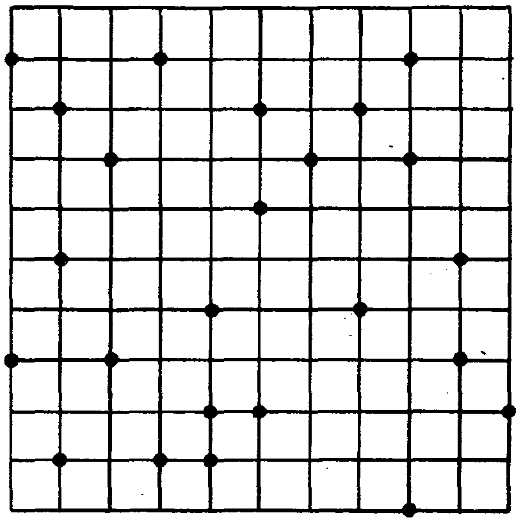
Besides the need for home range information to calculate population sizes and obtain density estimates, a brief study of home range was conducted to help evaluate the results of the extensive grid pattern selected (200 ft) for the final study.

An effort was made using a variety of grid, line, and random trapping methods to prevent the development of an animal-to-trap relationship described by Hayne (1950:34, 38) and others. Three methods used are presented diagramatically in Fig. 37. A fourth method, a standard grid with varying trap intervals, was used. The general appearance of the trapping area is shown in Fig. 38. Home range data available from the extended grid and random trapping within grid blocks was also studied.

Trap Spacing

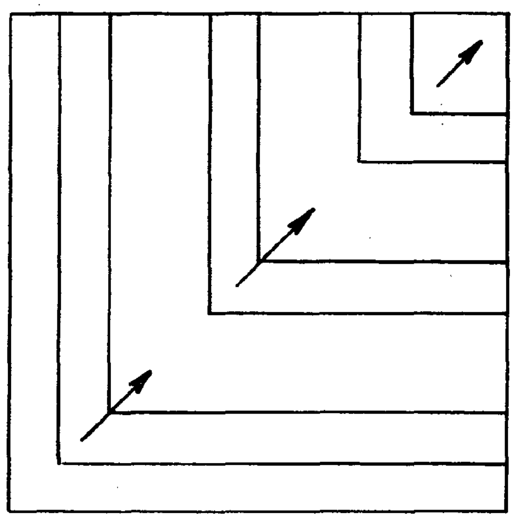
To study trap spacing and to reach a conclusion about an adequate number of traps per unit area is to study sample size. Several important factors have hindered the development of satisfactory mathematical models for small mammal populations. These factors include wide seasonal fluctuations in age and sex classes; mobile populations; complex interactions between and within populations, habitats, and land treatment; and lack of restriction in movements except in the case of islands.

These factors are largely responsible for the difficulty of sampling such populations for as Cochran (1953:1) pointed out: "when the material is far from uniform...the method by which the sample is obtained is critical, and the study of techniques that ensure a trustworthy sample becomes important." As in all sampling problems, the decision must be reached on desired precision of measurement. In assessing the gross effects of an application of malathion to an ecosystem, a precise measurement is needed. The more precise the measurement needs, the greater must be the sample. This study is designed to provide results for a conclusion on whether or not malathion effects woodland small mammal populations and to what



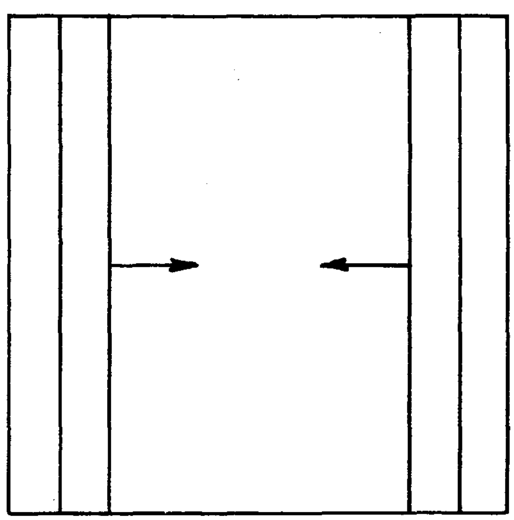
A

Random - 200 ft plot with 20 x 20 ft grid. 33 traps were randomly reset every night for 4 trap nights. 100 per cent of the area was covered every trap night.



B

Double V Lines - Area was swept with progressively smaller "V" trap lines. Trap spacing was 20 ft, distance between lines, 60 ft.



C

Bracketing Parallellines - Trap lines at 20 ft intervals overlapped at the center, then passed to the far side. Two 40 ft "steps" and one 20 ft step were used in 3 trap nights.

Fig. 37. Trapping patterns used in studying home range on plot no. 1 watershed no. 1, Dover, Ohio, study area, 1961.

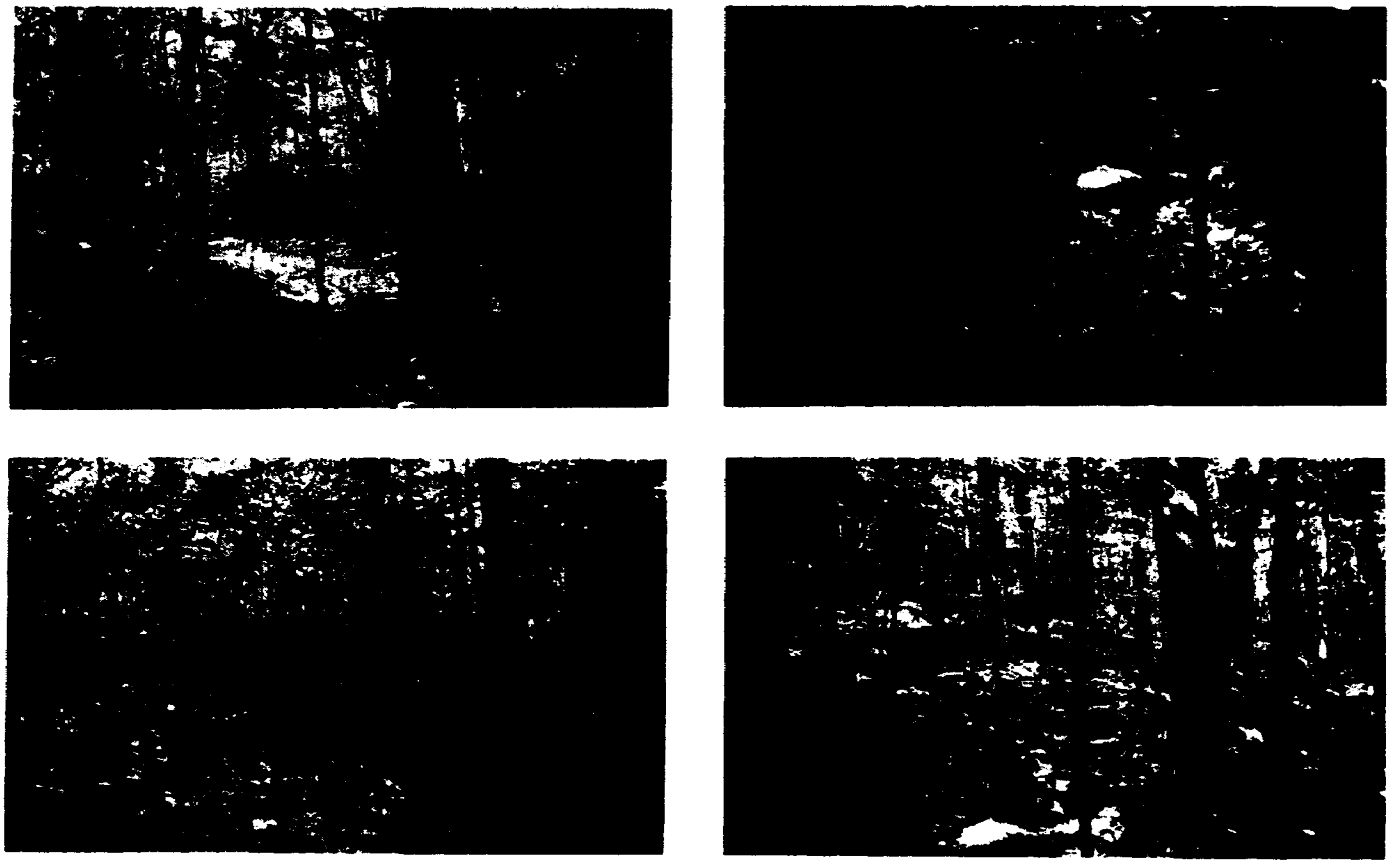


Fig. 38. Vegetation of plot no. 1, in Dover, Ohio, watershed no. 1 where intensive small mammal trapping was done in 1961.

extent results, if present, are evident. Based on needs and a study of the physical limitations to sampling intensity, the trap spacing recommended by Patric (1958:189) was adopted.

Patric recommended for sampling woodland small-mammal populations a trap line of "rosettes" of traps, each located 250 ft apart. This distance was based on available home range data and the desire to eliminate most trapping station interaction or competition for individuals. He provided evidence that traps closely spaced in a line (e.g. NACSM line) do not have equal probability of capturing an animal; those on the ends capture more. He explained that traps set 0.5 mile apart have equal chances of capture; traps 0.5 yard apart do not, since the end traps tend to "shield" the inner traps. His search was for a minimum spacing between these two extremes at which each trap had equal probability of capturing a small mammal. He called this interval of minimal spacing below which probability of capture would be unequal the critical trap interval (CTI) (Patric, 1958:160). He explained further that CTI is,

> the minimum spacing at which traps cease to compete for a small mammal with a given cruising radius. This interval is exactly the width of the theoretical home range (two times the cruising radius) because if the home range were slightly increased, two trapping stations would compete for the small mammal (Patric, 1958:163).

The rosettes of 6 to 10 traps which he proposed if beyond the CTI would provide an unbiased sample of the mammal population of the habitat in which it was placed since there are sufficient traps to provide a useful expression of abundance at each station and every trap at the rosette theoretically has an equal probability of capturing a small mammal (Patric, 1958:189).

A grid system of rosettes located 200 ft apart was selected as best meeting the needs of this study. See the map, Fig. 39. This system has the advantages of (1) allowing the maximum number of traps that can be checked once in 1 man-day (2) being an unbiased sample of each habitat (3) eliminating major trap-station interaction and competition (4) providing some home range information (5) meeting, for all practical purposes, the conditions of a random sample of the trappable mammals in the watersheds, and (6) allowing between and within-years and areas comparisons.

Following the recommendations of Patric (1958:188) it was found that a rosette of eight traps equally spaced in a 5 ft radius circle around each grid intersection trapping station was very desirable. See Fig. 40. In a single night the traps may catch five animals and one or more traps may be sprung without captures. Most other trapping programs, using one to three traps at a single location, restrict the trap response in that location after the available traps have been filled or sprung.

Since no more than six traps at a single station in this study have ever been sprung in 1 trap-night, it appears this is the minimum number of traps in each rosette needed to provide an unbiased sample of the trappable population at any one location.

Evaluation of the selected grid approach was attempted by the use of randomly located rosettes within each grid block. This amounted to proportional stratified random sampling of the study area. Random locations were selected by choosing from a table of random numbers coordinates (in ft) from a given corner of each block.

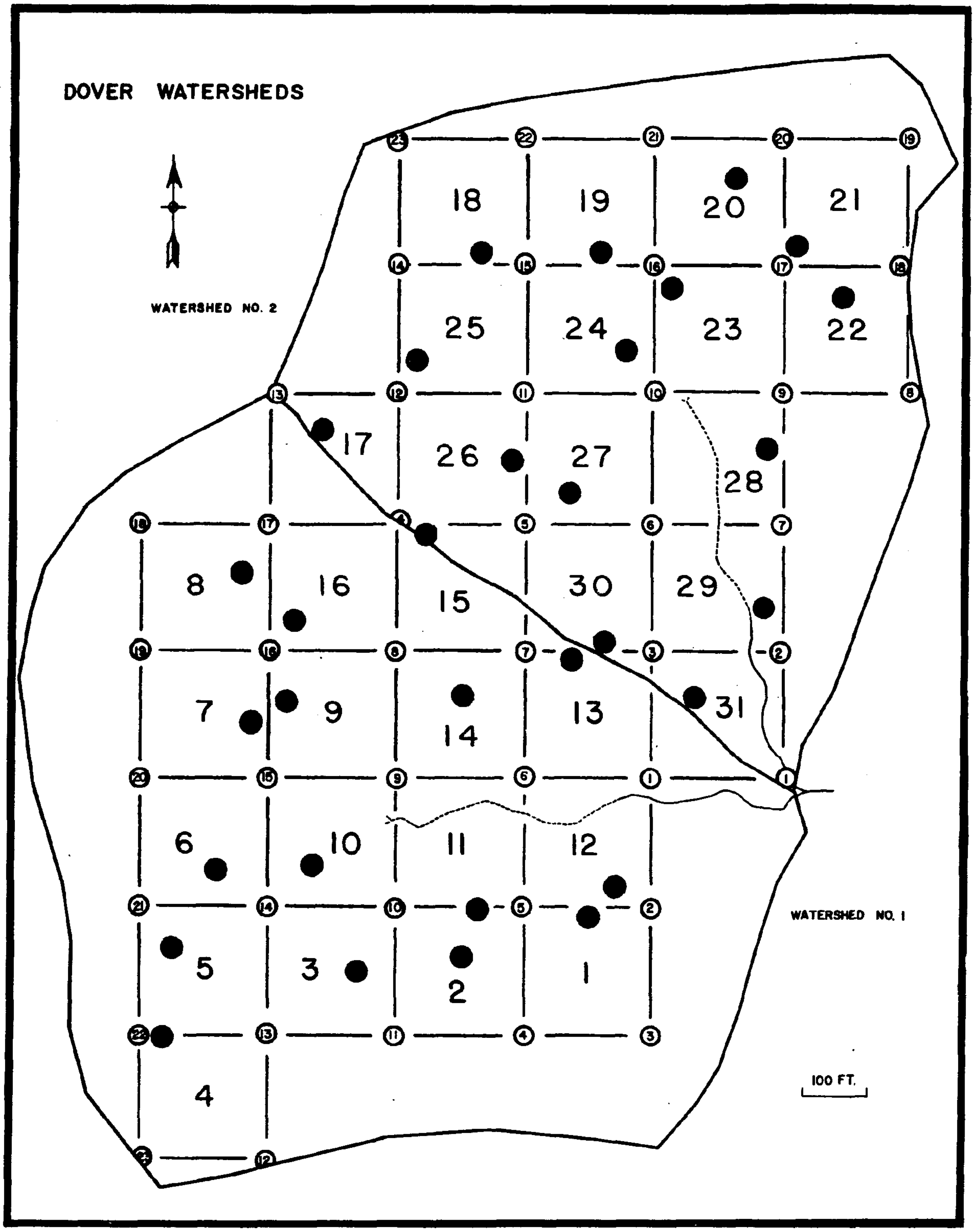


Fig. 39. Location of the B-1 series of random trapping stations used in 1961 on the Dover, Ohio,watersheds in connection with two other series, and used exclusively as random trap sites in 1962. Blocks between standard 200 ft grid markers are numbered for designating animal trapping locations.

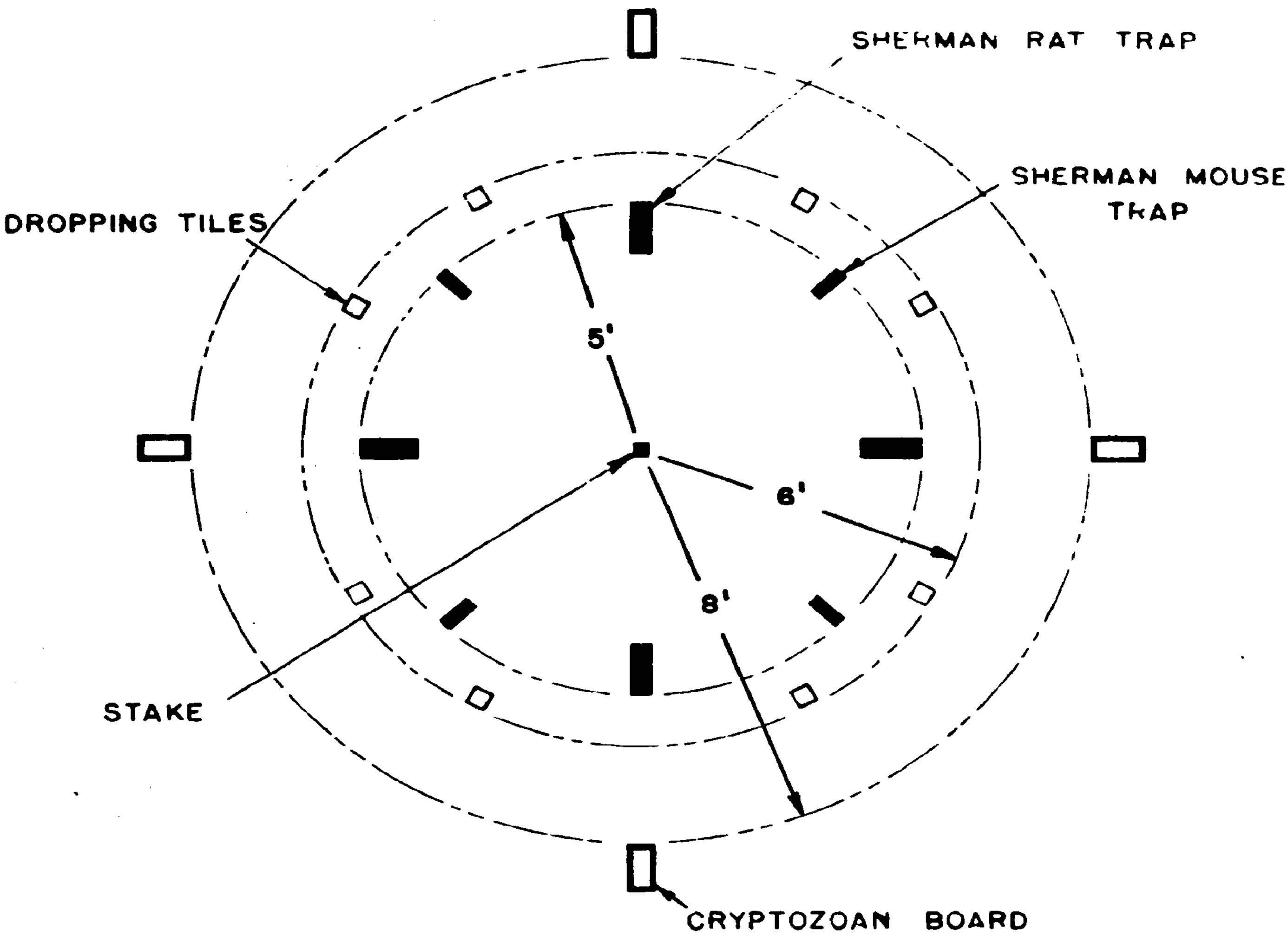


Fig. 40. Sampling units used around each of the 46 grid stakes in the two watersheds of the Dover, Ohio, study area in 1961 and 1962. In addition, one or two cat traps were placed within 4.6 m (15 ft) of the stake.

See Fig. 39. It appeared adequate that trap location be accomplished by compass and pacing. Chaining of distances seemed tootime consuming for the accuracy that was required. The trapping pattern essentially avoided animals whose home ranges overlapped the edges of the watersheds.

Four traps, two large and two small, were set alternately in a 4 ft circle around the selected random sampling point with doors pointing in one direction to make checking easier.

Initially, random placement of trapping sites was considered but rejected. The restrictions on placing a previously determined number of trap sites imposed by adhering to sampling strata such as elevation and aspect within each watershed and a minimum of 200 ft between trap sites were so rigid as to make the trap pattern essentially a grid. Since (1) a point of origin for the grid pattern was chosen without prior knowledge of the area (essentially at random), (2) for all practical purposes the gridded sites are within a few feet of where they would have been had they been located at random, and (3) the time and cost of placing and operating randomly-placed traps seemed prohibitive, the grid system was selected. It is believed to approach the conditions of a stratified random sample.

Trapping Methods and Field Procedure

Traps

Mammals ranging in size from shrews to raccoons were studied. The only other larger wild mammal, the white-tailed deer, occurred on the study area occasionally and effects on it, if any, would be unmeasurable. Two sizes of Sherman galvanized live traps were used, 2 x 2.5 x 7 in. and 3 x 3 x 10 in. Sealander and James (1958) found this trap the most effective of those studied. It has the advantage, besides effectiveness, of being commercially available (H. J. Spencer and Sons, Gainesville, Florida) and being relatively easily transported into remote areas. The larger Sherman trap is more costly but can trap mammals up to the size of a chipmunk. Some chipmunks were caught in smaller traps but were tightly confined and often suffered from "shock".

A third size mammal trap was used. It was described briefly and pictured by Allen (1943:80) and is known as the Biological Survey cat trap (see Fig. 41). It will be referred to as the "cat trap" hereinafter. The trap described by Linehan and Llewellyn (1957) would have been more satisfactory and should be used in similar future studies. Welded 0.5 in. woven wire should be used to cover either trap. Raccoon and woodchuck can escape from standard 0.5 in. hardware cloth though the latter is satisfactory for squirrels, weasel, chipmunks, and rabbits. The cost of repairing damaged wire, avoiding animal losses, and general trap adaptability justify the additional initial expense of welded wire. Twenty additional heavier traps were obtained early in 1962. They were evenly distributed throughout the area. A hardware cloth bait holder projecting into the trap from the rear reduced bait loss and trap closure due to under-the-trap activity of mammals to secure bait without entering. A piece of screen wire was placed in the bottom of the trap on top of the hardware cloth to stop weasel predation on captured chipmunks and rabbits through the

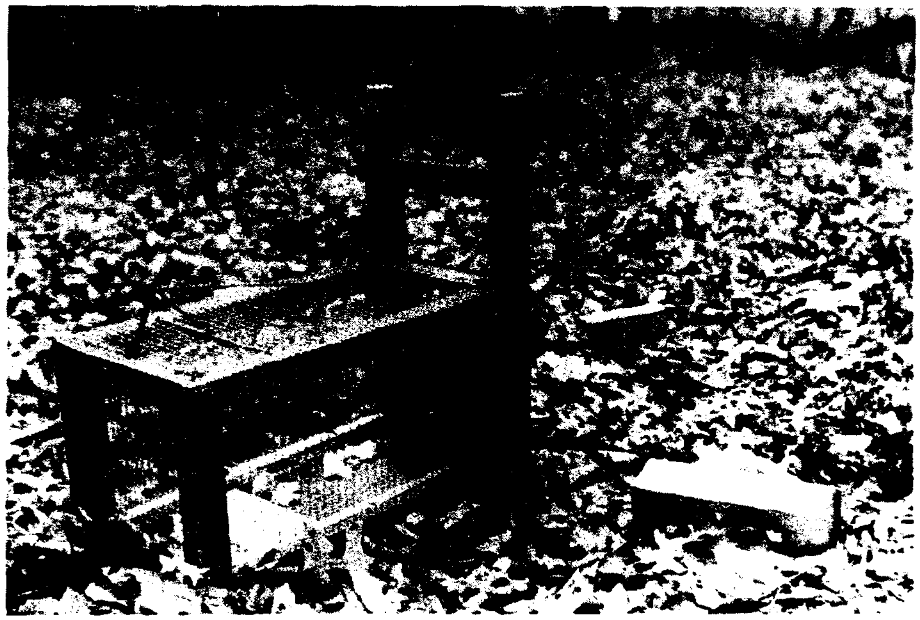


Fig. 41. The Biological Survey "cat trap" was used to capture large animals on the Dover, Ohio, watersheds in 1961 and 1962. A trapped cottontail rabbit is shown here. A Sherman rat trap is in the right front, a Sherman mouse trap right of center.

0.5 in. wire mesh. Infrequent capture of opossum and raccoons required replacement of this wire which was loosely wedged, unfastened, to the bottom. The closing door of the large Sherman traps occasionally stripped the skin from or snipped the tip of the tail off escaping chipmunks. Two escaping chipmunks were killed by the falling door of the cat trap.

In general it may be said that the traps used were effective in capturing animals. Their effectiveness depended not so much on skill of setting them or the sensitiveness of their trigger mechanisms but on the baits used. In this study, it was desirable that in the traps there be not only an attractant as part of the bait but also a survival food since animals may remain in a trap for 24 hr. Corn seemed to be best for the latter purpose.

From 15 May to 18 May 1961, 1072 trap-nights were accumulated. Bait was a mixture of equal parts crunchy peanut butter and rolled oats. The traps took 40 animals of 3 species. From 22 May to 25 May 1076 trap-nights took 87 animals of 4 species. The change in the trapping success was attributed to a new bait mixture of 2.5 parts (by volume) peanut butter, 4 parts rolled oats, 2 parts cracked corn, 2 parts seedless raisins, and 0.5 part bacon grease.

Whole corn grains placed in the traps for "survival food" frequently became wedged under the treadle and resulted in loss of trap-nights. Cracked corn mixed in the peanut butter seemed to serve the same purpose and reduced this problem.

During 10 to 20 October 1961 a 1 acre plot was trapped within 2-16, 17, 20, and 21 using a 6.1 m (20 ft) grid with alternate traps containing the standard bait mixture, and the mixture to which had been added 4 drops of oil of anise per liter of mixture. There were 40 captures in the 806 trap-nights. An F test indicates there is no significant difference between the capture of the two baits (F was 1.81; F at 0.05 probability for 4 d.f. is 6.39).

Just prior to the spray application in 1962 limited studies were conducted with artificial flavoring of a standard peanut butter bait mixture. It was found that 0.25 teaspoon of pure almond extract (oil of bitter almonds) added to 2 cups of standard bait mixture was most effective.

In the late spring and summer, baits became covered with a heavy growth of mold. Only during the first week were baits totally collected and replaced. Replacement of baits after this was done periodically as they became dry, excessively molded, or depleted by insects. Old baits were not collected but discarded within the vicinity of the trap. No attempt was made at prebaiting to overcome trap shyness (Leslie _et al_., 1953:142).

When only rodents were caught using ear and whole-kernel corn, in the cat traps, additional bait of Purina Fox Chow Checkers (Ralston Purina Co., St. Louis, Missouri) was used in each trap. Sanderson (1961:21) found that of the baits he tested, this was the most effective in capturing opossum.

In an effort to further test the influence of baiting on the capture of the larger mammals, a scent mixture was prepared by leaving a jar of 50 small fish and a dry beaver castor in the forest to decompose for a week. The fluids were poured onto a piece of cotton which was placed at the back of each cat trap with an ear of corn, during the 24 July 1962 trap period. Comparing the catch of the last previous trap week (17 July 1962) using corn only there appears no significant difference between captures except that a weasel, the only one in 1962, was caught on the fish scent bait. Chipmunk captures were reduced 52%.

Baits are extremely important variables in the measure of small mammal populations. Much research is yet needed in this one aspect of mammal sampling technique. Another question, yet unanswerable is, what is the influence on rodents of the annual addition of 4 bushels of ear corn and 6 quarts of peanut butter bait, quality food, to a 40 acre area?

Field Procedure

The traps were examined in the same order every day as shown in Fig. 42.

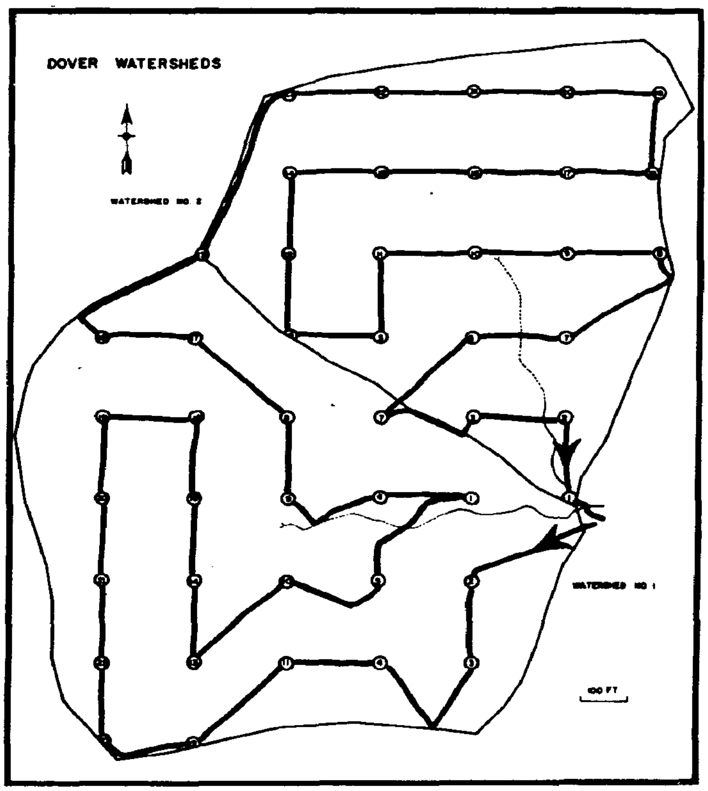


Fig. 42. Route normally taken for trapping and observations at permanent grid sites and reptile traps on the Dover, Ohio, watersheds, 1961 and 1962.

All observations were made by the writer except during the latter part of 1962 when Mr. Dilworth checked traps. Equipment carried was: bait, trap cover, 3 sizes of holding funnels, extra trap springs, pliers, thermometer, pencils, data cards, scissors, forceps, tags and tagging pliers, antiseptic, anaesthesia and hypodermic kit, 15 lb spring scales and lunch. Most items were carried in a home-make canvas vest with ample fitted pockets.

All closed Sherman traps were cautiously examined. Those empty were reset, rebaited if needed, and replaced. Those containing mice or shrews were emptied into a white 15 x 10 in. collecting bag. They were confined by the right hand then removed by grasping their skin behind the neck with two fingers. Chipmunks were occasionally handled in this manner but with some hazard of their biting the observer.

Chipmunks and other mammals caught in the cat traps were removed by placing a burlap bag over the cage top and sides. The cloth adapter to a wire holding funnel (Fig. 43) was placed over the closed door. When the door was raised and held open with the same hand holding the adapter, the other hand repulsed the animal from the trap into the funnel. The adapter was wedged into the funnel to hold the animal motionless as it was examined and either tagged or toe-clipped. Rabbits were removed by hand from the trap or dropped into a burlap bag for handling. Hardware cloth (0.5 in. mesh) was used for the holding funnel for chipmunks; 1 in. chicken wire was used for funnels for opossum, squirrels, and raccoons.

Mice and chipmunks were numbered by toe-clipping one or two toes.

Fig. 43. Chipmunks, flying squirrels, and weasels were handled in a wire funnel. The large white cloth, sewed to the funnel is an adapter for the opening of the traps. The cloth was forced in behind the animal or a trapping bag was used as shown here. Tagging, sexing, and weighing was done with the animal in the funnel. The numbered stake is grid location 1-23, Dover, Ohio.

Fore-toes represented units, 1-8; hind toes, tens, 10-100 (Baumgartner, 1940). By clipping only two toes, only one on each foot, and omitting all 9's, 131 different individuals could be marked. Dog-nail trimmers (Resco Co., Detroit 27, Michigan) were tried during the first week of trapping but did not provide as rapid and clean a cut as sharp, pointed, moderate weight scissors. "Negastat" a topical veterinary styptic, bactericidal, fungicidal, and protozoacidal solution supplied by Corn States Laboratories Inc., Omaha, Nebraska, was used on all toes clipped. The non-breakable plastic 4-oz bottles were handy for field use. The only disadvantage found with its use was that it was corrosive to clothing and equipment. This presented no problem when the bottle was capped tightly and the liquid used with care.

One monel metal tag (National Band and Tag Co., 721 York St., Newport, Ky.) Style 4-1005, Size 1, was used through the center base of the right ear of chipmunks, fore-edge of the right ear of weasels, rabbits and opposums. Style 4-1005, Size 3 tags were used on groundhogs and raccoons. Forceps were used to hold chipmunk ears through the funnel for tagging. Several chipmunks are known to have lost tags as evidenced from circular ear scars.

Myotol anesthesia (pentobarbital sodium with Sinan produced by Warren-Teed Products Co., Columbus 8, Ohio) was carried for use with skunks but none were trapped.

Traps were set sensitively but firmly enough to prevent frequent closure without captures. A trap-night normally consisted of a 24 hr period of availability to capture or hold an animal. Since traps were always checked in the same order, the first traps were operated from approximately 8 am to 8 am; those at the end of the line, 4 pm to 4 pm. Traps not counted in the total trap nights included those sprung by wind, leaves, insects, millipedes, escaping animals, those overturned, and those having an object wedged under the treadle making them inoperable.

Mammal Activity

Hole Lines

Four 300 ft long pieces of 16 ga. wire were staked across the north and south facing slope of each watershed. The one on the southfacing slope of watershed no. 1 was near 1-16 and 1-19; north slope, 1-2 and 1-4; in watershed no. 2 on the south slope, 2-7 and 2-8; north slope, 2-5 and 2-11. A board 0.44 m (1.45 ft) long was moved along the line to describe an area 0.01 acre in size in which all mammal holes were tabulated in four sizes. Inspection of the four lines took 35 min. The results are in Table 58. There appears to be a decrease of about 150% in mammal burrowing activity within the area treated with insecticide. Persistence of holes, nature and amount of leaf covering, and different observers produce crude data that have only general value.

Bait Lines

Along these same lines in 1962 were placed, every 20 ft, a plastic tile on which was placed a piece of corn and a fox checker. These lines were checked daily and corn and checkers that were moved or removed were tabulated. From the results in Fig. 44 it seems that regardless of the method of grouping the data, mammal activity on

Table 58. - Animal activity as measured by easily visible ground holes in four, 300 x 1.5 ft, 0.01 acre quadrats on the Dover, Ohio, watersheds, 1961-62.

	Diameter of hole in inches									Total		
	Less than 1			1 to 3			3 to 5					
Date (month-day-year)	6-28-61	5-4-62	7-24-62	6-28-61	5-4-62	7-24-62	6-28-61	5-4-62	7-24-62	6-28-61	5-4-62	7-24-62
Watershed 1												
North Slope	8	2	1	5	1	8	0	0	1	13	3	13
South Slope	13	1	4	18	2	7	0	0	0	31	3	11
Total	21	3	8	23	3	15	0	0	1	44	6	24
Watershed 2												
North Slope	4	6	4	16	4	5	0	0	0	20	10	9
South Slope	12	2	3	17	4	7	2	0	0	31	6	10
Total	16	8	7	33	8	12	2	0	0	51	16	19

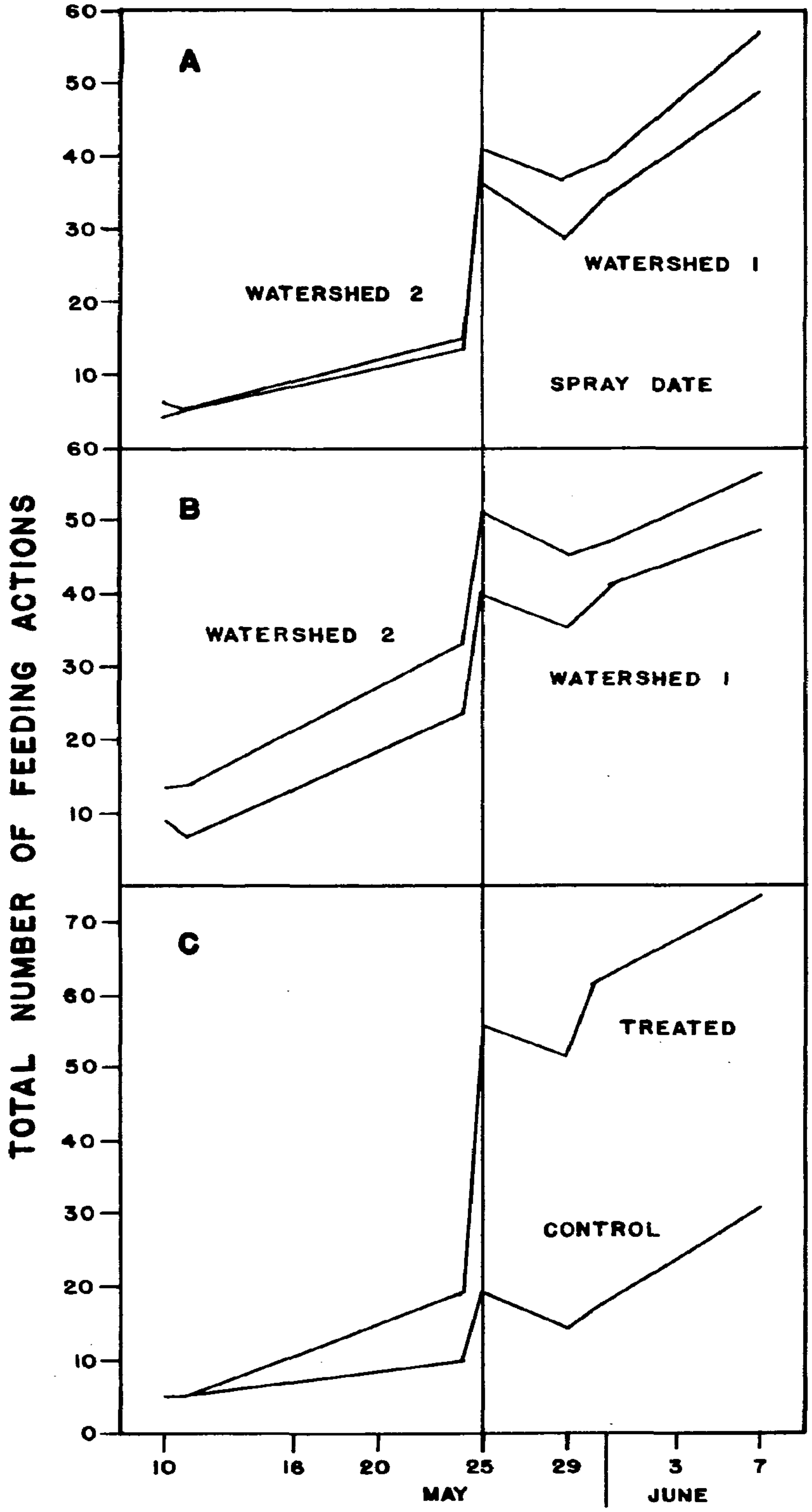


Fig. 44. Mammal activity as measured by corn and checkers eaten and moved from the center of 16 tiles along each of two transects in each Dover, Ohio, watershed, 1962. A - Corn and checkers totally removed, B - Corn and checkers removed or moved from the center of tiles, C - Corn and checkers totally removed from the three lines in the treated watershed and the contaminated north facing slope of the control area.

watershed no. 1 and the total area receiving quantities of insecticide (watershed no. 1 plus the north facing slope of watershed no. 2) differed significantly from the untreated area. Increase in feeding activity on the lines parallel high insect losses immediately post spray. General increase in feeding may be a conditioned behavioral response to the available food or a function of seasonal change.

Cryptozoan Boards

Counts were made of mammal holes under four cryptozoan boards (1600 cm^2) at each grid stake in 1962. There was no change in activity between watersheds or between the area contaminated and the south facing slope of watershed no. 2.

Dropping Tiles

In another attempt to obtain an index of mammal abundance for correlation with and possible verification of the trap-retrap data, dropping tiles were used (Tester and Emlen, 1960). Eight no. 82 beige polystyrene plastic wall tiles 11.4 x 11.4 cm (4.5 x 4.5 in.) of the Star Line, Meridian Brand were placed around each grid intersection as shown in Fig. 40. These were observed almost every day of the 1961 trapping and until 8 May in 1962. On the 368 tiles only two defecations by mice or chipmunks were seen during the entire study. Tiles (20) were moved to the field edge and examined every day for 4 days. No droppings were found. The method, successful for other workers, was useless in this study. Peromyscus leucopus may not be responsive to such areas. The tiles were not baited. The tiles were removed and some were used along the previously described bait lines.

Shrews

Six smoky shrews[1], were captured in 1961, four in 1962. Only one was captured alive in 1962. Four were trapped in watershed no. 1, six in watershed no. 2. During the night of 30 May 1961 a short-tailed and a smoky shrew were captured in the same small Sherman trap. Both were dead when found the next morning. There was no sign of fighting.

The captures of short-tailed shrews are shown in Table 59 and 60 along with a list of all mammal trap nights. The date represents the day on which the traps were checked.

Of 156 Blarina trapped, 97 or 62.2% were found dead in the traps. Because of this high trap loss and inability to return marked animals to the population, no reliable population estimate or index was obtained for this species. Table 59 presents the shrews captured per 100 trap nights. The trapping success is evidently low and very erratic. Shrews show bait preference. In 1961 after 1072 trap nights using peanut butter and oatmeal, no shrews had been captured. The next trapping period (1075 trap nights) when the bait was changed (as reported under the section on baits) to include bacon grease, 13 shrews were caught. Seven out of nine captured Blarina were captured with oil of anise flavored bait during a baiting experiment (10 October 1961) in watershed no. 2 (Table 59). On the multiple-flavor experiment, slight preference was shown anise bait, although the sample size is too small for a conclusion.

All of the shrews captured on the base grid were trapped at only

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[1]Scientific names are listed in the Appendix Table 2.

Table 59. - Summary of types and intensity of trapping programs conducted in 1961 and 1962 in the Dover, Ohio, watersheds and shrew captures with Sherman traps.

Type of trapping program	Date: Month-day-year	Sherman trap nights Watershed 1	Sherman trap nights Watershed 2	Sherman trap nights Total	Cat trap nights Watershed 1	Cat trap nights Watershed 2	Cat trap nights Total	Total All trap nights	Shrews Watershed 2 Dead	Shrews Watershed 2 Alive	Shrews Watershed 1 Dead	Shrews Watershed 1 Alive	Shrews Total	Shrews per 100 Sherman trap nights
Standard grid-peanut butter and oats	5-16-61	171	175	346	23	23	46	392						
	5-17-61	181	183	364	23	23	46	410						
	5-18-61	182	180	362	23	23	46	408						
Standard grid-complete bait	5-23-61	181	179	360	23	19	42	402		2	2	1	5	0.0139
	5-24-61	182	173	355	23	23	46	401	3		1	1	5	0.0141
	5-25-61	179	181	360	23	22	45	405	1	1		1	3	0.0083
Standard grid-complete bait	5-30-61	172	178	350	22	23	45	395			1	1	2	0.0057
	5-31-61	176	180	356	19	21	40	396	1		2		3	0.0084
Cat traps only-corn and lead-in bait	6-1-61				22	21	43	43						
Cat traps only-standard grid with lead-in bait	6-6-61				22	20	42	42						
	6-7-61				21	22	43	43						
	6-8-61				21	23	44	44						
Random-within 30 blocks (exclusive of no. 3) - 4 traps/station 1st coordinates	6-13-61			75				75						
	6-14-61			104				104		1	1		2	0.0192
	6-15-61			109				109		1			1	0.0091
	6-16-61			37				37	2		1		3	0.0811
Random trapping plot no. 1	6-16-61			57				57			1		1	0.0175

Table 59. - Continued.

Type of trapping program	Date: Month-day-year	Sherman trap nights Watershed 1	2	Total	Cat trap nights Watershed 1	2	Total	Total All trap nights	Shrews Watershed 2 Dead	Alive	Watershed 1 Dead	Alive	Total	Shrews per 100 Sherman trap nights
Random trapping plot no. 1 (cont.)	6-16-61			55				55				1	1	0.0182
	6-17-61			55				55				1	1	0.0182
Double-V sweep on plot no. 1	6-20-61			73				73			3		3	0.0411
	6-21-61			54				54						
	6-22-61			45				45						
	6-23-61			29				29			1		1	0.0345
	6-24-61			13				13						
Bracketing Parallel lines on plot no. 1 and cat traps only. Bait corn & checkers	6-27-61			78	17	17	34	112						
	6-28-61			73	22	22	44	117				1	1	0.0137
	6-29-61				21	19	40	40						
	7-1-61			65				65						0.0154
Cat traps-checkers only. Plot no. 1-1st 3 rows top 2 4 cat traps 32 Sherman in W.S. no. 2 for Ecology class	7-11-61			65	16	16	32	32						
	7-18-61			45	0	4	4	49			2	1	3	0.0167
Plot no. 1 misc. trapping 4 standards in W.S. no. 2 for Ecology class	7-26-61			52	0	4	4	56						
Plot no. 1 - 200' interval	8-1-61			8				8						
	8-2-61			6				6						
	8-3-61			4				4						

Table 59. - Continued.

Type of trapping program	Date Month-day-year	Sherman trap nights Watershed 1	2	Total	Cat trap nights Watershed 1	2	Total	Total All trap nights	Shrews Watershed 2 Dead	Alive	Watershed 1 Dead	Alive	Total	Shrews per 100 Sherman trap nights
Random trapping within 30 blocks, 2nd. series of random numbers	8-1-61			41				41						
	8-2-61			111				111						
	8-3-61			114				114	1	1			2	0.0175
	8-4-61			73				73						
Plot no. 1 - 100' interval	8-4-61			11				11		1			1	0.0909
	8-5-61			16				16		1			1	0.0625
Random trapping within 30 blocks-<u>3rd</u> series of random numbers	8-8-61			109				109		3		1	4	0.0367
	8-9-61													
	8-10-61			107				107						
	8-11-61			114				114						
	8-12-61			111				111		1			1	0.0090
Plot no. 1 - 50' interval	8-8-61			48				48	1				1	0.0208
	8-9-61			45				45	1				1	0.0222
	8-10-61			42				42		1			1	0.0238
Plot no. 1 - 40' interval	8-20-61			66				66						
	8-21-61			69				69						
	8-22-61			66				66				1	1	0.0152
Plot no. 1 - 20' interval	8-23-61			220				220			2	1	3	0.0134
	8-24-61			236				236						
	8-25-61			226				226						
Sunken traps-plot no. 3 anise standard bait	10-10-61			116				116						
	10-11-61			111				111						

Table 59. - Continued.

Type of trapping program	Date Month-day-year	Sherman trap nights Watershed 1	2	Total	Cat trap nights Watershed 1	2	Total	Total All trap nights	Shrews Watershed 2 Dead	Alive	Watershed 1 Dead	Alive	Total	Shrews per 100 Sherman trap nights
Sunken traps-plot no. 3 (cont.)	10-12-61			121				121	3	1			4	0.0331
Cat traps only-standard grid, corn checkers	10-31-61				21	18	39							
	11-1-61				22	21	43							
	10-17-61			113				113	1				1	0.0088
	10-18-61			117				117	2	1			3	0.0256
	10-19-61			117				117	1				1	0.0085
Top-of-ground traps	10-20-61			111				111						
Cat traps-standard grid-checkers only	4-18-62				22	22	44	44						
	4-19-62				22	23	45	45						
	4-20-62				18	19	37	37						
Bait preference studies 6 flavors 2 standard	5-1-62	129	38	167	22	0	22	189			1		1	0.0050
	5-2-62	180	160	340	38	33	71	411						
	5-3-62	175	174	349	37	40	77	426	1	1	1		3	0.0086
	5-4-62	177	176	353	38	39	77	430						
	5-5-62	8	164	172	2	38	40	212						
Standard grid-standard trap program	5-8-62	169	169	338	38	37	75	413						
	5-9-62	180	179	359	37	39	76	435						
	5-10-62	184	182	366	38	40	78	444						
	5-11-62	171	165	336	32	34	66	402						

Table 59. - Continued.

Type of trapping program	Date: Month-day-year	Sherman trap nights Watershed 1	Sherman trap nights Watershed 2	Sherman trap nights Total	Cat trap nights Watershed 1	Cat trap nights Watershed 2	Cat trap nights Total	Total All trap nights	Shrews Watershed 2 Dead	Shrews Watershed 2 Alive	Shrews Watershed 1 Dead	Shrews Watershed 1 Alive	Shrews Total	Shrews per 100 Sherman trap nights
Standard	5-13-62	168	172	340	35	38	73	413						
	5-24-62	174	164	338	30	0	30	368	2			1	3	0.0089
	5-27-62	163	170	333	32	38	70	403	3		1		4	0.0120
	5-28-62	112	173	335	31	39	70	405	2	1	1	2	6	0.0179
	5-29-62	170	177	347	35	33	68	415	2	1	2	1	6	0.0173
	6-5-62	160	154	314	36	29	65	379	4		3	1	8	0.0255
	6-6-62	155	172	327	33	37	70	397	1		1	1	3	0.0092
	6-7-62	171	170	341	35	38	73	414	2	1		1	4	0.0017
	6-19-62	169	166	335	33	32	65	400	1	1	1		3	0.0090
	6-20-62	177	172	349	38	34	72	421	2	1			3	0.0086
	6-21-62	176	172	348	38	36	74	422	1	2	1		4	0.0115
Sherman traps only, standard grid & traps/ station	7-3-62	150	156	306				306	10	4	7	3	24	0.0784
	7-4-62	152	169	321				321	3			1	4	0.0125
	7-5-62	143	172	315				315	2	1			3	0.0095
Random trapping within 31 blocks 4 traps/station	7-6-62			101				101						
	7-7-62			98				98		1			1	0.0102
	7-8-62			106				106	2				2	0.0189
Standard grid	7-10-62	80	86	166				166	1		1		2	0.0120
Sherman traps only 4/station	7-11-62	75	76	151				151	1	1	1		3	0.0199
	7-12-62	68	79	147				147	1	1			2	0.0136

Table 59. - Continued.

Type of trapping program	Date: Month-day-year	Sherman trap nights Watershed 1	Sherman trap nights Watershed 2	Sherman trap nights Total	Cat trap nights Watershed 1	Cat trap nights Watershed 2	Cat trap nights Total	Total All trap nights	Shrews Watershed 2 Dead	Shrews Watershed 2 Alive	Shrews Watershed 1 Dead	Shrews Watershed 1 Alive	Shrews Total	Shrews per 100 Sherman trap nights
Random trapping within 31 blocks 4 traps/station	7-13-62			105				105		1			1	0.0095
	7-14-62			100				100		1			1	0.0100
	7-15-62			98				98	1	1			2	0.0204
Cat traps-only corn for bait	7-17-62				36	34	70	70						
	7-18-62				37	36	73	73						
	7-19-62				35	37	72	72						
Cat traps only corn & fish beaver scent only	7-24-62				37	5	42	42						
	7-25-62				30	10	40	40						
	7-26-62				27	11	38	38						
Cat traps only-corn & fish fish scent	8-14-62	85	88	173	35	36	71	244						
	8-15-62	90	92	182	30	34	64	246	1	1			2	0.0109
	8-16-62	68	90	158	34	38	72	230						

Table 60. - Summary of captures of short-tailed shrews at various locations throughout the two Dover, Ohio, watersheds.

	Watershed 1					Watershed 2					Total	
	Dead		Alive			Dead		Alive				
Location	1961	1962	1961	1962	Total	1961	1962	1961	1962	Total	1961	1962
1					0		1			1	0	1
2		3		1	4		1		1	2	0	6
3	2				2	1				1	3	0
4		1			1				1	1	0	2
5		1			1					0	0	1
6	1	2			3		3			3	1	5
7					0		3	1	4	8	1	7
8	1	1		1	3		6		2	8	1	10
9					0		6		1	7	0	7
10				1	1		2			2	0	3
11					0		1			1	0	1
12		5		4	9			1	1	2	1	10
13		4		2	6	2	1			3	2	7
14					0		3			3	0	3
15					0		6		1	7	0	7
16					0		1			1	0	1
17		1			1		1		1	2	0	3
18	2		5		7					0	7	0
19					0	2		1	2	5	3	2
20					0					0	0	0
21					0				1	1	0	1
22		1		1	2		1		1	2	0	4
23	1	2		1	4		2		1	3	1	6
Total	7	21	5	11	44	5	38	3	17	63	20	87

33 of the 46 sampling points (Table 60). More than three shrews were captured at only 10 points. Table 61 summarizes the comparisons of mean numbers of shrews trapped within various environments. F tests of differences between the means of these groups indicate none are significantly different from the others.

Table 61. - Comparison of the means of the total number of Blarina shrews trapped at each Dover, Ohio, trap site, 1961 and 1962, having common environmental factors.

Description	n	mean	variance
North-facing slope	19	2.5	5.9
South-facing slope	14	2.2	7.4
Light grayish brown soil	2	2.3	14.9
Grayish brown soil	15	2.6	5.8
Very dark grayish brown soil	11	2.8	8.6
Very dark gray soil	5	1.2	2.4
Silt loam	14	2.4	4.9
Sandy loam	18	2.1	7.3
Silty clay loam	1	6.0	0

Species interaction at the 46 sampling points was investigated by multiple regression. Where

$$y = a + b_1x_1 + b_2x_2$$

and y = the number of individual shrews trapped at each location in 1961 and 1962,

a = the y axis intercept of the regression line,

b_1 = the partial regression coefficient of x_1,

b_2 = the partial regression coefficient of x_2,

x_1 = the number of individual white-footed mice trapped at each location in 1961 and 1962, and

x_2 = the number of individual chipmunks trapped at each location in 1961 and 1962, then

$$y = 0.08 + 0.52\ x_1 + 0.03\ x_2.$$

Tests of significance of the b's indicate only the mice were significantly correlated with the shrews. Recalculation of a simple linear regression produced

$$y = 0.45 + 0.50\ x_1$$

This positive correlation of mice and shrew populations, also seen in Table 62, may be explained by animal interaction or by similar responses of two mammals to environmental factors.

Table 62. - A correlation coefficient matrix for the number of individual shrews, mice, and chipmunks trapped at each trap site of the Dover, Ohio, watersheds in 1961 and 1962.

	Shrews	Mice	Chipmunks
Shrews	1.00	0.41	-0.04
Mice		1.00	-0.02
Chipmunks			1.00

Investigation of some measured environmental factors at each of the 46 trapping points by multiple regression analysis produced Appendix Table 3. Essentially the questions asked were: does y depend on x, if so, what is the best measure of this relationship, and can y be predicted from x? Absence of parallel correlations between shrews and mice as influenced by various factors suggests that the above shrew-mouse relationship is social interaction and not the response of the two species to similar environmental factors.

Table 63 was examined to try to locate the observed changes in mammal populations which occurred after treatment. The regression of y on 9 x-variables of the environment produced partial regression coefficients that rarely have statistical significance. Those that do, do not follow patterns between years that can be readily explained.

Of all mammals within the study area shrews should show effects from the insecticide. Their high metabolism, insectivorous feeding, and large food consumption would seem to make them highly subject to direct or secondary insecticide effects. The erratic capture per 100 trap nights, provides no evidence of such effects.

An investigation of the proportion of dead animals discovered in traps, Table 64, indicates that the number of dead animals in the traps after treatment was not significantly different than before treatment. ($\chi^2 = 0.239$ comparing the "expected" post-treatment 30 May 1961 with the "observed" post-treatment 25 May 1962).

On 31 July 1962, 20 sunken-can traps were placed 20 ft apart in a cross pattern on plot no. 1 within watershed no. 1 and left until 3 August 1962. They were checked daily but no shrews were captured. On 6 August the cans were baited with solidified ground beef, earthworms, and beef suet. Two Sorex were captured in the same can on the first trap night but none were captured before the cans were taken up on 10 August 1962. MacLeod and Lethiecq (1963) reported Sorex could jump out of 6 to 9 in. tall sunken can traps. The technique, used successfully by others for study of shrews, was unsuccessful. It has

Table 63. - Partial regression coefficients for multiple regressions of mammal observations on nine environmental factors. Level of significance: (*** = 10% ** = 20% * = 30%).

Mammal observations (y)	b_0	Elevation b_1	pH b_2	Organic Matter b_3	Phosphorus b_4	Potassium b_5	Distance from water b_6	Cover Density Rating: Tree b_7	Cover Density Rating: Shrub b_8	Cover Density Rating: Herb b_9
Total number of <u>Blarina</u> shrews (dead and alive) trapped in 1961-62 before and after spray application at each location.	4.050	-0.001	-0.110	-0.026	* 0.013	*** -0.013	0.004	** 0.464	0.208	-0.124
Number of individual mice trapped at each location in 1961	** 3.267	*** -0.020	0.109	0.112	-0.003	0.003	*** 0.006	* -0.197	** 0.273	*** -0.388
Number of individual mice trapped at each location in 1962	2.224	0.009	-0.119	0.149	0.001	*** -0.009	0.001	0.189	-0.202	-0.001
Total captures of all mice at each grid location in 1961	5.067	-0.039	0.236	0.484	-0.0004	0.003	* 0.011	* -0.667	** 0.860	-0.621
Total capture of all mice at each grid location in 1962	5.181	0.002	-0.247	*** 1.164	* -0.028	* -0.017	0.010	-0.247	-0.744	-0.229
Number of individual chipmunks trapped at each location in 1961	*** 11.457	* -0.026	-0.154	0.010	-0.004	-0.002	-0.002	-0.018	0.141	0.134
Number of individual chipmunks trapped at each location in 1962	*** 19.731	*** -0.044	-0.115	0.038	0.002	*** -0.013	0.004	-0.056	-0.093	* -0.376
Total capture of all chipmunks at each grid location in 1961	*** 19.311	*** -0.032	*** -0.503	0.199	** -0.019	*** -0.015	-0.004	-0.019	0.335	0.372
Total capture of all chipmunks at each grid location in 1962	*** 33.958	-0.050	0.009	-0.503	** -0.038	* -0.019	0.001	0.377	0.041	-0.204

Table 64. - Summary of shrews (Blarina) captured before and after treatment and the per cent of dead shrews found in traps on the Dover, Ohio, watersheds.

	Treated Watershed no. 1			Untreated Watershed no. 2			Total		
Date	Dead	Alive	%	Dead	Alive	%	Dead	Alive	%
Pre 30 May 1961	4	7	57	3	2	50	7	13	54
Post 30 May 1961	13	25	52	14	23	61	27	48	56
Pre 25 May 1962	3	4	75	2	3	66	5	7	71
Post 25 May 1962	39	59	66	19	29	65	58	88	66

its highest utility capturing ground arthropods.

On plot no. 3 in watershed no. 2, two small Sherman traps were buried in the ground to the level of their top and covered with a piece of roofing felt. These traps were operated every 20 ft for 3 trapping nights. No real difference in shrew captures were observed between buried and top-of-the-ground traps.

Table 65 presents the radioactivity of S^{35} in Blarina. Counts are variable and the pre-treatment count of 0.012 cpm for costal cartilage is difficult to explain. Costal cartilage, kidney, tibial caps, and heart appear to be capable of storing S^{35}. Kidney and heart may be satisfactory indicator organs for S^{35} assay. All collections were within the zone of insecticide contamination, so sharp comparisons can be drawn between concentrations in shrews of different areas.

Two Sorex were processed in July. Neither tibial caps nor costal cartilage contained S^{35}.

Mice

In 1961 in 6521 Sherman trap nights 63 individual (48 male, 15

Table 65. - Radioactivity of <u>Blarina</u> shrews collected from the Dover, Ohio, study areas in 1962. All samples taken before 15 May contained no radioactive sulfur.

Tissue or Organ	Date	Activity in cpm	Location
Costal cartilage	5-24	0	2-9
	5-24	0.012	2-9
	5-27	0.049	2-14
	5-27	0	2-15
	5-28	0	2-7
	5-28	0.085	2-6
	5-28	0.049	2-23
	5-28	0.037	1-23
	5-29	0	2-23
	5-29	0.049	1-12
	6-5	0	2-8
	6-5	0	1-12
	6-5	0	1-10
	6-5	0	2-14
	6-5	0	1-13
	7-16	0	1
Tibial caps	5-24	0.024	2-9
	5-24	0.037	2-9
	5-27	0.024	2-14
	5-27	0	2-1
	5-28	0	2-7
	5-28	0	2-2
	5-28	0	1-23
	5-29	0	2-23
	5-29	0.098	1-12
	5-29	1.22	2-6
	6-5	3.3	1-13
	6-5	0	1-12
	6-5	0	2-14
	6-5	3.1	1-10
	6-5	0	2-8
Kidney	6-5	2.5	1-10
	6-5	0	1-12
	6-5	0	1-14
	6-5	0	2-8
	6-5	2.5	1-13
Heart	6-5	0	1-13
	6-5	0	1-10
	6-5	0	2-8

Table 65. - Continued.

Tissue of Organ	Date	Activity in cpm	Location
Heart (cont.)	6-5	0	1-12
	6-5	3.2	2-14
Testes and Reproductive Tract	6-5	0	1-12
	6-5	0	1-10
	6-5	0	2-14
	6-5	3.1	1-13
	6-5	0	2-8
Brain	6-5	0	1-13
	6-5	0	1-10
	6-5	0	2-14
	6-5	0	1-12
	6-5	0	2-8
Lung	6-5	0	1-18
	6-5	0	2-14
	6-5	0	1-10
	6-5	0	2-8
	6-5	0	1-12
Liver	6-5	0	2-8
	6-5	0	1-12
	6-5	0	1-10
	6-5	0	2-14
	6-5	0	1-13
Hide and Hair from Back	6-5	0	1-10
	6-5	0	1-13
	6-5	0	2-14
	6-5	0	2-8
	6-5	0	1-12
Intestinal Tract	6-5	0	1-10
	6-5	2.5	1-13
	6-5	0	2-8
	6-5	0	2-14
	6-5	0	1-12

female, 320 males per 100 females) white-footed mice (*Peromyscus leucopus noveboracensis*) were trapped. Only 12 of these remained on the area in 1962, an 81% reduction. Fifty individuals 36 male, 14 female (257 males per 100 females) were trapped in 1962 in 9014 Sherman trap nights. Mice could escape from the cat traps and were probably responsible for many lost cat trap nights. Mouse no. 22, a male, was trapped most often, 42 times. Fig. 45 shows the varying trapability of *Peromyscus* with no real difference between years.

Studies of the effects of various trap spacing in plot no. 1 are shown in Fig. 46 and Fig. 47. Generally, trap success increases with trap spacing to a point, but this limit was not reached in this study. For such a study to have meaning it must be conducted many times over larger areas and different spacing intervals should be run consecutively in nearby plots.

In 1961 the random trapping within grids substantiated the effectiveness of the grid for capturing a majority of the mice on the area in a short period of time. When the random trapping was begun (13 June 1961) there were 33 marked mice in the area. At the end of the 3 nights, 38 captures of mice had been made, only four of which were new. During the second period (1 August 1961) after a lapse of over 1 month, there were 39 marked mice on the area; 30 captures were made, 10 were unmarked animals. During the third period (8 August 1961) there were 49 marked mice on the two watersheds; 44 captures were made, only 2 of which were new mice. These findings generally agree with Patric (1958:81) who quotes Manville, Burt, Hayne, and Mohr as saying most residents can be trapped in 3 to

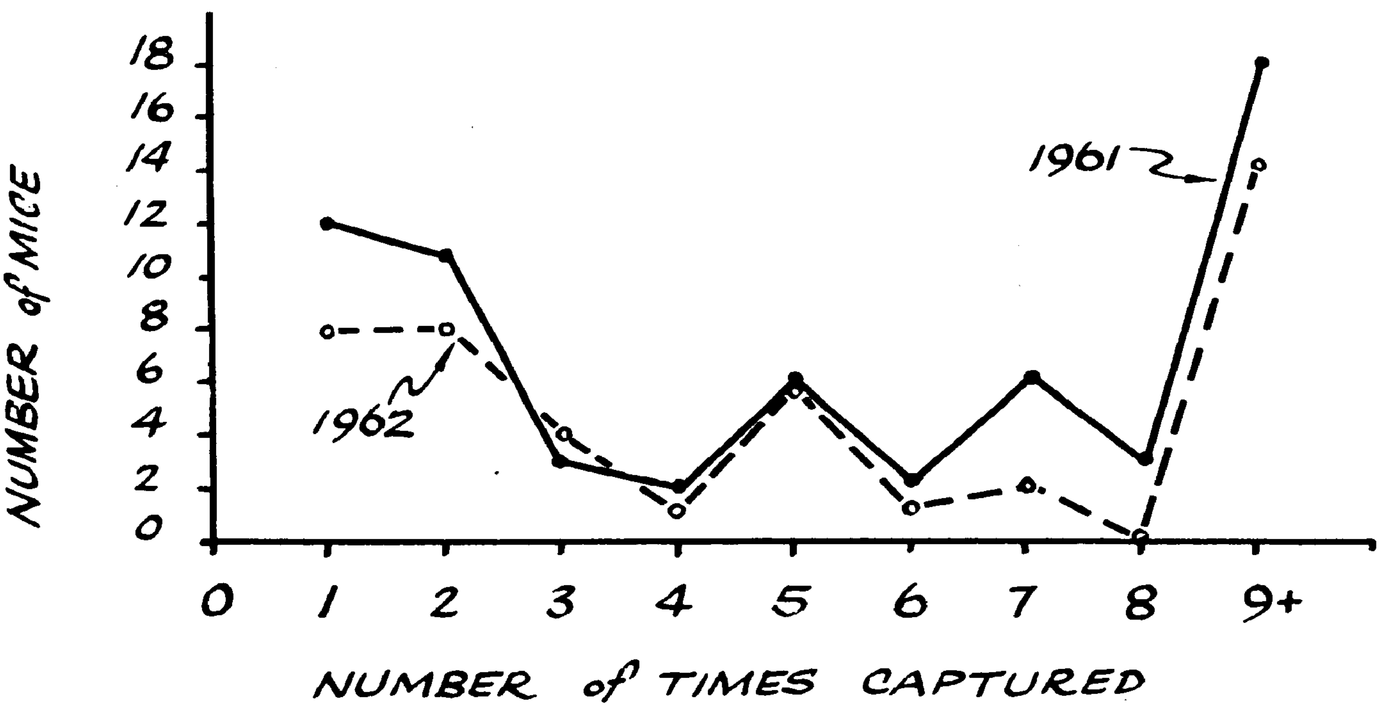

Fig. 45. Number of times individual mice were trapped on the Dover, Ohio, watersheds in 1961 and 1962.

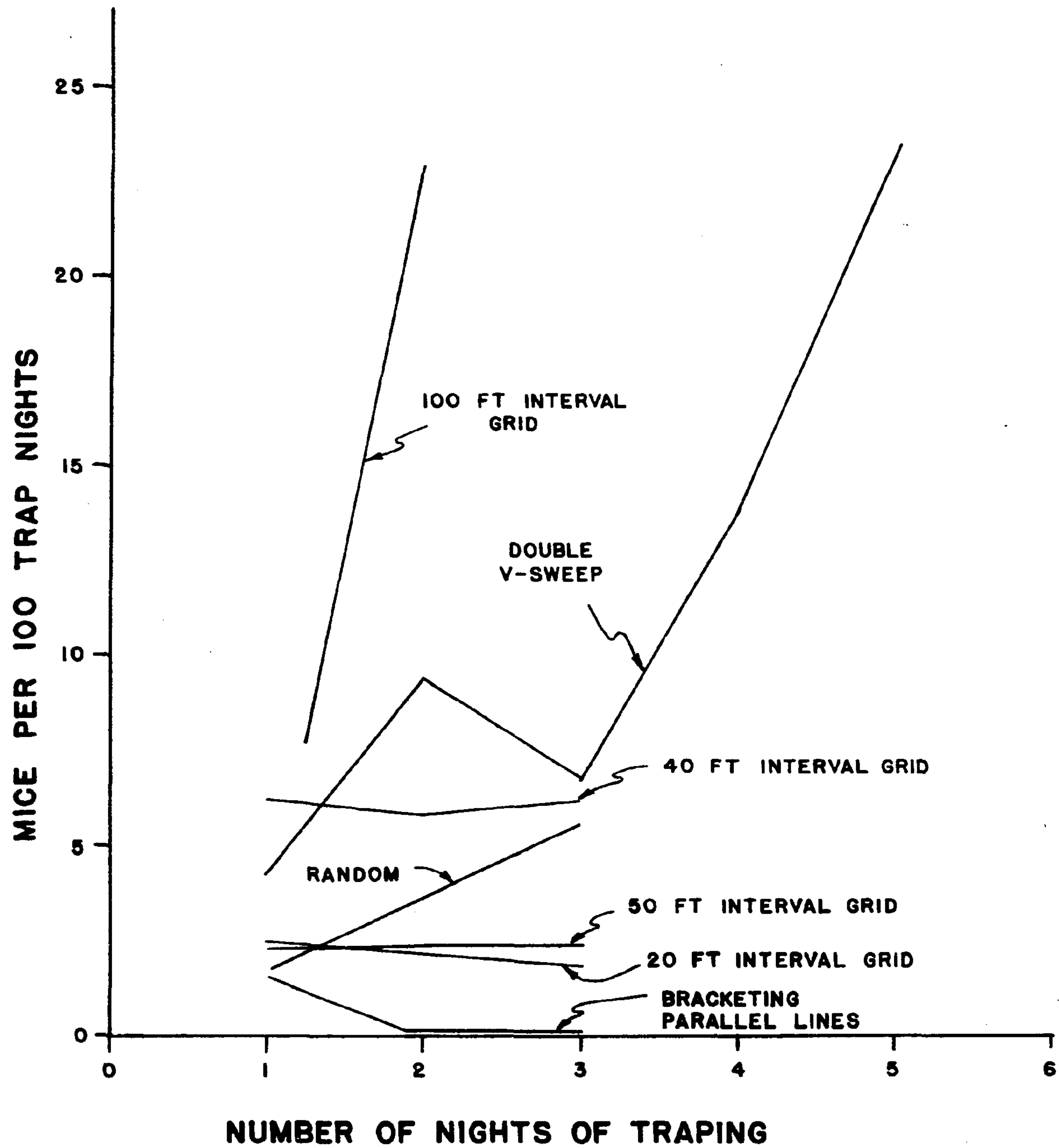

Fig. 46. - Summary of response of mice to various trap placement patterns within plot no. 1 of Dover, Ohio, watershed no. 1, 1961.

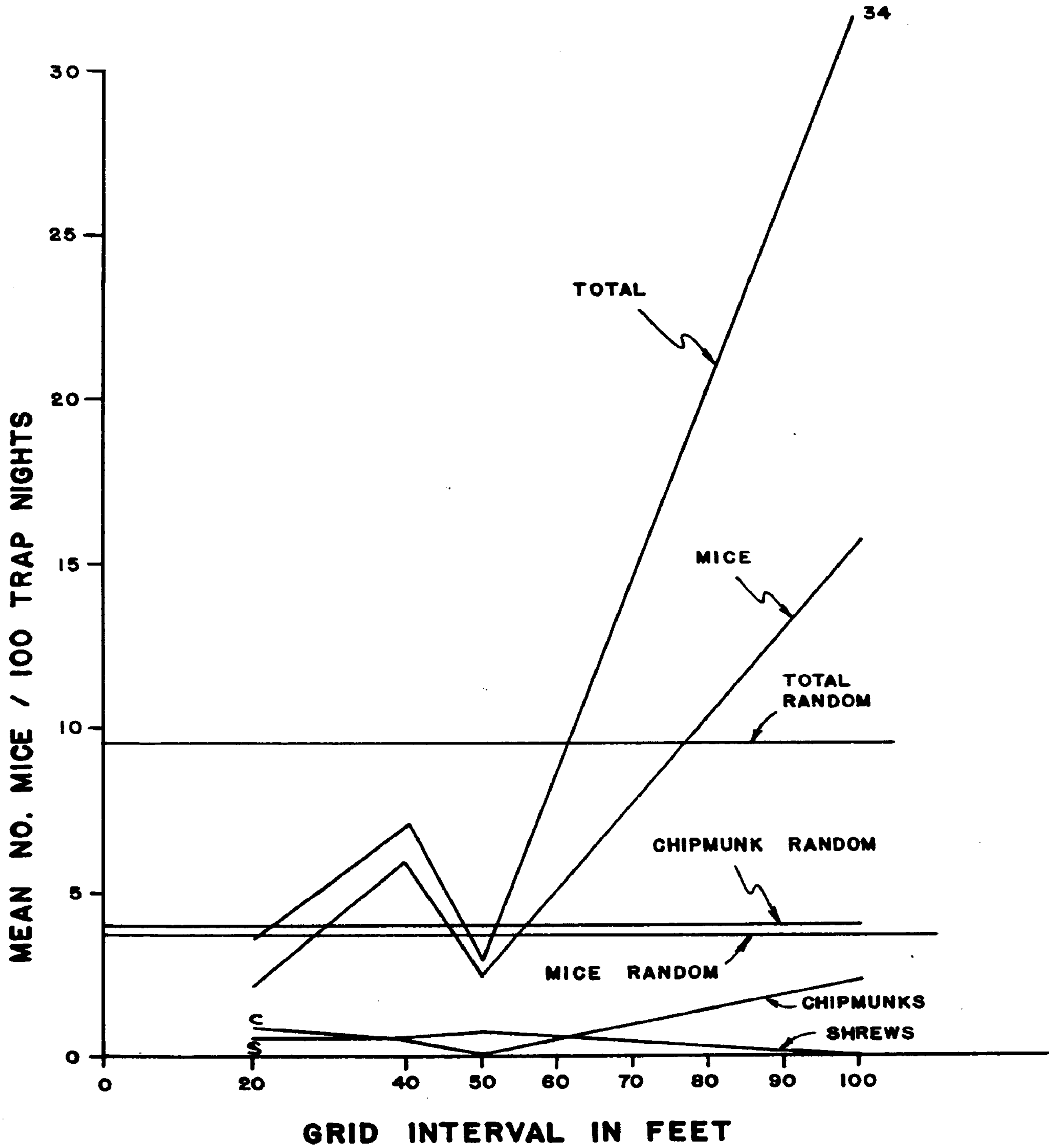

Fig. 47. Influence of trap spacing on trapping success in shrews, mice, and chipmunks based on studies in Dover, Ohio, plot no. 1, 1961.

5 days. However, Patric (1958:97) later casts doubt on this for in trapping on islands, only about half the known mice were taken in 3 or 4 nights.

Thirty-five out of 81 mice caught at grid sites were only caught at one site. Slightly less than half the members of the population are sedentary and appear to have an average home range radius of less than 200 ft. Patric's (1958) recommendation of a 250 ft grid appears valid for white-footed mice.

Fig. 48 presents population estimates based on the Schnabel method (Davis, 1963:109). The data is smoothed but was expected to show population changes that might result from treatment effects. Mouse populations on watershed no. 1 were supressed after the treatment. Where

$$\frac{\text{1962 treated area population/1961 treated area population}}{\text{1962 untreated area population/1961 untreated area population}}$$

$\times$ 100 = % remaining in the treatment area,

(Hoffman and Surber, 1948), the per cent remaining was

$$\frac{18.2/25.5}{20.8/16.0} = 55.0.$$

This descrepancy is too large to be due to chance and reflects treatment effects. These estimation figures integrate the factors of the trappable population, number of trap nights, trappability, food preference and others. Even if the denominator of the above equation is held as 1.0, the results, 71%, still amount to a significant reduction in mice on the treated area. This corresponds to burrowing activity reduction on the treated area as described previously. The stability

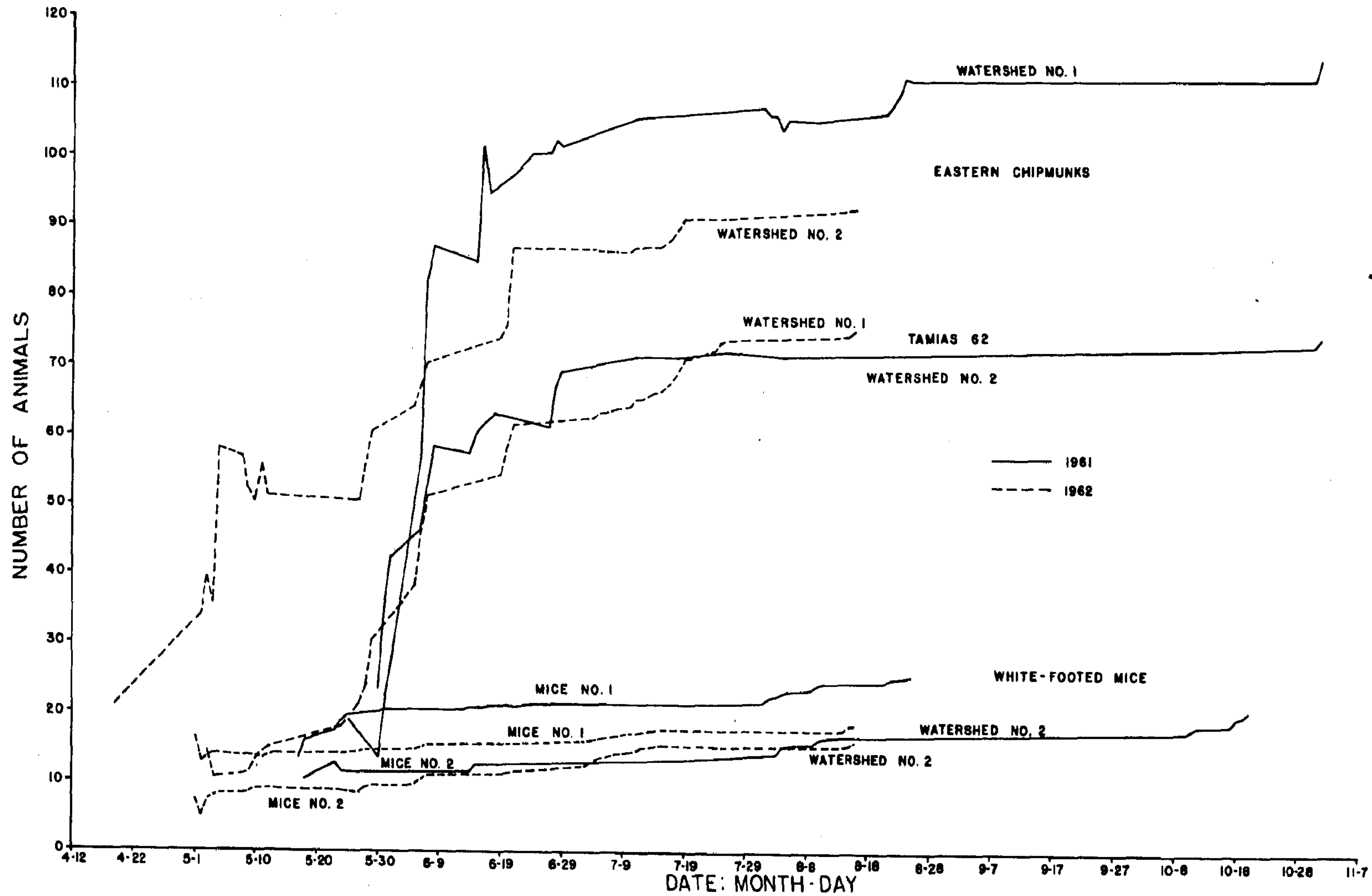

Fig. 48. A comparison of estimated mice and chipmunk populations on the Dover, Ohio, watersheds. Estimates were based on trap-retrap data manipulation by the Schnabel method. Dates are chronological. Cumulative trap nights for both years are shown. Chipmunks were caught in all traps, mice only in Sherman traps.

of the 1962 population on watershed no. 1 indicates no lethal effects of the treatment. The 1962 population simply failed to reach the levels that might be expected based on the previous year of study. If there were no treatment effects, the 1962 population on watershed no.1 would be expected to be located near the level of 1961. Such is not the case. Depending on the way the data is generalized there was a 20 to 45% reduction on the treated area.

Rather than use the estimates, the use of the total marked animals on each area indicates a 77% survival. Use of pre-treatment, 1962 and post-treatment, 1962 data present a conflicting picture, however. Keeping in mind there were only 11 Sherman trapping nights prior to treatment in 1962, the substitution of total marked animals on the areas in 1962 indicates a 60% survival or 40% reduction in mice after the treatment on the treated area as compared to the control. Using population estimates the survival is 70%.

Comparison of the means of mouse populations in various divisions of the watersheds produced only two observations (Table 66) worthy of note. Numbers of mice caught at trap sites on silt loam were significantly higher (5% level) in 1962. Other comparisons between and within years indicated no significant differences. Mouse populations do not seem to be significantly influenced by direction of slope, soil color, or soil texture.

A tally of the number of different trap locations at which each mouse was caught in 1962 (an indicator of home range size) revealed no difference in movement between mice of the two watersheds.

Trappability of mice does not follow an even pattern (Fig. 45)

Table 66. - Comparison of the means of the total number of mice trapped at each Dover, Ohio, trap site grouped according to environmental factors.

Date	Description	n	mean	variance	F value Between year comparison
1961	North slope	18	1.7	1.855	1.505
	South slope	11	1.0	1.636	1.517
	Soil color				
	1	2	0.8	0.916	1.092
	2	13	1.4	1.907	1.247
	3	8	1.4	2.108	1.837
	4	6	1.3	2.010	1.127
	Soil texture				
	1	7	0.6	0.650	3.588*
	2	21	1.7	1.946	1.382
	3	1	4.0	0	0
1962	North slope	21	2.6	2.792	
	South slope	19	2.1	2.481	
	Soil color				
	1	4	1.5	1.000	
	2	16	2.6	2.378	
	3	10	2.2	3.873	
	4	10	2.6	2.265	
	Soil texture				
	1	15	2.8	2.332	
	2	25	2.2	2.690	
	3	0	0	0	

*Significant at 5% level.

so no population estimate could be made as for chipmunks. There appears to be no real difference between the trappability or trap-proneness of animals between years. Reduction of populations does not seem to be related to a behavior change but to actual reduction in population size.

The live trap data was manipulated as if it were snap trap data, that is, the animal was "removed" from the population on it's first date of capture. (See Zippin, 1958). The unnaturalness of such a situation as regards territory, competition, etc., is evident, but the assumption, nevertheless provides another population estimate for eventually all the animals are "removed" and invasion phenomena are avoided. The results of this data manipulation are shown in Fig. 49. The post-treatment survival appears to be less than 66% since area divisions are not presented.

At the end of the 1961 tagging period there were 32 marked mice in watershed no. 1, 25 in watershed no. 2. After the trapping was complete in 1962 it was determined that there had remained on the areas from 1961 7 trappable mice in watershed no. 1, 6 trappable mice in watershed no. 2. This is, respectively, a survival or continued residency percentage of 22 and 24. Survival and continued residency were higher on the control area but not significantly. These figures coupled with those of predators trapped and seen eliminate the possibility of predation causing the observed population reduction on the treatment area. Radioactivity of mice from the tracer plots is shown in Table 67.

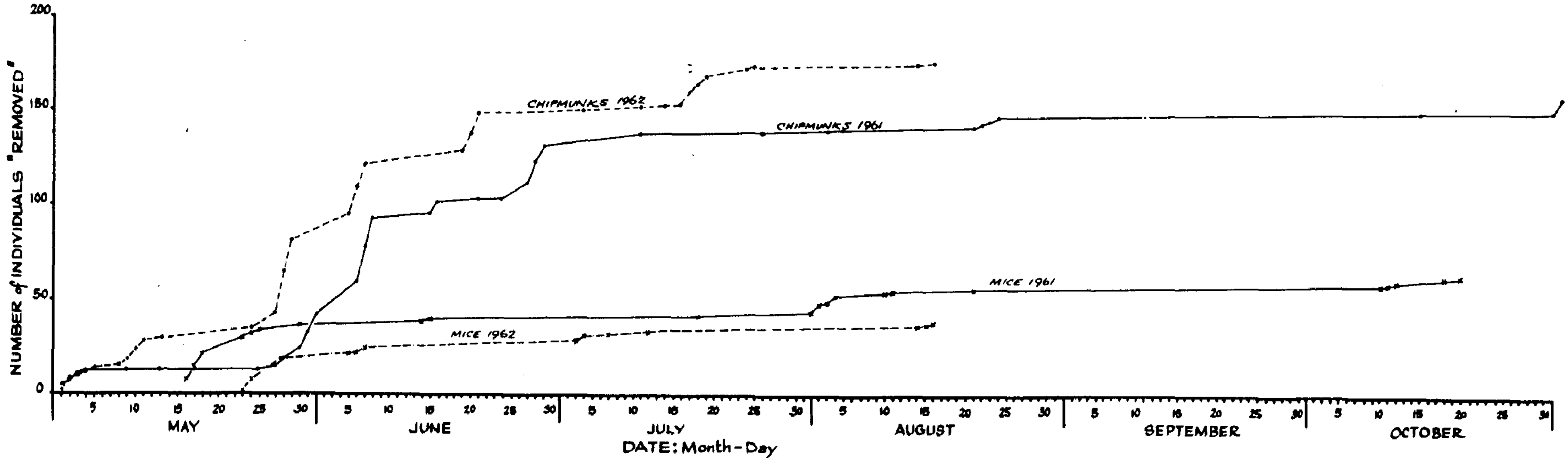

Fig. 49. Cumulative numbers of mice and chipmunks "removed" (as if live traps were snap traps) from the Dover, Ohio, study area in 1961 and 1962.

Table 67. - Radioactivity of mice confined in cages on the Dover, Ohio, tracer plots during and after treatment and collected on 14 September 1961 after 16 days exposure.

Location Plot number	Tissue	Wt. of sample in mg	Corrected total activity in cpm/mg
1	liver	322.6	0
1	tibial cap	47.5	0.04
1	skin	167.0	0.02
1	costal cartilage	107.5	0.04
1	kidney	34.4	0.06
3	intestine	607.3	0.015*
3	tibial cap	192.3	0.082*
4	tibial cap		0.12*
4	skin	173.7	0
4	costal cartilage	59.2	0
4	liver	224.2	0
4	kidney	24.6	0.18*

*significant at 10% level of confidence.

Chipmunks

A comparison of the population estimates between years and areas (Fig. 48) indicates a chipmunk reduction in excess of that found for the mice. There is only 45% survival of animals remaining on the area after treatment. As with mice this is not a lethal effect but apparently one of productivity and survival.

A population estimation was obtained based on chipmunk trappability (Eberhardt _et al_., 1963:45). By tallying the frequency of captures of individual chipmunks the solid line of Fig. 50 was obtained. By projecting the line to zero, the frequency, f(0), of the untrapped chipmunks was obtained. The sum of the frequencies, including the estimated frequency of untrapped animals, is the population estimate and was in this case 355 and 321 for 1961 and

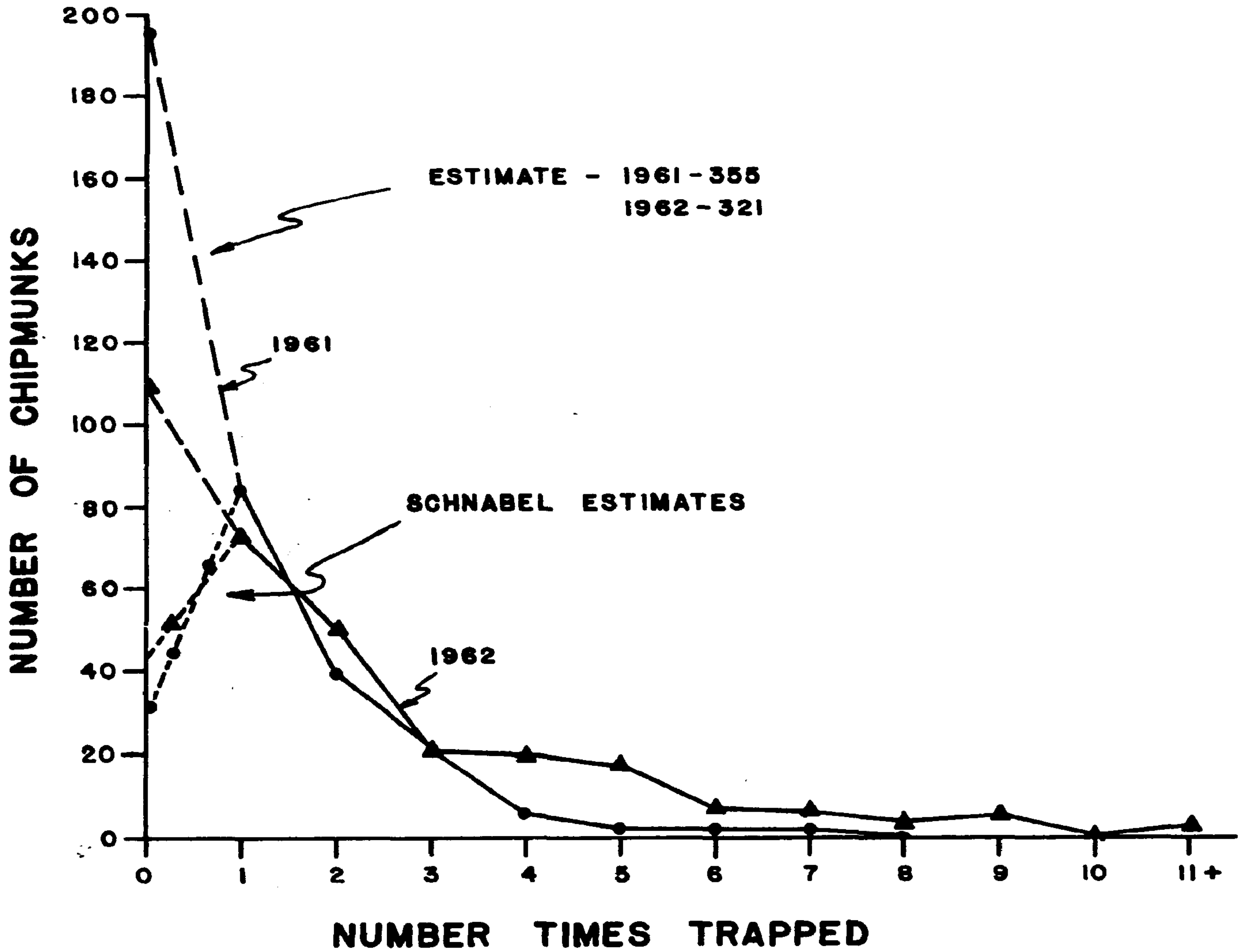

Fig. 50. Population estimates of chipmunks, Tamias striatus, in 1961 and 1962 on the Dover, Ohio, watersheds obtained by projection of the frequency of capture line and summing the counts including the projection on zero.

1962 (Table 68). However, if the Schnabel index is valid and it should approach actuality as trap effort increases, then the projection far overestimates the population. Accepting the results of the Schnabel index as the best estimate of the population, the subtraction of the sum of the capture frequencies provides the best estimate of the untagged population of the area. These are shown in Fig. 50 and refute one application of the projected frequency of captures as a means of estimating an intensively trapped population. The total estimates so obtained are 1.85 times higher than the Schnabel estimates for the same data. Therefore, there may yet be some basis for the use of this approach in population estimation with a correction factor.

Table 68. - Summary of number of times individual chipmunks were captured in 1961 and 1962 on the Dover, Ohio, watersheds.

Number of times captured	Number of animals 1961	1962
0 (estimated)	198	110
1	85	74
2	40	51
3	21	21
4	5	20
5	2	18
6	2	7
7	2	6
8	0	5
9	0	6
10	0	0
11+	0	3
Total Estimated Population:	355	321

At the end of the 1961 trapping period there were 79 marked chipmunks on watershed no. 1, 62 in watershed no. 2. After the tagging was complete in 1962, it was determined that there had remained on the areas 26 trappable chipmunks in watershed no. 1, 21 trappable chipmunks in watershed no. 2. This is respectively a survival or continued residency percentage of 33 and 34. Total survival and prolonged residency were slightly greater on the control area. This compares favorably with the results of the mouse studies.

As for mice, the means of chipmunks trapped at trap sites in various categories were examined for significant difference. Unlike mice, the only difference of real significance was that the mean number of chipmunk trapped on sandy loam was actually higher in 1962. All other comparisons (F tests) indicate no significant difference between or within years (Table 69).

Radioactivity of a tracer plot caged chipmunks is presented in Table 70 and for the 1962 study in Table 71.

Table 69. - Comparison of the means of the total number of chipmunks trapped at each Dover, Ohio, trap site having common environment factors.

Date	Description	n	mean	variance	F (between year comparison)
1961	North slope	20	3.3	4.235	2.717
	South slope	21	3.9	6.991	1.075
	Soil color				
	1	4	6.0	4.666	1.500
	2	15	3.9	6.335	1.197
	3	12	2.9	4.285	2.211
	4	10	3.3	4.452	1.037
	Soil texture				
	1	12	3.0	8.532	1.297
	2	28	4.0	4.000	2.081*
	3	1	3	0	0
1962	North slope	23	5.7	11.506	
	South slope	22	6.2	7.513	
	Soil color				
	1	4	10.5	6.996	
	2	17	5.9	7.585	
	3	14	4.6	9.474	
	4	10	5.8	4.618	
	Soil texture				
	1	15	5.0	11.062	
	2	29	6.4	8.323	
	3	1	5	0	

*significant at the 10% level of confidence

Table 70. - Radioactivity of a chipmunk fed water from a sprayed Dover, Ohio, tracer plot from 10 August to 9 September 1961.

Tissue	Dry weight of sample in mg	cpm/mg
Costal cartilage	220.6	0.0005 (not corrected)
Tibial cap	183.0	0.005 (not corrected)
Liver	905.0	0.018*
Kidney	201.0	0.010 (not corrected)

* Significant at 10% level of confidence.

Table 71. - Radioactivity of the tissues of a chipmunk collected from Dover, Ohio, watershed no. 1 on 26 July 1962, 62 days post-treatment.

Tissue or Organ	cpm/mg
Kidney	0.019
Heart	0.019
Lung	0.019
Costal cartilage	0.010
Testes	0.008
Liver	0
Tibial cap	0
Hide and hair from back	0

Flying Squirrels

Flying squirrels present an enigma. Only two were trapped in 1961, one on 31 May the other on 1 November, in 786 cat trap nights. In 1962 between 1 May and 10 May, 13 flying squirrels were trapped. No others were trapped until 16 August 1962 when one was trapped at 1-1. Of the total of 16 trapped both years, 4 were males, 10 were females, and 2 were of undetermined sex. Four of the 1962 captures were retrapped; only one was trapped three times. The female trapped three times was at 1-22, 1-5, and 1-12 from 3 May to 10 May.

The sudden disappearance or loss of trappability of the typically woodland animal is unexplained. It does not appear to relate to emigration as in rabbits, major food changes, or desirability of the bait. Since the animal was absent from the traps the week prior to the initial spray date as well as afterwards, treatment of the area does not seem to be the cause. The disappearance of the flying squirrel seems due to an undetermined behavior pattern.

Fox Squirrel

No fox squirrels were captured during the study. Eight were observed, 2 in 1961, one on each watershed; 6 in 1962 on watershed no. 1, 1 on watershed no. 2. Squirrels were observed feeding on the treated area after the spray. No gray squirrels were seen.

Rabbits

Rabbit droppings were abundantly evident throughout the study area but no rabbits were seen or captured during 1961. On 15 September 1961 the first rabbit was seen in the forest and they were seen periodically until the next spring when they were no longer seen. There is evidence of a field-to-forest-to-field population shift, probably due to more abundant mast, food, and cover during the winter and to succulent foods and breeding cover during the spring and summer.

Two rabbits were trapped in 1961, one of which was retrapped in 1962. From capture dates, locations, and movements no treatment effects can be concluded.

Opossum

Five opossum (*Didelphis virginiana*) were captured in 1961, all were males. Only one was recaptured after 15 days. It was at the same trap site. Sixteen were trapped in 1962, seven were females, nine were males. There were recaptures; one was recaptured 200 ft from the first trapping, one 460 ft, and the other was caught three times. The last animal, a male, was released on 26 July 1962 from 1-15 and moved 460 ft to 1-18 where it was found in a trap 30 min. after the first release. Not enough captures or recaptures occurred to allow conclusion about treatment effects on behavior. There appeared to be no effect. No significant radioactive sulfur was found in costal cartilage, skin, kidney, tibial caps, or liver of two opossum kept in cages on the tracer plots in 1961.

Raccoon

Only two raccoons, *Procyon lotor*, were trapped and ear tagged in 1961, one male and one female. In 1962, 9 were captured, 5 were females, 4 were males. All movement observations but one were made after the 25 May treatment of watershed no. 1. No behavioral changes can be concluded. One tagged raccoon was killed by hunters over a mile from the study area.

Woodchucks

Fig. 51 presents the distribution of woodchuck (Marmota monax) burrows within the study area. Watersheds were nearly identical in the number of burrows present. Watershed no. 1 had 34 active and 33 inactive holes; watershed no. 2 had 30 active and 34 inactive holes. The holes were located and mapped at a rate of 35 minutes per acre. See Gysel (1961:15).

In spite of the apparent large populations, only three woodchucks were captured during the study. All three were at 1-21 and 1-14 and all were caught on fox checkers or fish scent on cotton. Two others escaped by tearing out the side of the wire trap.

Weasels

Four weasels (Mustela frenata) were captured in 1961, only one in 1962. One moved 470 ft between traps in 24 hr. Weasels were believed responsible for loss of chipmunks in traps by eating off tails and legs through the bottom of the traps. Losses were reduced by placing screen wire in the bottom of the traps. Not enough weasels were caught to allow population change detection.

Bats

Bats of at least two species were seen in flight over the area but no identification or counts were made. These mammals, though

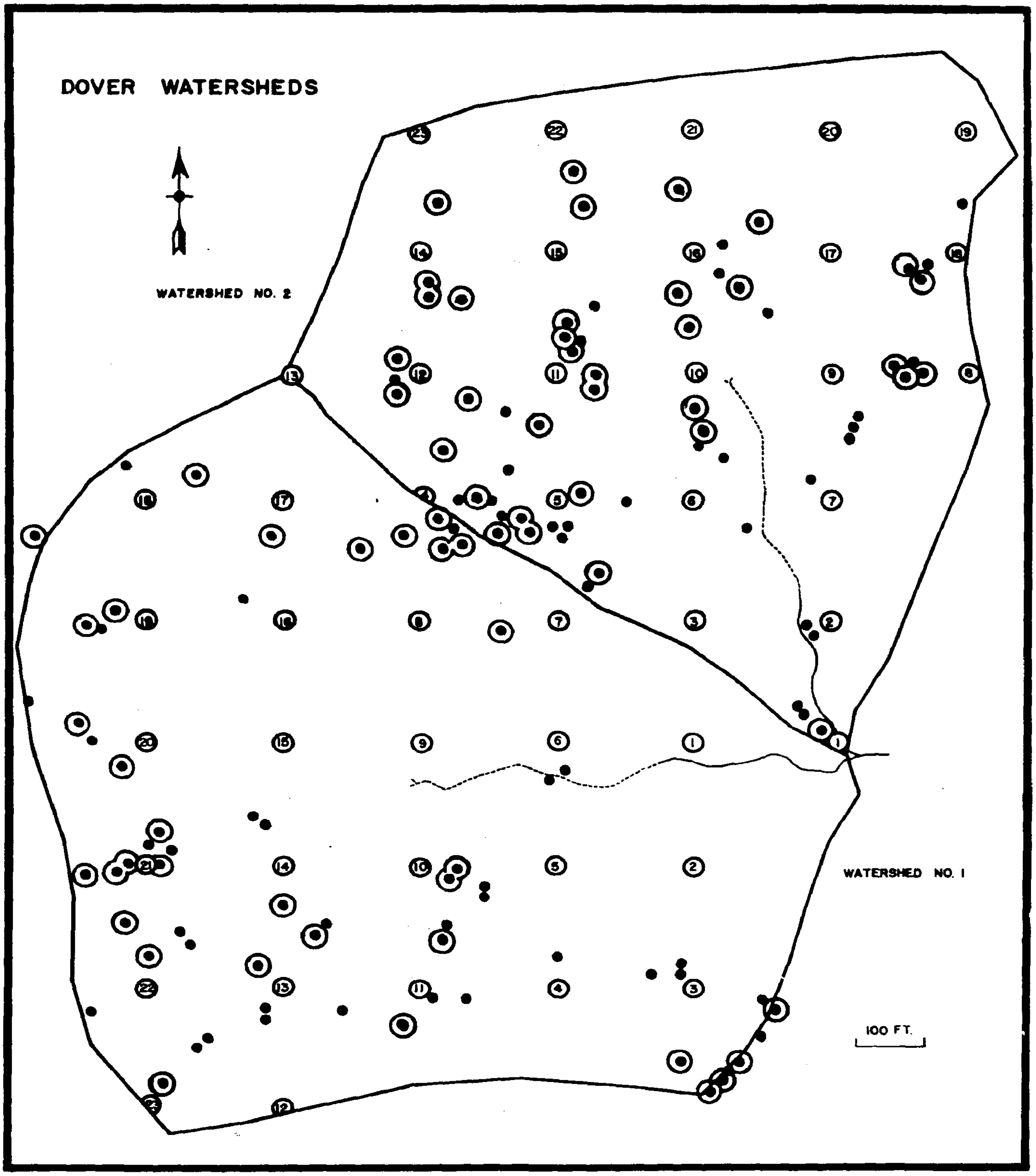

Fig. 51. Approximate location (within ± 10 ft) of visible burrows of the woodchuck, *Marmota monax*, based on size and appearance in early fall, 1961. Inactive burrows are encircled. No excavations were made to verify probable burrows.

probably unaffected by insect loss over 40 acres due to their wide home ranges, could conceivably be seriously affected when larger acreages are involved.

Other Mammals

Only three moles (*Parascalops breweri*) were observed but none were captured in traps. Two house mice (*Mus musculus*) were trapped; one was trapped on successive nights at the same location and was not recaptured. Only one woodland junping mouse (*Napaeozapus i. insignis*) was trapped near 2-16. One red squirrel (*Tamiasciurus hudsonicus*) was observed on the area.

Parasitism, Predation, and Disease

Ectoparasites of mammals observed are reported in Table 72. The first evidence of myiasis (*Cuterebra*) was observed 7 July 1962 in *Tamias*. No changes were noted in ectoparasitism after spraying the area. Sealander (1961:59) and Abbott and Parsons (1961) present the relationship of *Cuterebra* to populations and animal physiology. This suggests the possible benefits (at least temporary) of an insecticide application in reducing dipteran stress to a wild mammalian population. As Sealander (1961:59) said:

> If dipteran parasitosis should occur at a time of high population density, when members of the population are subjected to profound physiological alterations resulting from crowding stress which tend to reduce their survival potential ..., then at least part of any subsequent population declines might be triggered by the infection.

Predators of mammals observed were raccoon, snakes, hawks, feral cats, and weasel.

Table 72. - Observed ecto- and endoparasites of mammals on the Dover, Ohio, study area.

White-footed mouse, *Peromyscus leucopus novaboracensis*	
Hystrichopsyllidae	flea
Pulicidae	flea
Cuterebridae	
Cuterebra sp.	fly
Cottontail Rabbit, *Sylvilagus floridanus*	
Pulicidae	
Cediopsylla simplex (Baker)	
Eastern Chipmunk, *Tamias striatus fisheri*	
Hystrichopsyllidae	flea
Short-tailed Shrew, *Blarina brevicauda* - unidentified nematode	

Mammals often were found in "shock" in the traps. This condition has often been reported for trapping studies. Mammals so afflicted were left in the sunlight after attempts at pressure artificial respiration. One chipmunk in the early stages of shock was taken to a veterinarian but did not respond to epinephrine. Shrews were frequently found dead in the traps and their death is believed to be, at least in part, due to shock.

VEGETATION

Phytotoxicity of malathion has been reported in an earlier section. No evidence of such toxicity was observed following the application of 2 lb/acre of malathion. After most observations of the radioactivity of the litter around the crash site had been made it was noticed that cherry and red maple leaves had fallen and were still falling. These green leaves all had yellowish discolored spots on them that produced high readings on the field counter. The counts of the spots indicated a large quantity of malathion had been sprayed or splashed on them and had been toxic. Since the counts were higher than found elsewhere in the spray swath it was assumed this was a kill resulting from an abnormally large application associated with the crash. Six days after the crash as a result of the contaminated leaves falling, the ground downhill from the plane was 0.03 to 0.08 mr/hr more radioactive than at the time of the crash. In late August the trees were alive and had new leaf growth.

Only limited studies with vegetation were conducted to aid in interpreting buildups of radioactivity in the fauna. Crossley and Howden (1960) stated that sizeable fractions of the materials (radionuclides) taken up by plants may pass through the herbiverous insects in the system. Cook and Kettlewell (1960) labeled insect larvae by feeding them on leaves grown in a water culture of S^{35}.

The process of uptake by plants was verified when 1 out of 12 samples of new leaves and new stem growth taken from the treated tracer plots in 1962 showed relatively high radioactivity (15.6 to 60.0 cpm/mg).

On 3 August 1962, parts of blackberry plants growing in a 100 cpm or greater area of watershed no. 1 were collected and counted. Corrected total radioactivity in cpm/mg was: ripe fruit, 0.0650; green fruit, 0.0648; terminal stem, 0.0784; leaves, 0.0778; old stem, 0.0315; and major roots, 0.0162.

Timofeev-Resovskii *et al*., (1960) found that S^{35} uptake was higher in plants than in animals. Portable counter readings of leaves revealed that diseased portions of leaves and dried, curled edges had higher radioactivity. This phenomenon of selective absorption of sulfur has been studied and reported by Gottleib and Garner (1946) and Yarwood and Jacobson (1950). Part of the greater activity may be due to adsorption and retention rather than absorption, but in the light of the above past work it appears likely that absorption is involved. Gunther and Blinn (1955:141-42) discuss the mechanisms of insecticide fixation to leaves.

There was no evidence of the malathion washing down the tree or shrub trunks; *i.e.* the insecticide tended to stay where it hit. Based on portable field counter tracer plot monitoring on 11 September 1961, fungi were more active than surrounding plant tissue. Where background was 0.03 to 0.07 mr/hr, leaves had activity to 0.10 mr/hr and bark to 0.30 mr/hr. The field surveys were interesting, gave direction for subsequent work, but were unsatisfactory for quantitative work.

In conclusion, insecticides on and within plants are determined by and vary with characteristics of the leaf surface, pathology, and amount of insecticide applied to the area in which the plant grows. Metabolites of malathion occurred in new stem and leaf growth the year after application. Radioactivity of the fauna is influenced by the quantity of radioactive vegetation consumed and utilized.

DISPERSAL STUDIES

BASIC ISOTOPE CONSIDERATIONS

A new era in ecology has been opened by the multi-functional research tool, radioisotopes. Tracers, first used in biological studies in 1923 (Paneth, 1949:388), provide a means of investigating the dynamics of ecosystems, of ascertaining effects of extrinsic factors in the environment, of following cycling elements, of studying productivity, of investigating animal behavior, and of studying metabolic and many other processes.

The picture of the distribution and re-distribution of insecticides has defied accurate determination by classical analytical techniques. Insecticides are often toxic or active in quantities too small for detection by chemical techniques. Such techniques are frequently too crude, slow, and expensive, to allow analysis. Using isotopic tracers the fate of a chemical or its metabolites can be sensitively followed through all stages of most processes to quantitatively and qualitatively answer the questions of what, when, and how. Determinations can be made at concentrations far below those permitted by other means.

The parallels existing between environmental pollution from atomic energy by-products and insecticides are evident. The need for information on the role of natural consumer animal populations in the biological cycling of insecticides and radioactive wastes is great. Environmental pollution surely has effects on man, and investigations on radiation and insecticide ecology are necessary to maintain desirable man-land health. This study utilizes isotopes in studying an insecticide but suggests techniques that may be of value in studying ecological effects of other environmental pollutants.

Essentially all elements are available as isotopes for ecological studies, though ones having beta and gamma emissions are the only practical ones for use. Beta particles cause more ionization in tissues than do gamma rays. They are not highly penetrating and are stopped by a thickness of skin or 0.63 cm (0.25 in.) sheet plastic, yet they can be detected with beta-gamma field survey meters or thin window Geiger-Mueller (GM) tubes (Pendleton, 1952:24).

A tracer isotope was sought for this study that had the following characteristics:

1. It must be one of the component elements of malathion: P, S, O, C, or H.
2. It must have a half-life long enough to permit study over several years or at least the estimated period of insecticide effect.
3. It must have a half-life short enough so as not to preclude later tracer studies on the research area or create undue long-term health hazards.
4. It should, if possible, be a gamma emitter to increase ease of detection with available instruments.
5. It should not occur naturally within the area from fallout or waste disposal contamination.

Several basic assumptions were made in using an isotope:

1. An isotope will return to a stable state or will decay into different elements and in the process eject particles or

rays of energy which are detectable by instruments.

2. Decay or transmutation takes place at random at a constant rate - one unaffected by any environmental factors.
3. Radioisotopes become a part of the material under study, do not induce any unnatural responses in animals, and only differ from other atoms of the same substance in their ability to emit identifying energies.
4. Flow or movement of the radioactive element is entirely proportional to the amount present.
5. Isotopes are uniformly distributed in relation to non-radioactive elements.

The health hazards of isotopes as tracers have been studied. Godfrey (1954:951) warns of the possible long term and immediate effects on the health and consequently behavior of animals marked with isotopes. There are safety exposure limits published for humans but little is known of wild animal safety limits. No precise guides were followed in this study to arrive at safe quantities for use. Safety to animals was considered along with factors of isotope energy, half-life, expected deposition, expected contact, expected location, ease of detection, human safety, and others.

THE USE OF ISOTOPES FOR INSECTICIDE STUDIES

Jenkins in 1957 produced a comprehensive review of the isotope research in insecticides to that time. Dahm (1953, 1957) summarized use of isotopes with the organo-phosphate compounds. Since then the literature on the subject has more than doubled. Use of tracers to study insecticides, especially physiological effects, is widespread and has developed an established methodology. P^{32} exceeds all other tracers in use with insecticides (Dahm, 1953:164). Tracers are frequently combined with paper chromatography in studying metabolites of insecticides in target species as well as in mammals, birds, and *in vitro*.

SULFUR^{35}

Sulfur^{35} was selected for use. It is a negative beta emitter with upper limits of energy for particles between 0.12 and 0.17 MEV. It has an 86.35 $\pm$ 0.17 day half-life[1] (Cooper and Cotton, 1959). It is not present in normal atomic wastes (Auerbach, 1958:524). If ingested, it is potentially dangerous for it has a relatively long half-life, is in direct contact with tissue, moves to selective sites, and has a relatively long biological half-life. Selection of S^{35} provided a longer half-life substance but sacrificed the ease-of-detection characteristics of the alternative P^{32}. Pryor's (1962) book is a comprehensive treatment of the chemistry of sulfur.

THE SULFUR CYCLE

Of the approaches to ecological studies, the biogeochemical cycling of elements is currently of greatest interest, especially in

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[1]The commonly accepted S^{35} half-life value of 87.1 $\pm$ 1.2 days was used in computations throughout the study. This improper adjustment is constant and makes no significant differences in comparisons of levels of activity observed.

the cycling of the fallout elements, cesium and strontium. Study of the cycling of an element is complex. Only a superficial inquiry was made into the fate of the S^{35} of the malathion molecule. This does not, however, indicate the need is not great. As Neel (1961:1) pointed out, the health physicist needs methods for predicting both present and future distribution of isotopes in soil, animals, and plants since these, as part of the food chain, contribute to the internal radiation exposure of man. In order that such predictions can be made, new information is required on the "biogeochemistry and ecology of the chemical elements, and theoretical models that relate the accumulation and flux of these elements in different parts of an ecological system or 'ecosystem' " (Neel, 1961:1).

In the ocean, sulfur is present in excess of the needs of plants and is therefore not limiting (Redfield, 1958:207). Such is not the case on the land, for sulfur is 10,000 times more plentiful than phosphorus (Redfield, 1958:218). Distribution nor availability is not equal. In order to state whether sulfur is limiting to plants or animals, an average expression of the sulfur in plants and soil is needed as well as the amount to be added, i.e., the treatment. A limiting tracer element added to an ecosystem is rapidly and completely cycled; those that are non-limiting are not cycled as readily by the system.

Consider the addition of sulfur directly to a plant in an ecosystem. Kamen (1947:212) stated:

> The conversion of sulfur, administered as sulfur dioxide and sulfate in plants, has also been studied and, among other features, it has been noted that not only rapid conversion of most of the sulfur so administered to an organic form occurs, but also that, when sulfur is to be transported from leaves to roots or grain, it is changed to sulfate and then changed

back to organic sulfur on deposition in the roots and grain.

Ashby and Mika (1959:31) studied the influence of sulfur on basswood (<u>Tilia americana</u> L.) seedlings. Growth of those receiving a sulfur-deficient nutrient solution was markedly reduced over those on a complete nutrient. In the course of their studies they completely controlled an aphid attack on their test plants with 4% malathion dust. The sulfur of malathion in this case was not sufficient to modify detectable physiological response to a sulfur-deficient medium in which the plants grew.

Sulfur is converted to an organic sulfur and deposited throughout the plant. Ashby and Mika (1959:28) reported that sulfur approximates the phosphorus content when averaged over a number of crops.

They were unable to find reference to the status of sulfur requirements on forest lands. They stated (1959:31) that "under certain environmental conditions the amount of sulfur applied via the atmosphere may be sufficient for those plants having a low sulfur requirement." Species variability and thus community variability become evident as factors influencing the sulfur cycle. Upon leaf-fall or general plant-part fall, the part is set-upon by microorganisms and other decay producers. In a symposium on "Biogeochemistry of Sulfur Isotopes" (Jensen, 1962) many workers discussed use of sulfur by microorganisms. Generally speaking, sulfur cannot be used by higher plants in its elemental form; it requires biochemical change by micro-organisms (Pelczar and Reid, 1958:487). Several bacteria oxidize sulfur to sulfates, a useable form. Pelczar and Reid (1958:487) continue, outlining the reaction performed by <u>Thiobacillus</u>

thiooxidans. The bacteria Desulfovibrio, e.g., reduces sulfates to H_2S. The oxidation of H_2S which resulted from either of these two reaction pathways or through amino acid decomposition provides elemental sulfur and completes a cycle.

Bollen (1961:88) reported that parathion had a stimulatory effect on soil bacteria, nitrifiers as well as nitrogen-fixers. He reported studies with the insecticide thimet and other compounds and bacterial actions upon them. Thiobacillus thiooxidans was unable to utilize the sulfur from the thimet molecule. Another variable, the chemical form of the element, is evident, and it is affected by microorganisms, environmental conditions such as light and temperature, and by available CO_2. One source of CO_2 is as a byproduct of microbial activity.

Rather than fall to the ground, the plant-part may be consumed by an animal.

Most organisms presumably can satisfy their sulfur requirements for the synthesis of sulfur-containing amino acids, biotin, etc. by the utilization of organic sulfur compounds (Fruton and Simmonds, 1958:909).

Kamen (1947:212) stated:

> The distribution and retention of sulfur in the animal organism depends markedly on the form in which it is ingested. Thus, the rat cannot use elementary sulfur in place of cystine or methionine for incorporation into tissue protein. Ingestion as sulfate results in rapid excretion of most of the dose, a small fraction appearing in the animal, with the highest concentration in bone marrow. Sulfate also is not utilizable for amino acid synthesis. Ingestion as sulfide is rather inefficient, because most of the sulfur in this form is oxidized to sulfate in which form it undergoes the same fate as ingested sulfate.

The physiology of sulfur within animals is beyond the scope of this section. With the thioamino acids and their interaction (Binkley _et al_., 1952:112; Anslow, _et al_., 1946) it is intimately related to Coenzyme A synthesis, vitamin F (Olson, 1953:253); the skeleto-muscular system (Christman _et al_., 1958:460; Denko and Bergenstal, 1955:76); the endocrine system (Thomas, 1956:251); circulatory and digestive systems (Cember _et al_., 1956:175); and others.

Fitzgerald _et al_.,(1954) studied the distribution of ethionine-S^{35} in rats and found it concentrated in kidney, pancreas, gastro-intestinal tract, and skin. They observed primarily urinary excretion of S^{35}, little fecal excretion. The cycling of sulfur within the animal varies with the initial form of the sulfur from the plant, its conversion, the health or condition of the animal, and specific organism needs that vary not only with species but with the state of the animal as influenced by his environment-conditioned behavior. The report of Miller and Price (1956:564) that growth depression in rats produced by molybdenum could be reduced by additions of sulfate of Na and K presents a near-complete block to studies of cycling phenomena. Interactions such as this are almost impossible to predict and measure. Sulfur could be a usually non-functional constituent of an organism that would be brought into play only when challenged by an intake of a compound or an element (such as molybdenum). The active cycling of an element would therefore take at least two pathways, one active and functional, the other passive and in reserve.

Electronic computers offer hope for analyzing the multiple variables and interactions of the cycling of an element within an ecosystem. Neel (1961:95) conditioned the use of computers on the organization of necessary ecological information in terms of a compartment model. He (1961) developed such a model assuming that the rate of movement of material out of an ecological compartment (e.g. air, water, soils, and organic litter) is proportional to the quantity of the material in that compartment. He was thereby able to describe a system of simultaneously linear differential equations.

Sheppard's (1962) book offers valuable leadership in tracer cycling investigations.

FACTORS AFFECTING ISOTOPE DISPERSAL

There are so many factors of unknown magnitude affecting isotope dispersal that a realistic appraisal of such dispersal within an ecosystem is presently impossible. The immediate and real problems of studying dispersal of fallout nuclides such as strontium-90 or cesium-137 are extremely complex. Work with a tagged insecticide whose complex natural breakdown products are yet unnamed, whose *in vivo* characteristics are yet incompletely understood, and whose complete *in vivo* metabolism is unknown, is many times more complex. Schmidt and Weidhaas (1958:643) commented that radioactivity in insects may represent the original insecticide, its metabolites, or both, and at a specific time an equilibrium between the amount taken up and that excreted. These vary with the insecticide and the insect.

Auerbach (1958:523) presented some factors needed to evaluate the effects of a radionuclide. These are all related to dispersal and measurement of dispersal. The factors are: quantity of nuclide, uptake, mode of entrance into the body, body retention, critical organ retention, biological half-life, radioactive half-life, and the energy and type of the radiation.

Comar (1955:6) warned that care must be taken in measuring time of transfer from one point to another within a system. Measured time of arrival is "a function of the amount of radioactivity used, the sensitivity of the measurement, and the volume of circulating fluid with which the isotope becomes diluted."

Nelson (1960:81) listed factors affecting accumulation of radioisotopes by the biota. Related to uptake, the specificity of organisms, organs, and tissues for elements used in the structure and metabolism of species are factors. Elements may be either concentrated or diluted in passing through the food chain, depending upon the organisms involved. Ophel (1960:21) for example, stated that "food is not necessary for Sr^{90} uptake by fish. They can accumulate high concentrations by direct absorbtion through their gills." Davis and Foster (1958:531) found that bottom animals, particularly herbiverous insect larvae, were even more radioactive than fish in the same water. They stated (1958:531) that:

> the relative proportions of the several isotopes differ from one organism to another not only because of dissimilarities in the chemical composition and physiological demands of the different forms but also because of the different sorption characteristics which vary with morphology.

When similar pairs of elements occur together, one element in abnormally high concentrations may tend to reduce the metabolism or uptake of the similar element by an organism. For example the presence of Ca or Mg in water reduces the uptake of Sr^{90} (Ophel, 1960:21)

Isotope uptake is also affected by the physiological condition of organisms as influenced by food, temperature, and other factors. It appears that food chains tend to "select for" essential isotopes and "select against" non-essential elements. Nelson (1960:81), though speaking of the aquatic environment, generalized that for any given chemical element, there will eventually be found at least one plankton species capable of spectacularly increasing without it. Concentration is related to exposed absorptive surfaces, absorption into tissues, and assimilation of ingested material. Davis and Foster (1958:530) noted that since the bulk of the essential elements of an organism is obtained from its food, assimilation is the major avenue of radioactive material accumulation. Pendleton (1952:127) warned, however, that "radioactive content in an aquatic animal is not conclusive evidence that the radioactivity was acquired as a result of food taking."

Ophel (1960:21) pointed out the environment differential that occurs in the uptake of isotopes: "It is evident that many of the barriers to Sr^{90} transfer that are found in dry-land communities are not present in fresh-water communities." Davis and Foster (1958:530) agreed that concentrations many times those of the environment can be built by some organisms, but comment that apparently there are many radioisotopes not biologically concentrated.

Nelson (1960:80) listed retention as a second factor. Retention of radioactive elements is dependent upon the metabolic turnover of the particular element with the organism (biological half-life), biochemistry of the particular element, and site of deposition. Kornberg (1956:20) listed three major processes in biological systems accounting for uptake, retention, or deposition as (1) flow, (2) diffusion, and (3) ion exchange. Ion exchange is a combination of absorption and chemical reaction.

Mode of elimination is a third factor, and includes ion exchange, diffusion, excretion, defecation, and unknown methods such as reported by Pendleton (1952:35). In studies of ant and aphid response to P^{32} labeled thistle plants he noted that:

> ...two insects have different methods of removing excess ions. The aphid apparently has a mechanism whereby excess materials coming out of the plant juices can be by-passed and eliminated through the honey dew when the supply necessary to the body has been achieved. The ant, however, appears not to lose this material except through natural defecation and transfer to others. This differential concentration offers a basis for future experiments on the aphids involving the mechanism by which such metabolites are by-passed.

Nelson (1960:81) stated that isotopic dillution effects probably cause decreased concentration by the biota. A non-linear relation for isotopic dillution has been found. At low and high concentrations of phosphorus with certain aquatic plants and animals, dillution was less effective in causing decreased biotic concentrations than at intermediate concentrations.

Davis and Foster (1958:533) found that:

> The specific activity of the river biota should be appreciably lower than that of the water not merely because of the time required to incorporate the isotope into the organisms but also because of the 'pools' of the elements fixed in the biota and sediments.

They noted that (1958:533) "with a single addition of an isotope into a 'static' environment, the specific activity will eventually become uniform throughout the biota." Crossley and Howden (1960) made similar findings in a terrestrial environment. Soil isotopes were so high that the radioactivity of the insect populations was a very slight part of the total biomass or radio-count.

Isotopic Measurement of Insecticide Distribution

Ladd *et al*., (1946) and Jenkens and Davis (1952) used isotopes of Mn^{54} and Au^{198} for studying dissemination of airplane sprays. Isotopes as compared to dyes are more accurate, more rapidly assessed by fewer personnel, and depositions on vegetation and uneven surfaces can be measured without panels or other sampling devices. In addition, they are independent of light, temperature, pressure, and molecular state of the nuclide; present no solubility problems as with dyes; and have well known dillution phenomena. The method is applicable with volatile chemicals. In their tests, Ladd *et al*., (1946:1) found that dye and isotope methods agreed within 20%, the isotope tracer giving higher values. They state that the sensitivity of the tracer method is more than 100 times that of the dye procedure.

The disadvantages of the use of isotopes are the expense, variable decay rates, and handling difficulties and hazards. A half-life of 1 to 30 days is most desirable for simple dispersal studies. Where factors of mollecular-tagging or other project objectives must be considered these limits do not apply. If the half-life is too short there is not adequate time for suitable field work with its customary delays due to weather and other factors. If the half-life is too long, the area and the equipment may be so contaminated as to prevent subsequent tests.

Ladd et al. (1946) used 60 mc of manganese-54 napthenate in a DDT formulation to test spray dispersal. They used two 15.4 x 91.6 cm (6 x 36 in.) blotter panels and one 27.9 cm (11 in.) enamel plate every 30 ft along two 375 ft strips. They could detect quantities to 0.001 lb/acre of DDT by rolling blotters around the G.M. tube and counting activity. Jenkins and Davis (1952) used gold-198.

Many factors modify the dispersal of aerial spray. Any list would be incomplete. From the nozzle where droplet size and production are relatively unknown for the conditions of flight, to the ground where all factors have had their effect, knowledge of spray particle size and movement is needed.

Like most studies, this one was concerned with final spray size and amount of application per unit area. The observed particle size is of a droplet that has undergone the action of all the environmental factors and has escaped interception except by the sampling device, which also modifies its size expression. Davis and Elliott (1953) stated that:

> The indicated deposit may be expected to show some reduction due to evaporation. The amount of reduction will be somewhat proportional to the vapor pressure of the insecticide at the ambient temperature and to the time required for the spray to settle, and inversely proportional to the square of the drop size.

Most aerial applications have shown a loss in insecticides before they reach the ground or sampling devices. Sailer and Rozen (1958:4) observed that malathion applied by biplane at 2 lb/acre was deposited at 0.063 and 0.034 lb/acre; at a rate of 1 lb/acre, deposited at 0.040

and 0.054 lb/acre. These measurements were from oil-sensitive spray assessment cards placed at 66 ft intervals along diagonal sampling lines in each plot under study. Cope (1960:18) reported that an application of 1 lb/acre of malathion on alfalfa near Jackson, Wyoming, resulted in 0.53 and 0.73 ppm reaching the ground. He noted that up to 0.04 ppm (about 0.1 lb/acre) was in nearby water although direct spraying of waterways was avoided. Davis et al. (1956) listed 18 comparisons of lb/acre applied and lb/acre deposited. The mean ratio was 1:0.2.

The deviation of spray from the flight path has been noted by Isler and others (1955, 1957, 1961). Carleton et al. (1960:75), for example, stated:

> Research today indicates that spray discharged along the lateral axis of an airplane will follow the air currents set in motion by its passage through the air, until these forces will no longer support the weight of the spray droplet. These principal airstreams, which affect spray distribution, are generated by an airplane in flight. These are the right and left wingtip vortices and the propeller vortex. These vortices, plus the effect of gravity, determine the path of the spray discharge and the resultant deposit pattern.

They noted that sprays are rotated to the side by the plane propeller. The results of a plane crash on 15 May 1962 in the study area after one spray swath is shown in Fig. 52. The wide variability of the deposition pattern can only generally be explained. Wind was less than 2 mph. The above mentioned aerodynamic forces offer the only explanations. The high concentration area to the west at 1-21 is the most difficult to explain. It indicates variability and points up practically the unlikelihood of avoiding waterways by paralleling

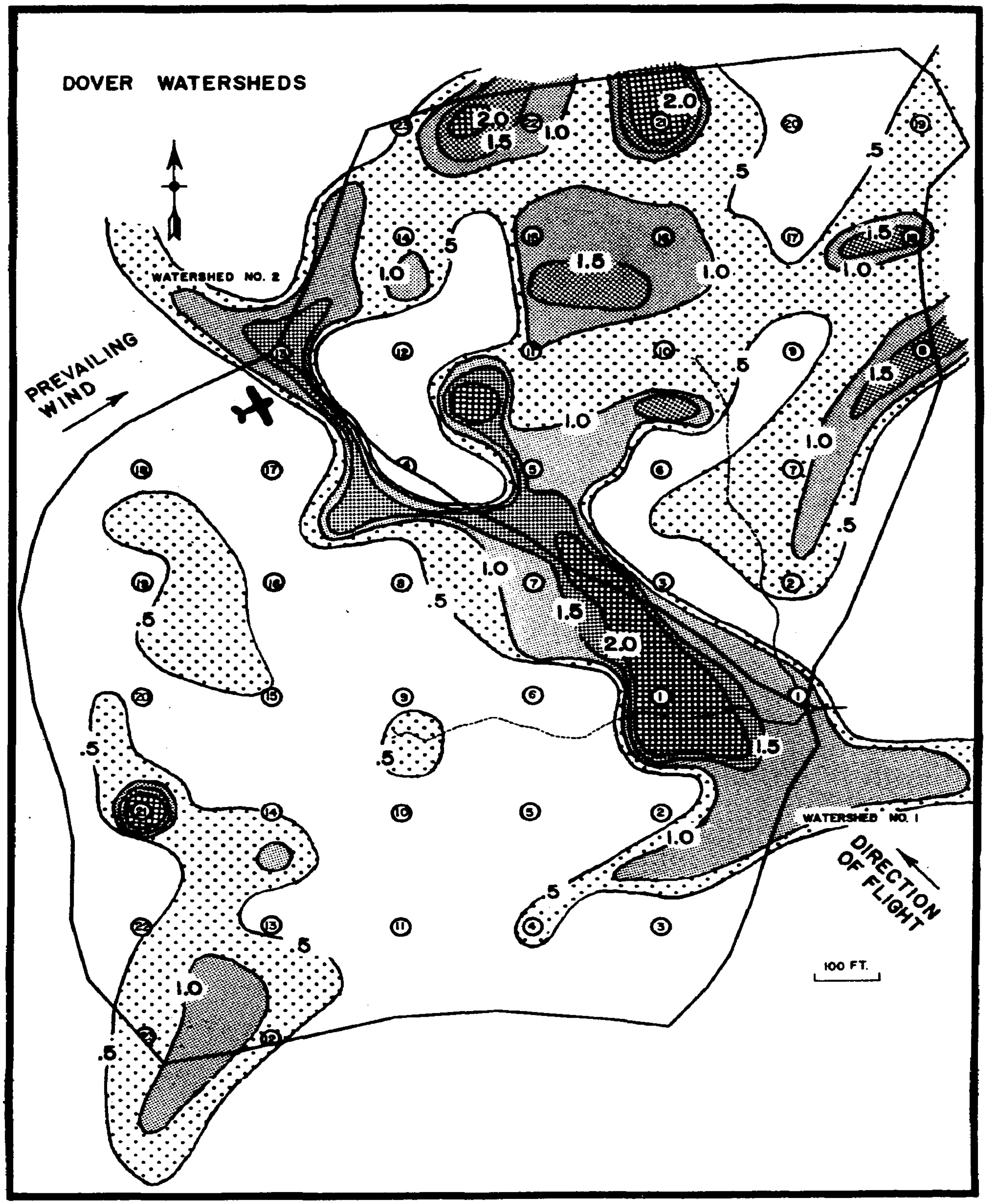

Fig. 52. Dispersal of the 15 May 1962 one-swath application measured by the mean of two frosted glass discs on the ground at all grid intersections, every 100 ft along the path between intersections, and every 50 ft along lines running at right angles to the boundaries. Position of the crashed plane is shown near 1-17.

them "a reasonable" distance away.

Spray Atomization and Dispersal Studies

Carleton et al. (1960:82) stated that:

> The problem of measuring chemical distribution is one of our most critical because all other research hinges upon it. This will include highspeed measurement of particle size distribution, quantitative measurement of chemical distribution on the soil, and quantitative mass distribution of chemicals on plant surfaces. Until we have these methods available, all other research on pesticide application equipment will have a corresponding lack of precision.

Hickey (1961:3) contended that ecological hazards seem more closely related to manner of use than to volume used. Certainly the two are related, for as Carleton et al. (1960:70) pointed out, since methods of application are relatively crude, control operators must use the simple expedient of applying more than minimum amounts of pesticides needed to control pests. These application excesses run up costs and residue hazards. For a single method of application (such as aerial, ground mist spray, etc.) it seems that hazard is related to volume used. However, total ecological hazards are related to volume and the number of habitat strata affected by the spray. Aerial application affects all strata so presents the most complex ecological problems.

In forest aerial spraying, control effectiveness depends largely on two factors, timing and atomization (Carleton et al., 1960:71 and Davis et al., 1956:338). Forces acting on particles are conditioned by weather and time of day. Pest control generally is inversely related to particle size, but fine particles are difficult to place.

An uneven, wasteful distribution is more common with the finer sprays. The balance of the factors of low cost, low gallonage, small particle size, and increased effectiveness is difficult to obtain. Failure to obtain such a balance has one outcome - ineffective control. Residue longevity can account for some gaps in control of motile pests, but insecticides such as malathion with short residual action and potentially small ecological hazards do not provide safeguards against non-treated gaps in area sprays.

In forest operations where large gallonage is required, fine droplet spraying is desired to materially reduce the load, and consequently to reduce the costs and the effects of weather and other factors on insecticide loss. Coarse atomization results in less vaporization and loss of spray but finer atomization allows wider spray swaths and more uniform deposits.

Spray deposition and assessment throughout the study was made on 2.54 cm (1 in.) diameter circular glass discs frosted on one side. After air drying, these were collected in pill boxes or envelopes, placed on planchets, and counted as described for the filtered samples.

Because the glass disc method of determining spray distribution was considered very reliable, attempts were made to correlate disc readings with more standard aerial spray assessment techniques. Rates of application have been measured by glass plates and blotters; oil, chemical, and moisture sensitive papers; isotope and dye techniques; and pilots' statements. Davis and Elliott (1953) have summarized the methods usually used and the disadvantages. The assessment of water-emulsion sprays is more difficult, expecially when droplet size is desired, than assessment of oil sprays. Dr. J. S. Yuill, USFS (pers. correspondence 15 March 1962) suggested assessment cards of woodbine enamel paper. The Appleton Coated Paper Co., Appleton, Wisconsin, donated thirteen 63.5 x 76.2 cm (25 x 30 in.) sheets of different color enamel paper for testing. Water droplets from a hand atomizer were sprayed on test cards. There appeared to be no difference in spotting by water and spray formulation. Emerald color, 25 x 38 - 160M, BS 80# base was judged best by three people to provide the most visible and distinct spots. Orange color, 25 x 38 - 160M, BS 80# was next best. These two were far more suitable than any of the other 11 samples tested.

For the 15 May flight, 2 emerald cards, each 10.2 x 10.2 cm (4 x 4 in.), were placed in previously installed wire holders which were located on opposite sides of and 30 cm (1 ft) from each grid stake. The wire holders were those described by Maksymiuk (1959b) and were of 16 ga. wire which, though useable, was too light. The cards were put out less than 1 hr before the flight and removed 6 hr later. After the crash, orange cards were used for the second flight. A light rain (unrecorded on the gauge) ruined 40% of the cards, most of them on watershed no. 2 following the 25 May 1962 application. Results of disc and assessment card counts are shown in Table 73 and 74.

The cards at each location were picked up, numbered and placed in a plasticine paper bag. The radioactivity of the cards was determined in a way perhaps more expedient than statistically sound.

Table 73. - Dispersed insecticide radioactivity in corrected cpm as measured from frosted glass discs and spray assessment cards from Dover, Ohio, watershed no. 1, 1962.

	25 May 1962				Maximum rainout post spray in cpm		25 May card
Location	Mean cpm	Max. cpm	Min. cpm	Median cpm	26 May 1 day	28 May 3 days	max. cpm
1-1	74.9	166.5	7.4	50.8	7.0	3.5	29.3
1-2	85.4	147.3	16.9	92.1	12.8	0	136.5
1-3	100.0	129.9	60.7	109.5	6.8	3.3	74.4
1-4	12.8	21.5	5.3	11.5	14.5	1.9	36.6
1-5	15.8	42.5	0	4.9	6.7	0	51.8
1-6	8.7	14.0	5.5	6.5	9.0	0	44.4
1-7	14.2	26.4	7.7	8.5	5.8	1.9	8.0
1-8	22.9	29.5	19.5	19.6	189.0	2.7	40.1
1-9	12.4	25.4	1.3	10.4	9.1	0	35.2
1-10	7.0	10.3	2.9	7.7	21.3	0	25.2
1-11	94.8	197.3	4.2	82.8	6.2	2.9	83.4
1-12	2.3	3.4	1.3	2.3	2.2	0	63.9
1-13	1.9	3.9	0	1.7	3.5	0	18.5
1-14	4.2	6.8	1.6	4.3	4.7	0	16.5
1-15	16.7	34.2	7.8	8.1	1.8	0	27.1
1-16	14.7	23.4	8.9	11.9	2.4	0	60.5
1-17	7.0	8.4	4.5	8.0	1.7	2.4	12.8
1-18	4.0	4.3	3.5	4.1	4.2	3.4	9.5
1-19	7.4	15.7	1.6	4.9	5.8	0	35.4
1-20	13.2	32.4	3.1	4.2	2.3	1.8	35.1
1-21	7.9	13.4	3.1	7.3	2.7	0	132.2
1-22	295.5	381.8	167.0	337.8	96.0	3.3	359.0
1-23	3.3	4.8	1.6	3.6	4.9	2.2	103.7

Table 74. - Dispersed insecticide radioactivity in corrected cpm as measured from frosted glass discs and spray assessment cards from Dover, Ohio, watershed no. 2, 1962.

Location	25 May 1962 Mean cpm	Max. cpm	Min. cpm	Median cpm	Maximum rainout post spray in cpm 26 May 1 day	28 May 3 days	25 May card max. cpm
2-1	3.7	5.8	0	5.4	0	0	3.1
2-2	2.0	2.3	1.8	2.0	29.0	0	3.2
2-3	2.0	3.4	0	2.5	31.0	0	5.7
2-4	4.8	6.8	3.2	4.4	0	3.5	12.7
2-5	12.0	13.2	11.4	11.5	0	2.2	13.9
2-6	3.9	4.6	3.5	3.7	0	0	2.7
2-7	3.0	6.1	0	3.0	0	2.4	0
2-8	0.9	2.6	0	0	0	0	0
2-9	0.6	1.7	0	0	0	0	13.4
2-10	1.8	3.1	0	2.3	0	3.1	3.5
2-11	3.3	6.0	0	3.8	0	1.7	4.5
2-12	2.0	3.0	0	2.9	0	0	9.3
2-13	6.5	9.1	2.8	7.7	31.0	2.1	11.3
2-14	0	0	0	0	0	0	3.2
2-15	0	0	0	0	0	2.6	0
2-16	0	0	0	0	0	2.4	0
2-17	0.5	1.5	0	0	0	0	0
2-18	0	0	0	0	0	0	0
2-19	0	0	0	0	16.0	0	2.2
2-20	0	0	0	0	0	3.1	2.3
2-21	0	0	0	0	0	0	0
2-22	0.6	1.9	0	0	0	1.7	0
2-23	0.5	1.4	0	0	31.0	0	0

In the laboratory a circular disc of 2.98 cm (1.25 in.) diameter, with an area of 7.93 cm^2 (1.23 $in.^2$), was cut at random from one quarter of each card, placed in a planchet and counted for radioactivity. This method seemed to meet the secondary interest of the study in comparing visual spray deposit assessment methods with known radio-activity counts from the same cards or from other sampling devices.

Pirie's reagent did not digest the paper as it did biological samples. The planchet-size paper disc was considered an infinitely-thick sample and counted without further treatment. There are some unmeasured absorption properties of the paper for beta activity which were known to influence the readings.

Counting numbers of droplets and measuring droplet sizes on assessment cards is a tedious, difficult task with much possibility for error. While the comparison of cards with previously prepared standards is a desirable method (Davis and Elliott, 1953), no such standards were available for water emulsion on Woodbine enamel paper.

Thornton and Davis (1956) recognized the difficulty of direct counts for measuring mmd and determined a sampling technique for counting card spots. A constant-area sampling intensity was used (7.93 cm^2 or 1.23 $in.^2$) which was well over that specified as desirable (0.5 $in.^2$) when droplet sizes exceed 312 microns.

Cards were selected from both flights that had obvious dense spotting from the spray. Five were selected from the green cards of the one swath flight; seven were selected from the orange cards. A circle of the same diameter (2.98 cm) as that removed from each card was drawn with a compass slightly off center of the remaining card. The spots within the circle (7.7% of the total area of the card) were measured and tabulated.

Using a microscope with reticle 20 droplets were measured of malathion formulation suspended on the tip of a finely drawn glass thread. The droplets were placed on each of the two colored assessment cards and allowed to dry. The spots were then measured with the same microscope scale and the measurements converted to microns. A spread factor or ratio of the mean spot size to the mean droplet size was obtained (Table 75). (See Maksymiuk, 1959a.) Maksymiuk and Moore (1962:698) said that drop size is the source of the greatest variation in spread factor, hence all spread factor determinations should cover a range of drop sizes. Very small drop sizes are missing from this study so the factor obtained is slightly large.

$$\text{Spread factor} = \frac{\text{mean spot diameter on card}}{\text{mean spherical droplet diameter}}$$

The D-max reading was obtained from the entire card as described by Maksymiuk (1959a). The five largest droplet spots were picked by eye, circled with a pencil, measured to the nearest 100 microns, and tabulated in descending order of magnitude. One spot, termed D-max, was the largest spot diameter with not more than 200 microns difference between it and the next smaller spot.

The mean spot size was determined by dividing the total of the spot diameters by the number of spots. The median spot size is that one above or below which one-half the remaining observations fall. In the event of even numbers of spots, the larger observation was

Table 75. - A comparison of methods of assessing spray droplet sizes and actual rates of application on enamel coated paper cards taken from the Dover, Ohio, study area, 1962.

Flight description card type and location	Median spot diameter B	Weighted mean spot diameter C	D-Max A	Activity of card in cpm	Maximum activity of glass disc	Spread factor
Emerald: 15 May						
1-1	83	134	1300	14.0	8.0	
2-4	825	853	1300	0	0	
2-16	495	539	2000	0.8	2.6	
2-17	825	701	1000	0	0.3	
2-23	110	124	600	0	0	
$\bar{x}$	468	467	1240	2.96	2.73	2.91
Orange: 25 May						
1-3	770	798	1700	95.4	129.9	
1-11a	1100	1062	3300	83.4	197.3	
1-11b	1100	1093	1800	110.7	197.3	
1-14	495	619	1700	18.2	6.8	
1-16	1430	1368	2000	60.5	23.4	
1-22a	743	847	2000	235.6	381.8	
1-22b	963	914	2900	359.0	381.8	
$\bar{x}$	943	957	2200	137.5	188.3	2.66

selected as the median.

The conversion factors (D-max divided by the mean of the median spot diameters) of 2.6 for the emerald paper and 2.3 for orange paper are very close to 2.2 ± 0.08 reported by Maksymiuk (1959a). A similar manipulation of figures provides almost identical conversion factors of 2.7 and 2.3 when the mean D-max is divided by the mean of the weighted mean spot diameters. The conversion factor makes use of the measured relationship between median diameter and D-max to convert subsequent D-max observations to the more meaningful mass median diameter (mmd).

$$\text{Estimated mmd} = \frac{\text{spot D-max}}{\text{spread factor x conversion factor}} .$$

The estimated mmd for the 15 May 1962 flight was 177; for the 25 May flight, 360. In comparison the weighted mean diameter (wmd) was 161 and 360 for the two flights. This observation substantiates the use of D-max in estimating the mmd.

A comparison of the radioactivity of the cards and the maximum glass disc reading in cpm yielded a significant correlation coefficient, r, of 0.97 for both spray flights. Measurement of isotope labeled aerial spray therefore seems equally suitable by either cards or glass discs.

The activity of the discs was 1.37 times that of the cards. Though not significant, the correlation coefficient of the D-max and card activity of the 25 May flight was 0.41. This seems to indicate that the system of D-max though mechanically accurate, does not accurately estimate the actual amount of deposition. This observation can only be interpreted in the light of the relationship of desired droplet size for a particular spray application. The ratio of D-max to cpm is 1:16. The data indicates that D-max overestimates actual quantity of deposition more frequently than it underestimates it.

Isler and Maksymiuk (1961:8) found that test helicopter flights provided an estimated 144 micron mmd compared to a measured mmd of 137. They classified this droplet size as within the medium atomization range of fixed wing spray planes used on forest insect control jobs. Davis <u>et al</u>. (1956:340) set the bounds of coarse, medium, and fine atomization as 452, 291, and 151 respectively. They found that a medium atomization and 150 mmd proved more consistently effective than coarser or finer atomization of DDT sprays for spruce budworm control. Isler and Thornton (1955) found that a mmd of 150 microns provided the most efficient swath pattern for forest spraying. Finer sprays gave a wider, more uniform swath than medium sprays, but more spray did not reach the forest floor. Coarse sprays resulted in narrow swaths, high deposit peaks, and reduced uniformity of distribution across the swath. These observations are supported by Courshee (1960:330).

Figures 53 and 54 show the variable pattern of spray distribution on the treated watersheds and drift off the areas as measured from spray assessment cards for both flights. The difference between Fig. 52 and 53 reflects a difference in sampling intensity - there were more discs than cards and they were more closely spaced. A comparison of Figures 55B and 56B suggests the difference in results caused by two different sampling intensities. In Fig. 55 only those discs at 200 ft intervals were considered for purposes of comparison.

Fig. 55 shows the difference in the use of raw data (Fig. 55B)

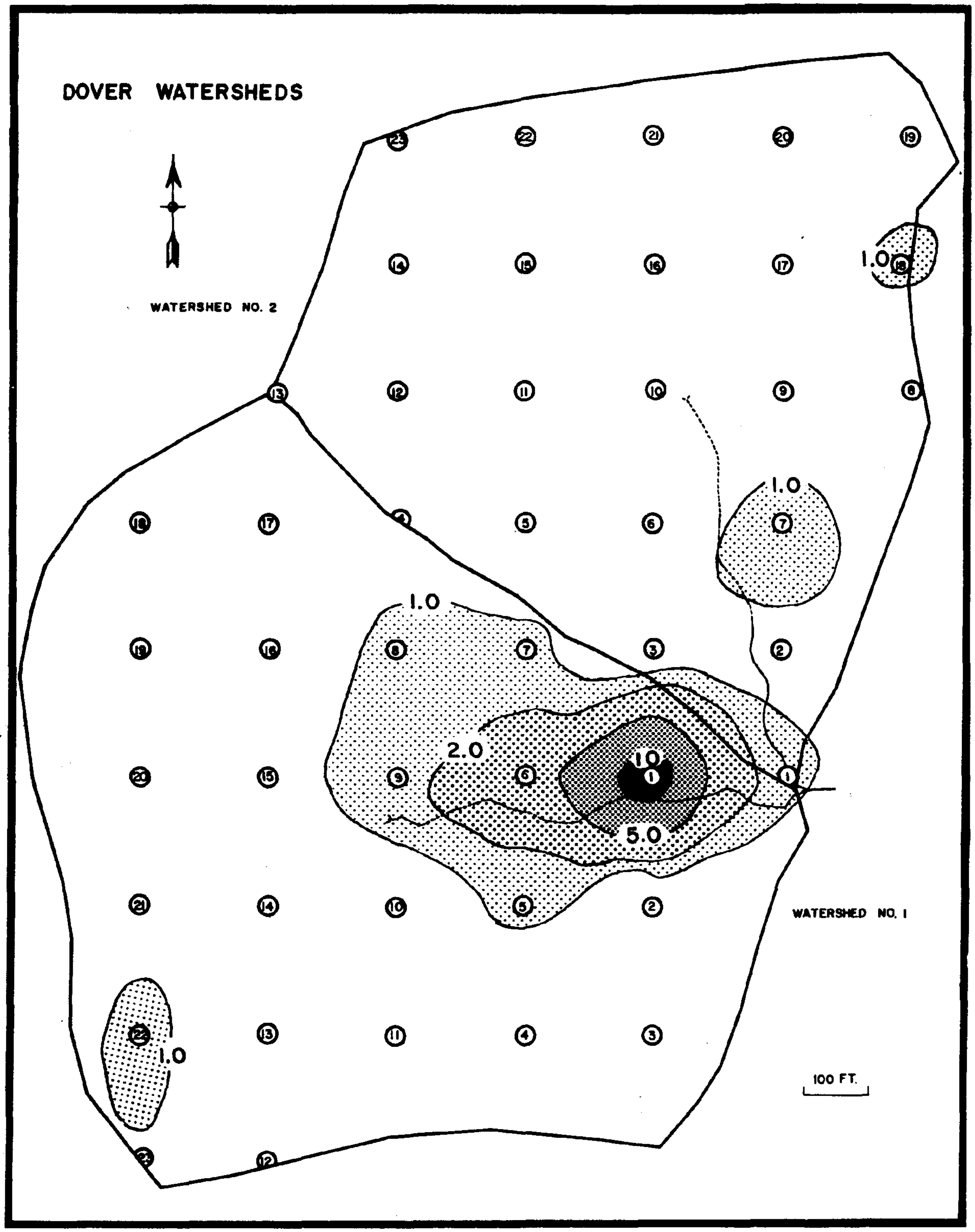

Fig. 53. Dispersal of the 15 May 1962 one-swath application as measured by the mean of two green enamel-coated papers 15 cm above the ground at all grid intersections.

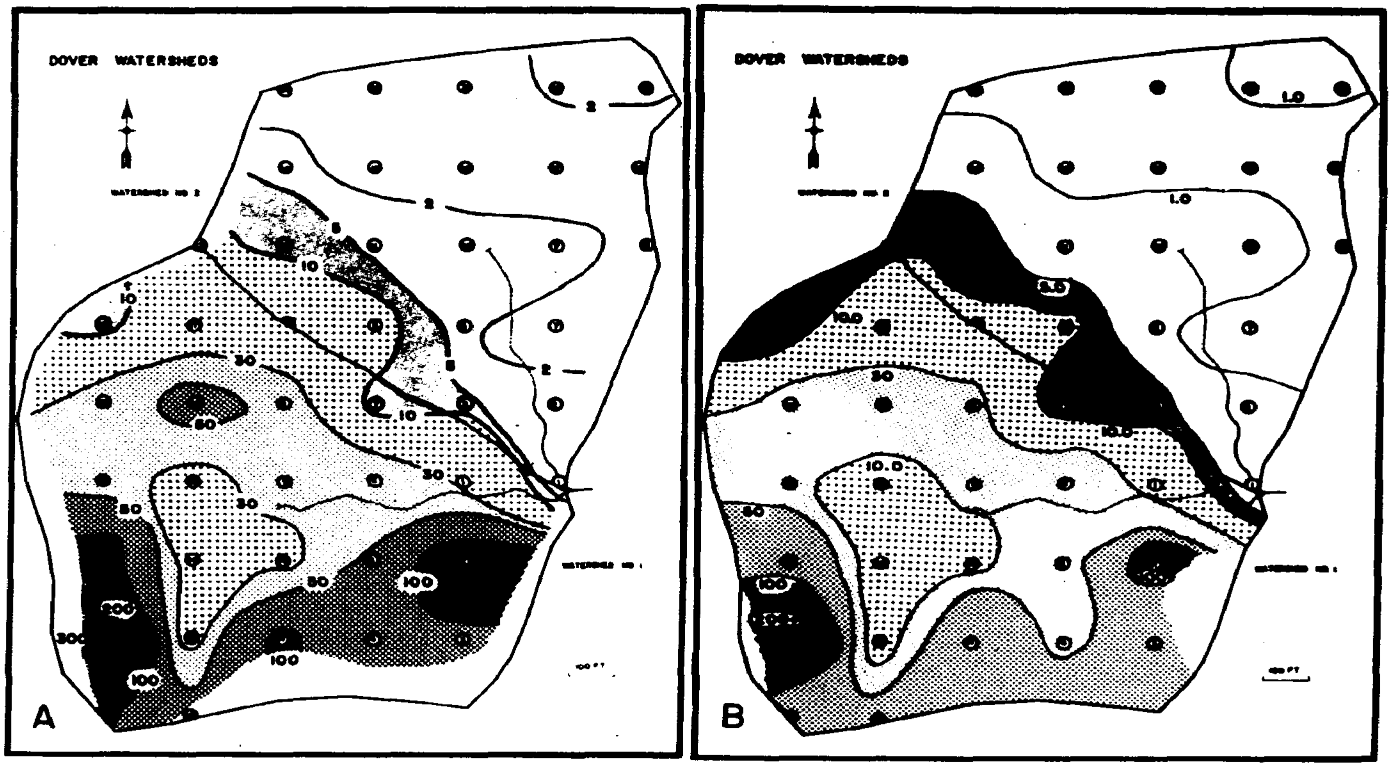

Fig. 54. A. Ground dispersal of malathion applied 25 May 1962 as measured by the maximum cpm of two orange enamel-coated cards at each intersection of the grid. B. Dispersal pattern measured from the mean cpm of the cards.

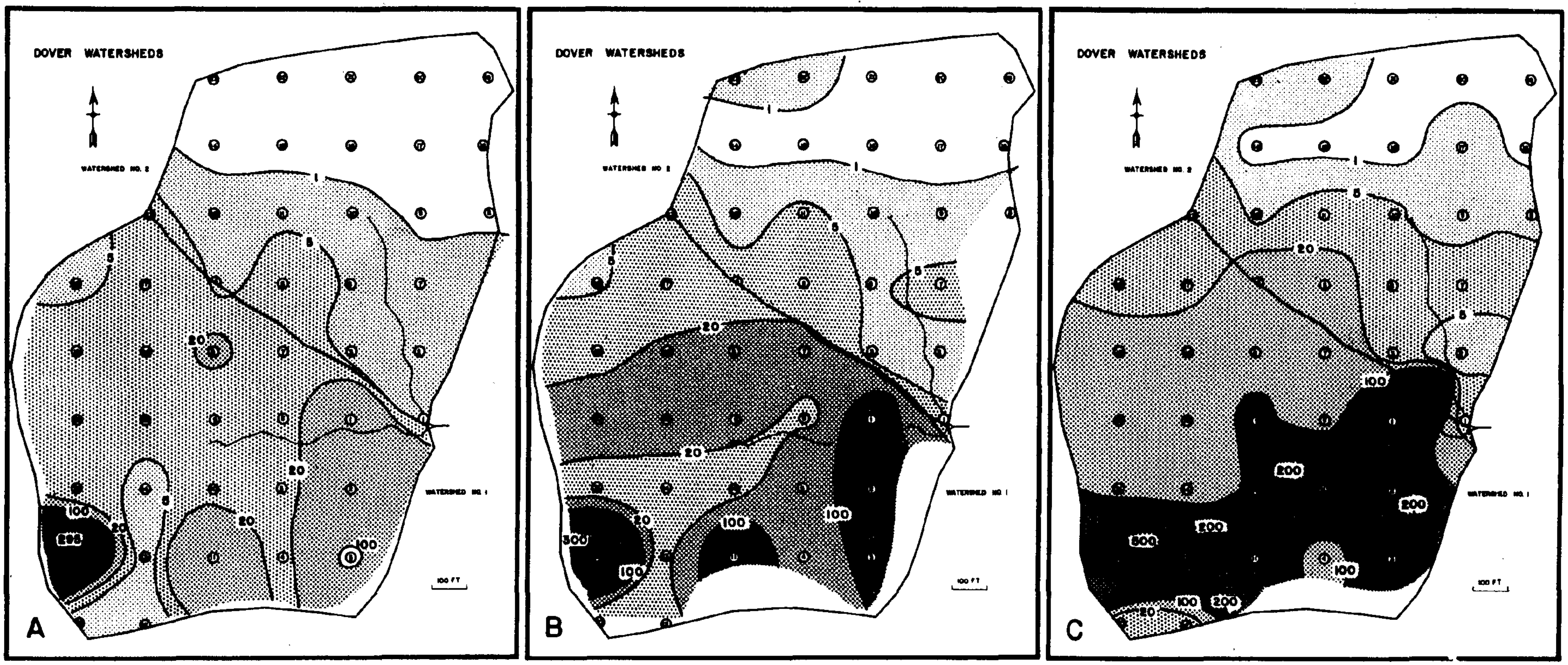

Fig. 55. A comparison of initial ground dispersal patterns of malathion on 25 May 1962 as measured by (A) the mean cpm, (B) the maximum cpm, and (C) the maximum cpm corrected for vegetative density. Only discs at grid points where vegetation was rated are considered.

DOVER WATERSHEDS,

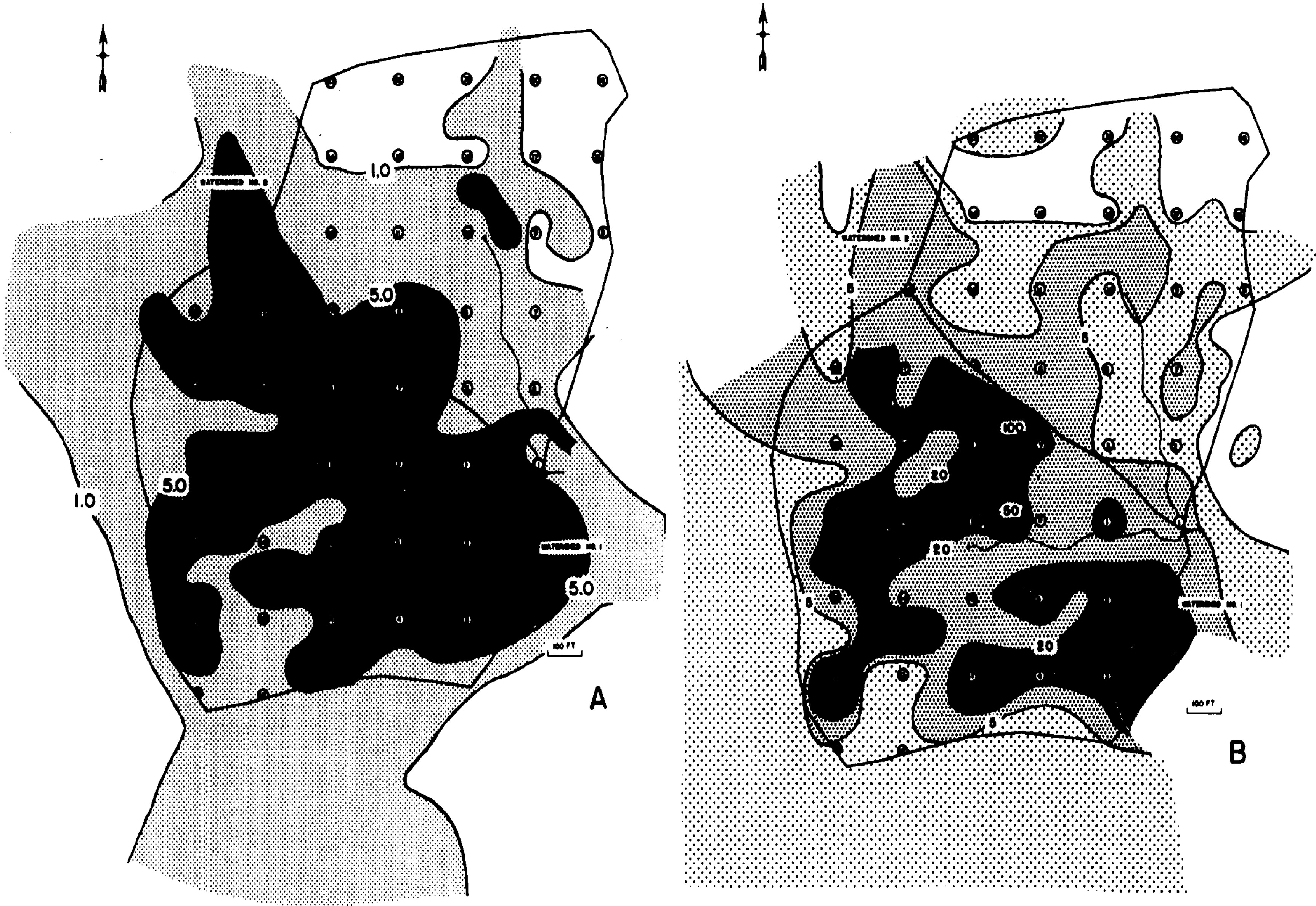

Fig. 56. Initial ground dispersal of malathion on 25 May 1962 as measured by (A) the mean cpm and (B) the maximum of three frosted glass discs on the ground at all grid points, 100 ft between all points, and every 50 ft along lines running at right angles to the boundaries.

and transformed data (Fig. 55A and 55C) to plot the distribution of spray. There appear real differences and these will be discussed in the next section. Figures 56A and B show the amount of insecticide initially reaching the ground.

Malathion was "applied" to the watersheds for over 3 days after the airplane had departed. Figures 57 and 58 show this secondary application or "rainout" from the forest measured from glass discs. Discs, picked up on 25 May were replaced with two discs and picked up again one day later, 26 May. These were replaced and picked up 2 days later, 28 May 1962. There had been 0.2 in. of rain on 26 May and 0.2 in. on 28 May. These rains may account for the generally negative correlation of the rainout on the two dates. The differences observed are somehow related to topography.

This secondary application is of interest in coordinating aerial applications with foliation, with insecticide half-life, and life histories of insects whose control is sought. The insecticide deposited, especially for long residual insecticides is the sum of the initial measured deposit, all subsequent rainouts, the amount volatilizing, the amounts adsorbed and absorbed by the vegetation, and the amount remaining airborne - never reaching the target area.

DISPERSAL

It is well known that amounts of spray applied and deposited within an area vary widely. Ground assessment cards or discs present a picture of the amount applied minus all the effects of the airplane-to-canopy and plant-to-card environment. The forest is the most

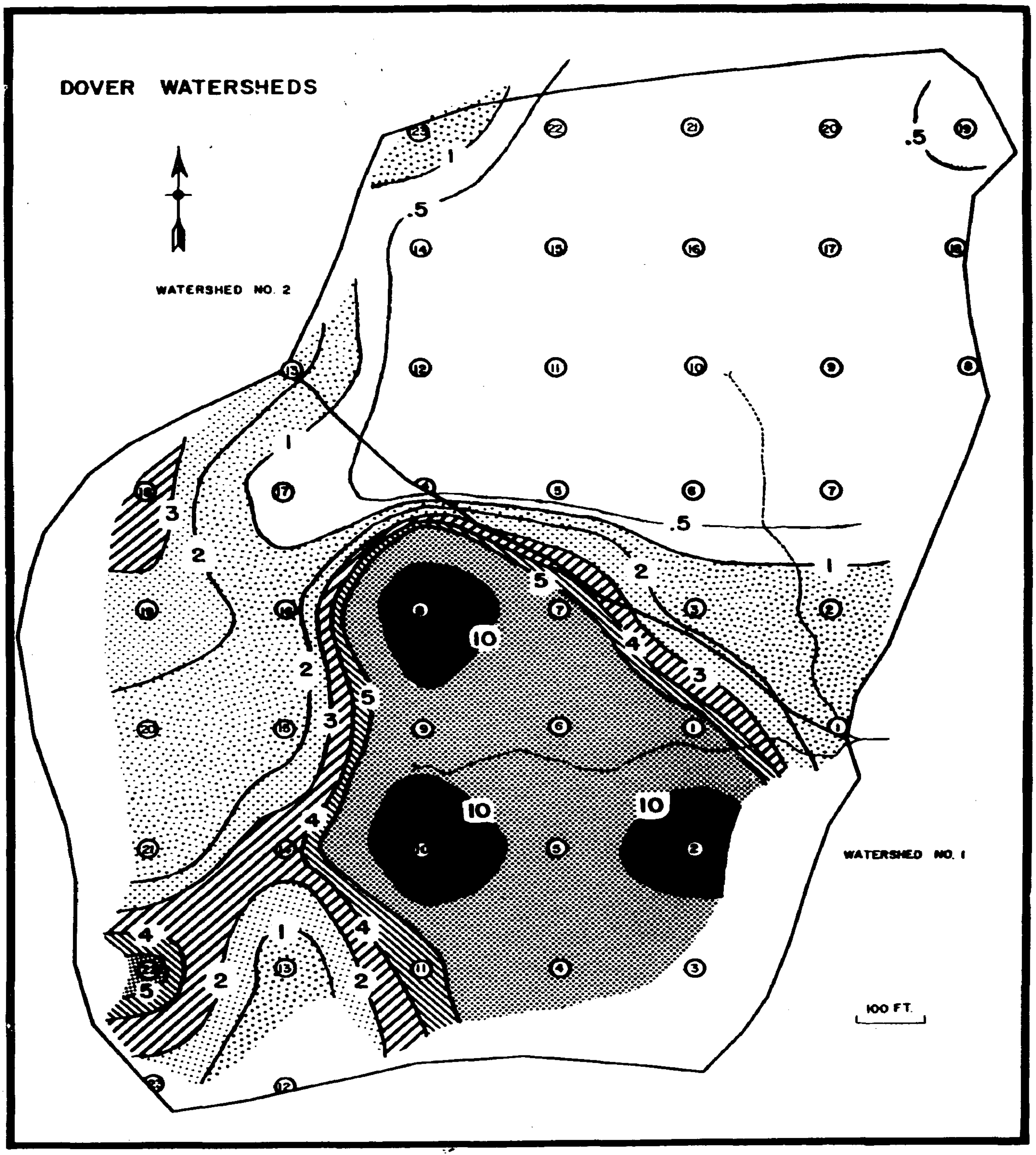

Fig. 57. "Rainout" of malathion from the forest as measured by mean cpm of two frosted glass discs on the ground at each grid intersection. Discs were in position from 25 May to 26 May 1962.

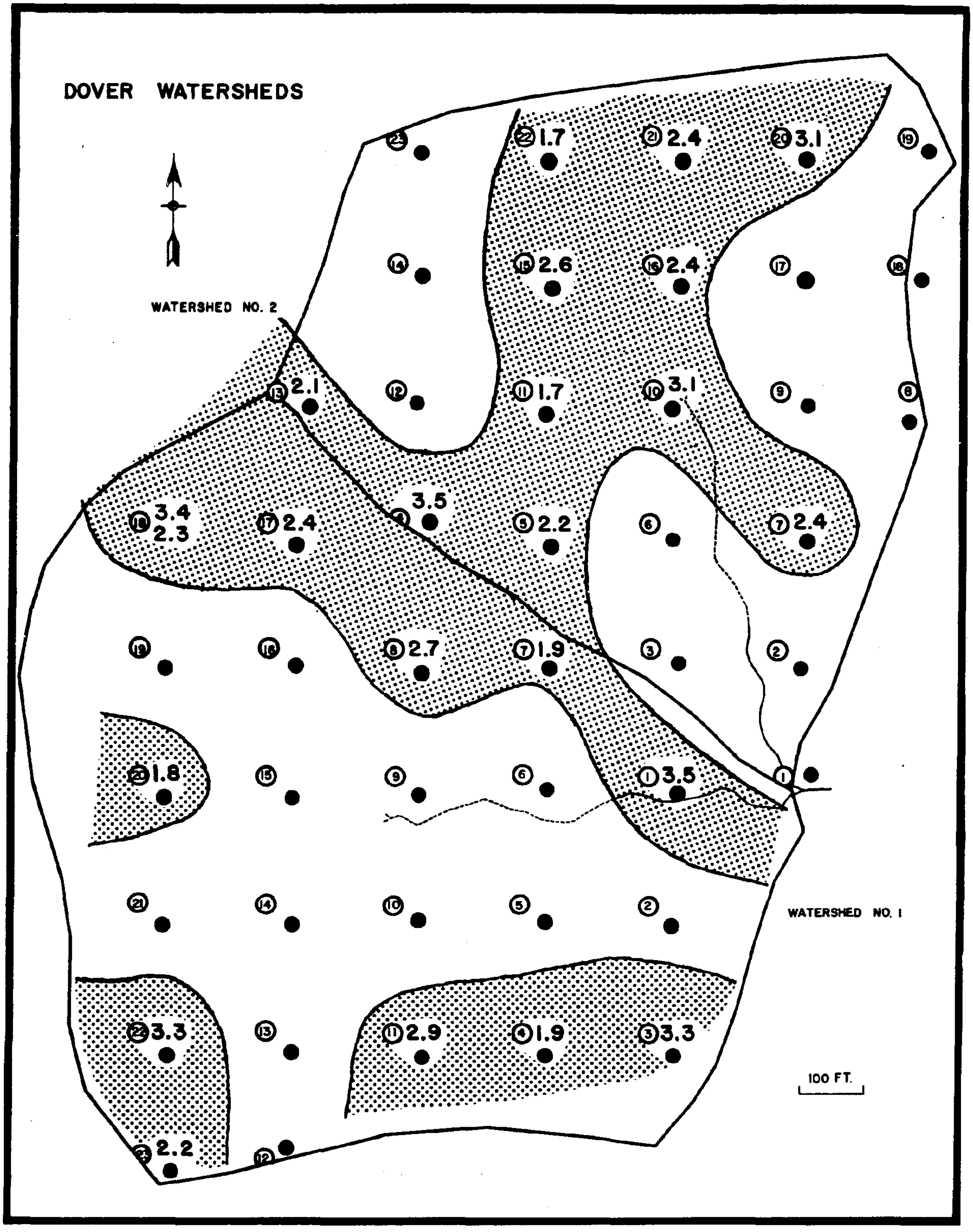

Fig. 58. Distribution of "rainout" from the 25 May spray flight as measured by the maximum cpm of radioactivity of two glass discs at 200 ft grid intersections. Black dots are zero readings on the discs, all of which were removed from the field on 28 May 1962 after being in place 2 days.

complex of such environments and its effects, varying from direct interception to micro-turbulence, greatly influence the measured ground deposition.

Dale's (1962:827) calculations of approximate crown-surface area suggest that a canopy surface area can be calculated for an area and that it is in excess of ground acreage by a factor of 2.2 for paraboloid-shaped tree crowns. This coupled with increased lateral surface area as compared to horizontal acreage measurements, increases the surface acreage and decreases the actual amount applied per acre.

Maksymiuk (1963) reported that at distances shorter than three tree heights away from the nearest tree, the deposit on cards is not a true measure of the application on a surrounding forest area. Tree spacing is therefore a function of deposition measurements.

It is obvious that canopy density influences the amount of ground-deposited sprays. An attempt was made to reduce numerically the effects of the forest on the glass disc deposition readings. In a meter diameter column over each of 46 sample points within the watersheds, the vegetative density was estimated by two workers and a density rating given to each of the three major strata-tree, shrub, and herbaceous. A rating of 1 was equivalent to "heavy" or 70-100% canopy closure; 2, moderate, 30-70%; 3, light, 1-30%; and 4, canopy absent. See Table 76 and 77.

Vegetative layers or strata within the forest do not have equivalent interception characteristics nor equal effects on insecticide re-distribution between strata. This is believed to be partially due to the distance between strata canopies; the greater the

Table 76. - Rated density of three forest strata and calculated TFR or throughfall rating for Dover, Ohio, watershed no. 1, 1962.

Location	Tree	Shrub	Herb	TFR
1	2	3	2	0.53
2	4	1	3	0.41
3	2	4	3	0.74
4	1	1	3	0.05
5	1	2	1	0.20
6	1	3	3	0.43
7	1	1	4	0.07
8	1	3	4	0.45
9	1	1	2	0.03
10	1	1	1	0.01
11	4	3	4	0.81
12	3	4	1	0.82
13	1	1	1	0.01
14	2	2	2	0.32
15	1	4	3	0.62
16	2	1	4	0.19
17	1	1	4	0.07
18	4	1	3	0.41
19	4	1	2	0.39
20	2	1	4	0.19
21	4	1	1	0.37
22	3	4	2	0.84
23	1	1	3	0.05

Table 77. - Rated density of three forest strata and calculated TFR or throughfall rating for Dover, Ohio, watershed no. 2, 1962.

Location	Tree	Shrub	Herb	TFR
1	4	1	3	0.41
2	1	4	4	0.64
3	1	2	3	0.64
4	1	1	2	0.03
5	1	1	3	0.05
6	1	1	3	0.05
7	4	1	4	0.43
8	2	1	4	0.19
9	1	4	3	0.62
10	4	1	3	0.41
11	1	3	4	0.45
12	1	3	4	0.45
13	2	1	4	0.19
14	2	1	1	0.13
15	4	2	3	0.60
16	2	2	4	0.38
17	1	1	4	0.07
18	1	1	4	0.07
19	3	1	3	0.29
20	2	1	3	0.17
21	2	1	3	0.17
22	1	1	4	0.07
23	1	1	3	0.05

distance the more homogeneous will be the unintercepted spray. Where I is the total amount of spray intercepted by vegetation and I_T, I_S, and I_H the amounts intercepted by each strata, tree, shrub, and herbaceous, then

$$I = I_T + I_S + I_H \cdot$$

As the spray falls, and the functional density of the forest, D, i.e. the vegetation intercepting the spray then may be represented by I. The amount of spray reaching sampling devices (S) approximates the amount applied (A) minus that intercepted (I) or

$$S = A - I .$$

By weighting the vegetative density of each stratum a more accurate picture is gained of the total influence of the forest on the amount of spray measured by ground assessment devices. The vegetative density observations can range from 1,1,1 (all layers heavy) to 4,4,4 (all layers absent). A Throughfall Rating (TFR) can be calculated which is the mean of the weighted density observations minus 0.32, a conversion which allows an easier mental ranking of throughfall on the basis of 0.01 to 1.0. This is an expression of the contribution of the density of each of the three strata to the numerical TFR. In order to determine the amount each stratum should be weighted, a multiple regression was calculated with the maximum cpm of the three discs on 25 May set as the dependent variable, y, and the three strata set as independent variables. The following equation resulted:

$$y = 25.6\ T + 36.7\ S + 7.9\ H - 86.5.$$

This equation and others in Table 78 indicate that in terms of

Table 78. - Partial regression coefficients for multiple regressions of insecticide deposition on vegetative strata densities from Dover, Ohio, watershed no. 1, 1962.

	Sum	Mean	Standard Deviation	b_o	Cover Density Tree b_1	Shrub b_2	Herb b_3
Maximum of 3 discs on 25 May	1343.1	58.4	90.6	-86.5	25.6	36.7	7.9
Mean of 3 discs on 25 May	827.0	35.9	64.6	-57.5	15.8	26.2	3.8
Minimum of 3 discs on 25 May	335.4	14.6	35.5	-23.2	4.3	14.0	0.6
Median of 3 discs on 25 May	802.5	34.9	73.0	-62.7	17.4	27.9	2.8
Maximum of 2 discs 1 day after spray (rainout)	160.3	6.9	5.3	10.3	-1.0	-0.3	-0.3
Maximum of 2 discs 3 days after spray (rainout)	29.3	1.3	1.4	-1.3	0.1	0.3	0.6
Maximum of 2 orange spray assessment cards on 25 May	1439.1	62.6	74.0	-2.6	2.2	1.9	-6.3

relative contribution of (T) tree, (S) shrub, and (H) herb strata to the total amount of throughfall, y, the proportions should be 0.36, 0.52, and 0.11 (Table 79). These proportions were obtained by

$$\frac{b_1}{\sum b_{1,2,3}}, \quad \frac{b_2}{\sum b_{1,2,3}}, \text{ and } \frac{b_3}{\sum b_{1,2,3}} \quad \text{(Table 79).}$$

Based on the number of times the regression coefficient for the herb strata influence was insignificant by t tests, it appears that the regression on the median cpm provides the best estimate of appropriate values for calculation of TFR. They are therefore, 0.36, 0.58, and 0.06. The proportionate influence of the three strata is therefore 0.12, 0.19, and 0.02 and TFR becomes

$$TFR = 0.12\,T + 0.19\,S + 0.02\,H - 0.32.$$

Table 79. - Proportional influence of the three forest strata of Dover, Ohio, watershed no. 1 on the amounts of insecticide actually reaching the ground.

	Proportion of influence		
	Tree	Shrub	Herb
Assumed strata influence on maximum cpm	0.18	0.22	0.60
Actual strata influence on maximum cpm	0.36	0.52	0.11
Actual strata influence on mean cpm	0.35	0.58	0.08
Actual strata influence on minimum cpm	0.23	0.74	0.03
Actual strata influence on median cpm	0.36	0.58	0.06

It is expected that the higher ground disc readings will correlate positively with TFR; the higher the throughfall, the higher the deposition; the greater the vegetative density the greater will be the interception. Lack of correlation is the influence of variable amounts of spray applied above the canopy and the inadequacy of the rating to properly account for insecticide loss. Though the assumption may not be entirely true, it is thought to allow a more meaningful expression of dispersal than the raw data. Vegetative density rating based on light intensity would improve the estimates of actual application.

Comparing TFR with the maximum glass disc reading, a signficant correlation coefficient of 0.61 was obtained. Compared with mean disc readings r was 0.19 but was not significant.

From glass discs left out 1 day post spray, a negative correlation would be expected since higher quantities of interception would "apply" the spray. Such was the case.

The total ecological effects of insecticide deposition are believed to be more closely correlated with the maximum reading on the sampling units (card or glass disc) than with the mean of the readings at any one point. Spray assessment units on the ground at any small area would be expected to receive near identical deposits. Such is not the case. For example, at one sampling point in watershed no. 1 three glass discs within a 1 ft area received 217.4, 42.7, and 16.3 cpm of insecticide from the 25 May flight. If A is the amount applied to the top of the tree canopy; D, the amount deposited in a 900 cm^2 (1 ft^2) area on the ground; and I the amount of spray intercepted by trees, evaporated, etc., then D = A - I. If A is assumed constant for a given small area such as the 900 cm^2, then D varies with I, not A, and therefore the best estimate of the A is the maximum D reading or the D with the minimum I influence. The best estimate of the actual amount of spray applied to a large portion of a forest or to an entire forest is obtained by:

$$\frac{\text{maximum observation at each assessment point}}{\text{TFR's at each assessment point}}$$

This is simply the average maximum deposition increased by a factor (the average of the TFR's) to account for the interception effects of variable forest strata canopy densities.

PATTERNS OF DISTRIBUTION

Above the Forest

Glass discs were glued to the top of helium filled balloons tethered above the center of the treated area. Activity of the discs was 277.0, 217.6, and 206.1 cpm. This represents the approximate rate of deposition of 2 lb/acre of malathion on top of the forest canopy.

Two electric air samplers were used about one-fourth mile from the area to measure air contamination by the spray. One was a Gelman Nuclear Air Sampler, Gelman Instrument Co., Chelsea, Michigan, using a circular Type AM-5, 100 ct., polypore membrane filter; the other was a General Metal Works high volume air sampler, Model 2000, Cleves, Ohio, using a glass fiber filter web NOCT 75428, Mine Safety Appliance Co., Pittsburg, Pennsylvania, sampling respectively 100 ft^3

per hr, and 4000 ft^3 per hr. The high speed sampler had a rectangular glass fiber filter (8 x 10 in.) with 394 cm^2 area. Four planchet-size circular samples were cut from the center of each quadrant of each filter and counted for radioactivity. There was some self absorption by the sample so results reported are of minimal air pollution by insecticide.

The slower machine was operated from a farm house one-fourth mile to the west of watershed no. 2. The circular polypore filter was 13.8 cm^2 in area. A 50.7% sample was cut from the center of each filter to fit into a planchet. The time of operation, volume of air sampled, and cpm/ft^3 air sampled are presented in Table 80 and Figures 59 and 60. There are fluctuations in the concentrations that cannot be explained except by variable wind currents and sampling and counting irregularities. The inverse correlation of the radioactivity of the air on 25 May is accounted for by directional changes of the moving, contaminated air masses. The lower activity of the western moving air is thought to be due to the valley-position of the sampler; the eastern sampler was at a higher elevation and on an exposed knoll.

Since there are no standards and since the total volume of air involved is unknown, it appears impossible to calculate the proportion of air-borne spray. The pre-spray peak may represent natural radioactivity or a serious counting or tabulation error.

By conjecture only, if an air mass 50 ft above the canopy of the treated area were contaminated at the rate of 0.2 cpm/ft^3 of air, there would be 5.1×10^6 ft^3 of air with 1.0×10^6 cpm of activity. Since there were approximately 32,500 cpm per ml of formulation on

Table 80. - Results of air sampling one-fourth mile east and west of the Dover, Ohio, treatment area for both spray flights, 1962. Time of spray for 15 May flight was 8:32; for 25 May, 6:40.

Date and Length of Operation	Ft^3 air sampled	cpm per total filter	cpm/ft^3
Crash Flight			
Western Air Sampler			
14 May, 11:20 am to 15 May 5:10 am	1810	20.3	0.0112
15 May, 5:10 am to 9:48 am	440	40.0	0.0909
15 May, 9:48 am to 12:09 pm	200	7.3	0.0365
15 May, 12:09 pm to 1:11 pm	10	7.3	0.0730
Eastern Air Sampler			
15 May, 5:14 am to 5:29 am	1000	515.2	0.5152
6:14 am to 6:29 am	1065	638.4	0.5994
8:24 am to 8:39 am	1020	296.8	0.2909
9:24 am to 9:39 am	1050	140.0	0.1333
9:34 am to 9:49 am	1035	33.6	0.0325
9:49 am to 10:04 am	1125	184.8	0.1643
10:11 am to 10:26 am	1050	100.8	0.0960
10:32 am to 10:47 am	1050	162.4	0.1547
11:47 am to 12:02 pm	1005	201.6	0.2006
12:47 pm to 1:02 pm	1005	140.0	0.1393
Western Air Sampler			
24 May, 9:40 am to 8:30 pm	1090	19.3	0.0177
8:38 pm to 3:54 am	740	56.1	0.0758
25 May, 4:00 am to 12:08 pm	810	46.9	0.0579
12:10 pm to 3:50 pm	360	35.5	0.0986
3:50 pm to 9:15 pm	540	41.4	0.0767
9:15 pm to 8:47 am (26 May)	1150	24.6	0.0214
Eastern Air Sampler			
25 May, 4:09 am to 4:24 am	975	190.4	0.1953
4:25 am to 4:40 am	975	151.2	0.1551
4:44 am to 4:59 am	975	156.8	0.1608
7:04 am to 7:19 am	975	196.0	0.2010
8:12 am to 8:27 am	975	28.0	0.0287
8:29 am to 8:44 am	975	302.4	0.3101
8:46 am to 9:01 am	975	156.8	0.1608
9:02 am to 9:17 am	975	95.2	0.0976
9:19 am to 9:34 am	975	128.8	0.1321
9:36 am to 9:51 am	975	151.2	0.1550
9:53 am to 10:08 am	975	168.0	0.1723
10:32 am to 10:47 am	975	151.2	0.1550
11:05 am to 11:20 am	975	67.2	0.0689
11:45 am to 12:00 am	975	134.4	0.1378
12:45 pm to 1:00 pm	975	140.0	0.1436
1:45 pm to 2:00 pm	975	190.4	0.1953
3:00 pm to 3:15 pm	975	123.2	0.1264

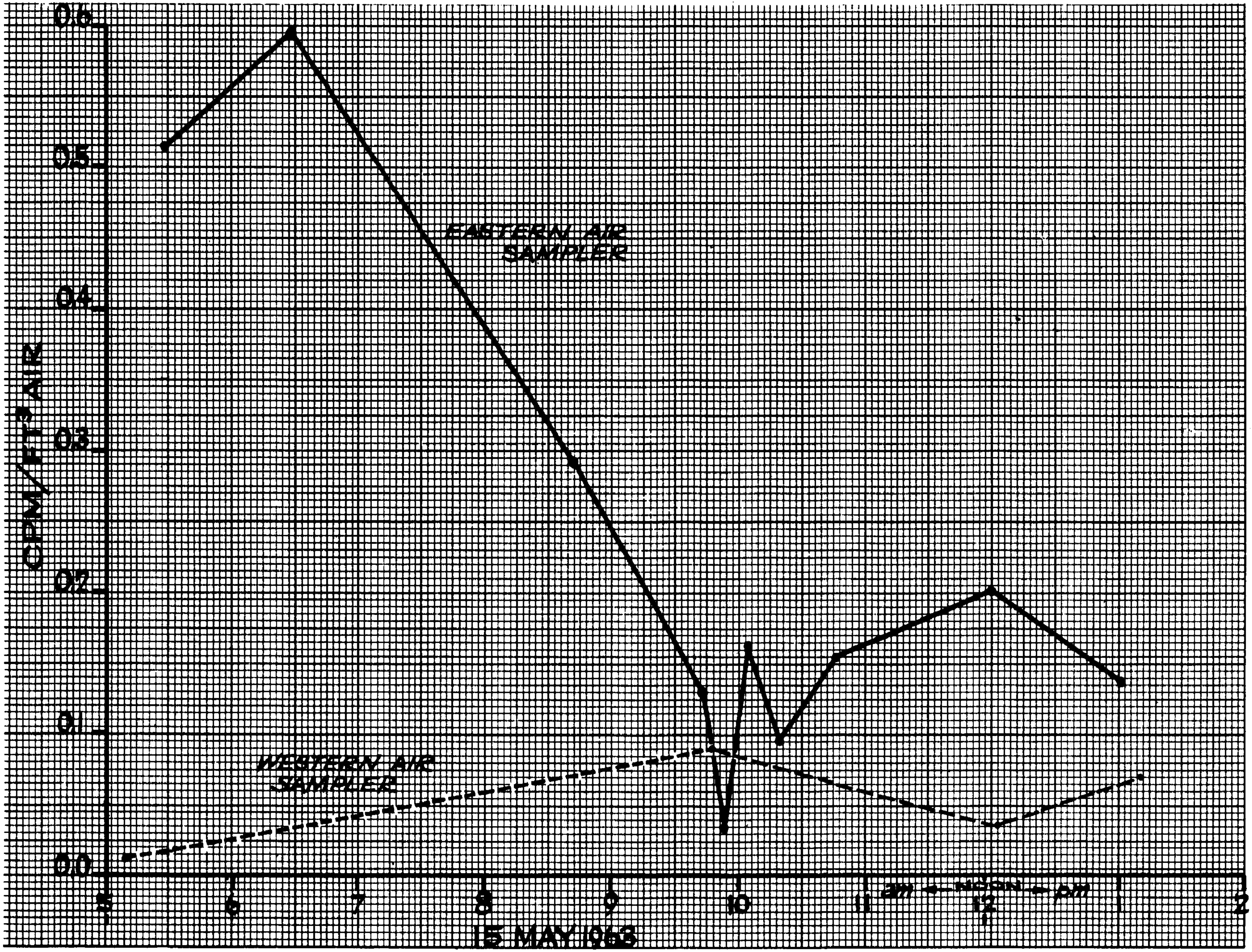

Fig. 59. Radioactivity of air samples taken from two locations near the Dover, Ohio, watersheds on 15 May 1963. Time of application was 8:32 am.

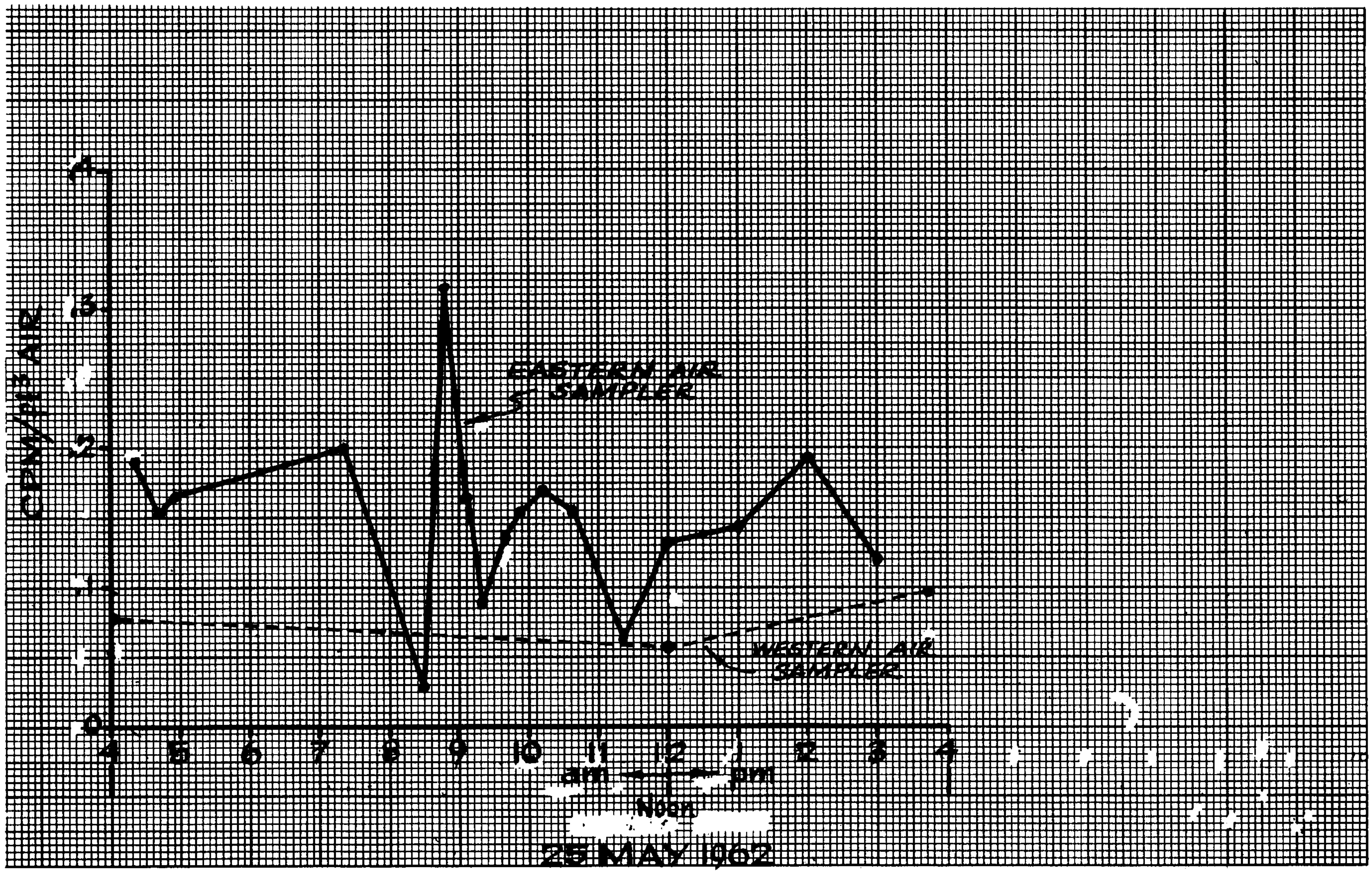

Fig. 60 Radioactivity of air samples taken from two loactions near the Dover, Ohio, watersheds on 25 May 1962. Time of application was 6:40 to 8:25 am.

25 May, there would only be 31 ml of formulation air-borne. Even if this were multiplied 10 or 100 fold, the amount of air-borne insecticide in relation to the total volume of formulation applied was very small. Reductions in amount applied in this study as measured by ground assessment apparatus is largely therefore a function of interception and not the amount blown away.

Within the Tree Layer

Glass discs were suspended on pulley-attached ropes within six trees in a transect through the forest (Fig. 61). Discs were at intervals of 5 ft. The uppermost 10 to 15 ft of the trees were not sampled. At no position within the tree canopies were readings as high as those recorded on the balloons over the forest. The upper 10-20 ft of canopy apparently intercepts a large percentage of the

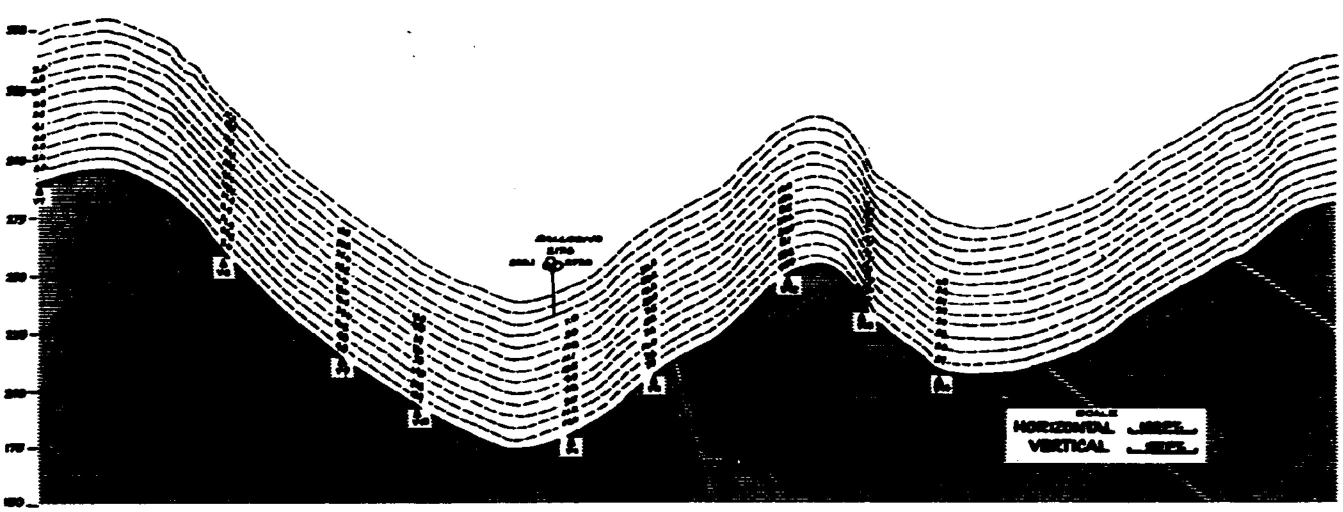

Fig. 61. Locations of suspended glass discs in trees along a transect across Dover, Ohio, watershed no. 1 and part of watershed no. 2. Numbers are cpm of radioactivity of the 25 May 1962 application. Trees were given V numbers.

spray. The remaining spray is intercepted irregularly throughout the tree layer. There is no apparent correlation between height above the ground and amount of spray (cpm) delivered. An hypothesis that trees on the upper slopes would receive more spray laterally than trees in the valleys was not verified by the data.

Bark samples were collected at heights within the trees in which the glass discs were suspended. The sampling of bark after spray would be easier for vertical spray distribution assessment than the use of discs but only a slight negative correlation existed between the radioactivity of the bark in cpm/mg and the glass discs in the five trees sampled. Bark sampling is ineffective for measuring vertical distribution of the aerial application of an insecticide within a forest.

Within the Shrub Layer

In an effort to discover the distributional pattern of insecticide within and under the shrub layer, glass discs were again utilized. Frosted glass discs were affixed with rubber cement to blocks spaced 15.24 cm (6 in.) apart on a 254 cm x 7.6 cm x 24 m (1 in. x 3 in. x 6 ft) pine board set at a 45° angle to the ground. (See Fig. 62.) Three of these devices were placed in the center of watershed no. 1 on 23 May 1962. Each was set up as a tripod under (1) a 2.4 m (8 ft) maple coppice, (2) a 1.8 m (6 ft) spicebush and (3) a 3.1 m (10 ft) tulip poplar sapling. The base of the pine board was placed on the ground at the base of the shrub. The technique was designed to sample the insecticide passing through the segments of the

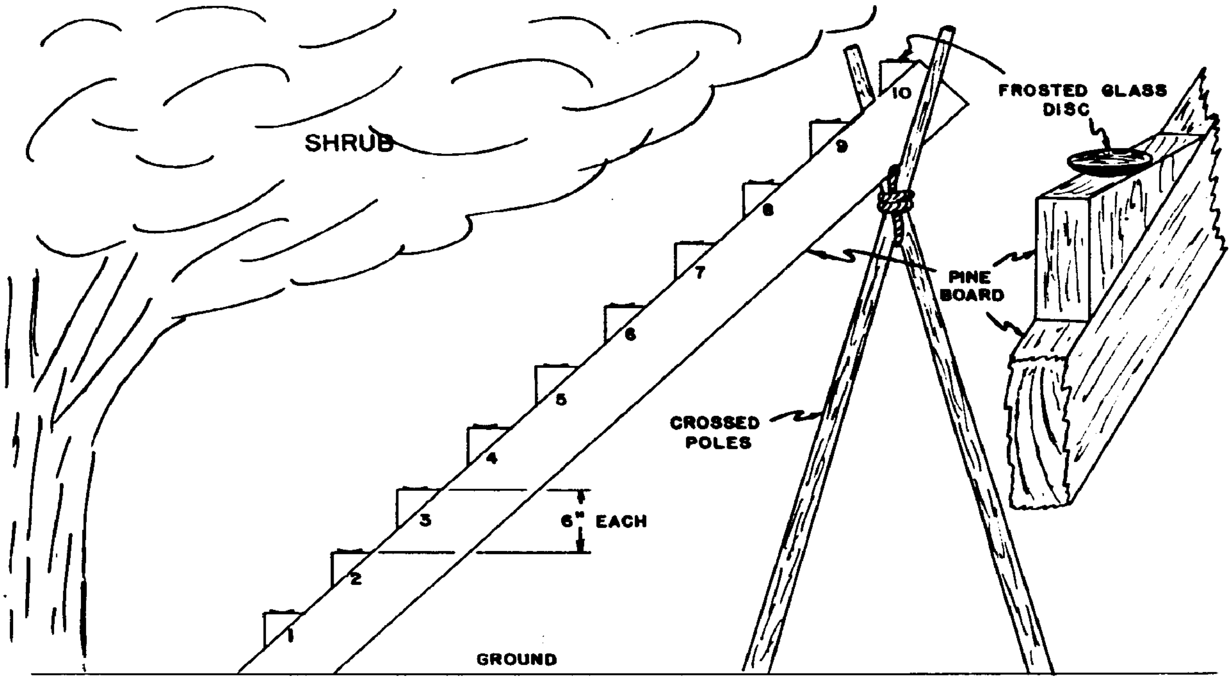

Fig. 62. Glass discs were stair-stepped under the shrub layer in Dover, Ohio, watershed no. 1 to measure spray distribution of the 25 May 1962 application.

shrub crown before the droplets reached the forest floor where they might be affected by ground winds. In Table 81 it can be seen that the irregular pattern of spray dispersal noted within the tree layer prevails. There is a slight tendency for higher amounts to be deposited above 1 m.

Within the Litter

Field GM counter readings presented in Table 82 indicate that radioactivity and thus malathion at 2 lb/acre does not penetrate more than four layers of dead A_1 horizon leaves. Background was about C.04 mr/hr.

Table 81. - Distribution of malathion-S^{35} in counts per minute under small shrub or sapling canopies as measured from glass discs on Dover, Ohio, watershed no. 1, 1962.

Shrub or Sapling species	Distance from ground (inches)									
	6	12	18	24	30	36	42	48	54	60
Spicebush *Lindera benzoin*	13.7	7.8	5.7	10.4	13.8	31.1	9.4	17.9	40.0	28.9
Tulip Poplar *Liriodendron tulipifera*	0.0	19.8	6.1	14.3	6.2	6.7	9.4	14.5	35.4	12.7
Red Maple *Acer rubrum*	9.9	13.3	3.9	5.2	24.6	7.3	38.7	6.0	5.7	14.6

Table 82. - Portable field counter (GM tube) readings of radioactivity of leaf and soil layers under a point near stake 20, of Dover, Ohio, tracer plot no. 2 taken 15 September 1961 from 10 to 12 am.

Description	mr/hr
First layer	To 0.2
2nd layer	To 0.17
3rd layer	0.09
4th layer	0.05
5th layer	0.04
6th layer	0.03
Layers indistinct 0.25 in. below leaf layers	0.01
Soil - 1 in.	0.04 - 0.05
Soil - 3 in.	0.03 - 0.02
Soil - 4 in.	0.01 - 0.02

In another experiment glass discs were placed under varying numbers of leaves held in place with a wire pin. The discs were removed periodically and counted. The results, much more sensitive than the GM readings, are shown in Table 83. The results are variable due to wind blowing the leaves, molestation by animals, and continued rainout of insecticide from the overhead tree canopy. These data seem to indicate that higher disc contamination occurs under fewer leaves. The data are not reliable enough to differentiate absorptive properties of leaves for the insecticide.

Another approach was used to measure sub-surface movement of insecticide. A 30.5 cm (1 ft) deep, meter-long trench was dug near 1-20 and frosted glass discs were inserted horizontally into the uphill trench-face at increasing depths. They were placed diagonally across the face. A knife was used to carefully provide a smooth soil contact surface with the upper frosted glass disc surface. A small sharpened stick was placed below each disc to hold the disc firmly up against the soil face. The pit was filled loosely with moist leaves and the top covered with a piece of sheet plastic. The upper trench edge was left uncovered; the plastic was to reduce the occurrence of unnatural air-soil interface phenomena. The results, inconclusive, are shown in Table 84. The presence of activity at a depth of 15.2 cm (6 in.) is of interest. It may indicate channel movement with rain along roots, air space, or animal burrows.

Over the litter surface application rates varied widely. Using the field counter, activity of sectors of cryptozoan boards on the tracer plots was measured. Representative ranges and variability are

Table 83. - Distribution of malathion-S^{35} or its metabolites through varying numbers of leaves on the forest floor, Dover, Ohio, 1962.

		5-27	6-6	6-15	7-13	8-7
Plot 1 (1-21) Number of hickory leaflets	0	-	3.2	4.4	6.6	-
	1	-	5.5(2.3)	3.5	6.1	5.8
	2	-	2.6(7.3)	4.2	3.2	4.0
	3	-	3.1(2.8)	7.2	3.6	3.8
	4	-	6.2(2.6)	-	-	4.4
	5	-	1.8	3.3	-	3.4
Plot 2 near (1-14) Number of beech leaves	0	7.2	3.8	10.0	8.4	-
	1	-	9.6	4.7	4.6	-
	2	1.5	3.9	-	2.1	-
	3	3.0	3.7	3.1	3.1	-
	4	-	2.6	3.6	2.0	-
	5	-	4.4	-	3.4	-
Amt. of rainfall since spray		0.09	2.5	3.25	4.7+	4.7+

Table 84. Radioactivity of glass discs buried in a pit near 1-20, Dover, Ohio, 1962. Dash indicates no observation.

Depth in inches (cm) of discs in soil	Dates: Month-day		
	6-7	6-15	7-13
1 (2.54)	3.2	0	2.14
2 (5.08)	4.2	0	0
3 (7.62)	4.2	0	7.65
4 (10.16)	-	0	2.49
5 (12.70)	0	-	-
6 (15.24)	5.4	0	0
8 (20.32)	0	-	-
12 (30.48)	-	0	-

shown in Table 85 and Fig. 63. Such board measurements were found to be the only satisfactory reference for field measurements of activity. By marking counting areas in pencil the GM tube could be returned to the near-identical positions on later dates. A flat smooth, absorbent surface is needed to prevent damage of the tube's thin-window and to allow constant counting. Even the slightest change in tube position changed the measured activity. Earphones with the GM counter were found beneficial in scanning areas, vegetation, and soil. The correlation (r) between cryptozoan board activity throughout watershed no. 1 and the maximum disc readings at the same locations was only 0.20.

Results of monitoring tree trunks, vegetation, and soil were inconclusive because of wide variability both vertically and horizontally in activity as recorded by the portable counter.

Sixteen gauge wire probe holders were made by hand and placed before spray near select stakes on all tracer plots and nailed to

Table 85. - Radioactivity of cryptozoa boards on Dover, Ohio, tracer plots before and after a light rain 9-14 and 15, 16 days post spray, 1962.

	mr/hr							
	Plot 1		Plot 2		Plot 3		Plot 4	
Board No.	Pre rain	Post	Pre	Post	Pre	Post	Pre	Post
5	0.01	0.02	0.04	0.05		0.01		0.02
8	0.02	0.02	0.03	0.04		0.02		0.03
11	0.03	0.03	0.04	0.07		0.03		0.03
14	0.01	0.02	0.04	0.04		0.04		0.03
17	0.03	0.02	0.05	0.04		0.01		0.03
20	0.02	0.03	0.04	0.03		0.03		0.02
23	0.02	0.03	0.04	0.02		0.03		0.05
26	0.03	0.03	0.14	0.12		0.02		0.03
29	0.02	0.03	0.38	0.33		0.01		0.03
32	0.02	0.02	0.11	0.07		0.03		0.03

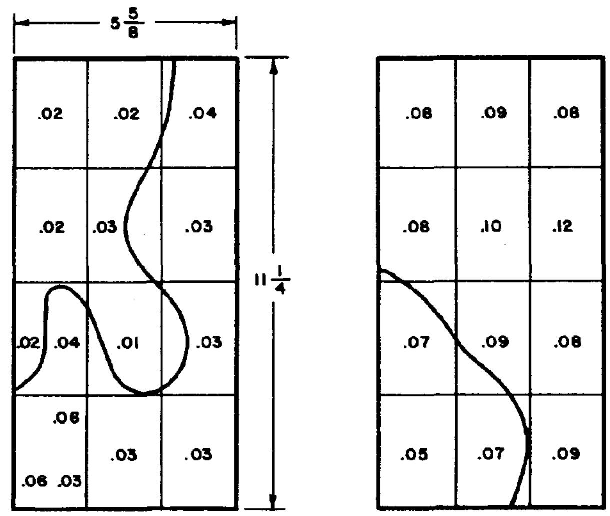

Fig. 63. Variability of radioactivity in mr/hr as measured with a portable GM tube on cryptozoa boards from the Dover, Ohio, tracer plots 2 weeks post spray. Location of the figure to the left, 4-14; to the right, 2-32, 1962.

trees. The holder was an attempt at obtaining in the field a standard reading from a given area of soil, leaf litter, or tree bark on which malathion had been sprayed. The open nature of the holder allowed contamination of the surface to be measured and reduced possible disturbance of the ground after the spray application. The extreme variability of readings within a small area as shown in Fig. 63 made such a holder very desirable for field monitoring isotope activity and its change due to leaching, insect feeding, tracking by animals, absorption by plants, and other factors. One set of readings was taken but the probe could not be held firmly enough to allow any degree of accuracy. The holders were too limber and their construction allowed lateral movement of the probe of several millimeters with consequent wide differences in readings. The technique has merit if the holders are manufactured with long stakes, stable supports, and close-tolerance inside diameter. With a gamma source they would be valuable even if constructed as above.

By comparing readings from discs and probe holders a correlation, $r = 0.60$, was obtained which suggested no profit in continuing their use.

Within the Soil

The previous section shows that the absorptive properties of the forest litter retard malathion or its metabolites from entering the soil but that it does occur irregularly below the litter.

Dean (1960) discussed the relationships of insecticides and soils. He showed that the fate of the pesticide molecule depends not only on its properties but also upon those of the soil and its physical environment. Prediction of the fate of a pesticide requires the integration of adequate knowledge about the interactions of pesticide; environmental, physical, and chemical factors; the soil, and biological or enzymatic processes. Many years of study will be required for such prediction of insecticidal persistance and will require a knowledge of the biological or chemical reactions which accomplish or retard the molecular alterations (Dean 1960:66). Dean suggested soil may be a necessary catalyst for breakdown: "Literature reviews reveal that pesticidal behavior cannot be predicted from studies with a single soil or from a single pesticide on several soils" (Dean, 1960:63).

Clay content of soils influences retention of organic molecules. Under similar conditions, organic matter content of soils increases with clay content, suggesting clay contributes to persistance of organic matter in soils (Dean 1960:65). Dean (1960:63) stated that "one common feature of soils is the accumulation or persistence of varying quantities of organic residues." Surface area, ion-fixation, molecular size of pesticide, and lattice structure of the clay crystals are all involved. Lichtenstein (1958:381) has studied movement of parathion-P^{32} in soils. He found it moved laterally and up as well as down slope under non-leaching conditions. Soil type altered movement; it moved most rapidly in sandy soil, less in loam, and least rapidly in a muck soil with the latter retaining the insecticide longer.

Tracer Plot Pits

Five pits dug with a bucket auger were unsatisfactory for determining the presence of radioactivity and malathion in subsurface water.

Circled Stakes

On 2 May 1962, 2 weeks prior to the spray date, stakes were driven at five sites within watershed no. 1 to measure, if possible, the downward movement of S^{35} within the soil. Site no. 1 was in a sandy, low humus, dry southern exposure soil having a slope of 45 to 55%, half way between 1-8 and 1-17. Three stakes were driven, each made of 1 3/4 x 3/8 x 20 in. finished fir lumber with 18 numbered 0.5 in. radius circles drawn in India ink along one surface. Three were placed in muck soil below 1-10, site No. 2; three in well drained, deep humus soil half way between 1-21 and 1-20, site No. 3. Three were placed 25 ft SW of 1-15, site No. 4. Eight were placed one above the other on a steep slope along stream No. 1 below 1-2 starting 1 ft above the stream. All stakes were inserted in a hole prepared by a similar-size stake. Six inches of each stake was exposed above the surface, 18 in. were below ground. All circles on the stakes were faced uphill. Readings were made periodically by pulling the stake, scraping off adhering earth with a knife and and placing the end of the GM tube tightly within each numbered circle on the stake. The results are shown in Table 86. Although background was 0.04 mr/hr the audible monitor signals indicated activity above background. Field counter readings, though unsatisfactory, indicate

Table 86. - Mean radioactivity of eight soil stakes on Dover, Ohio, watershed no. 1 on stream slope below 1-2, measured 7 June 1962 after 2.3 inches of rain. Readings are in maximum mr/hr observed within 15 seconds after tube contact.

Inches above ground	Max. mr/hr
6	0.047
5	0.045
4	0.049
3	0.042
2	0.044
1	0.045
Inches below ground	**Max. mr/hr**
1	0.042
2	0.043
3	0.044
4	0.046
5	0.041
6	0.040
7	0.040
8	0.047
9	0.032
10	0.036
11	0.035
12	0.037

some activity down to 8 in.

The airplane crash on 15 May provided an unusual opportunity to closely study the movement of a large quantity (30 to 40 gal) of radioactive insecticide in a Wellston silt loam soil. The spray not applied in a one-swath flight leaked from the tank within a 2 m^2 area.

On 21 May 6 days after the airplane crash, a grid of stakes and string 35 x 75 ft was laid over the immediate vicinity of the plane. Fig. 64 is a topographic map of the plot prepared with tape, abney

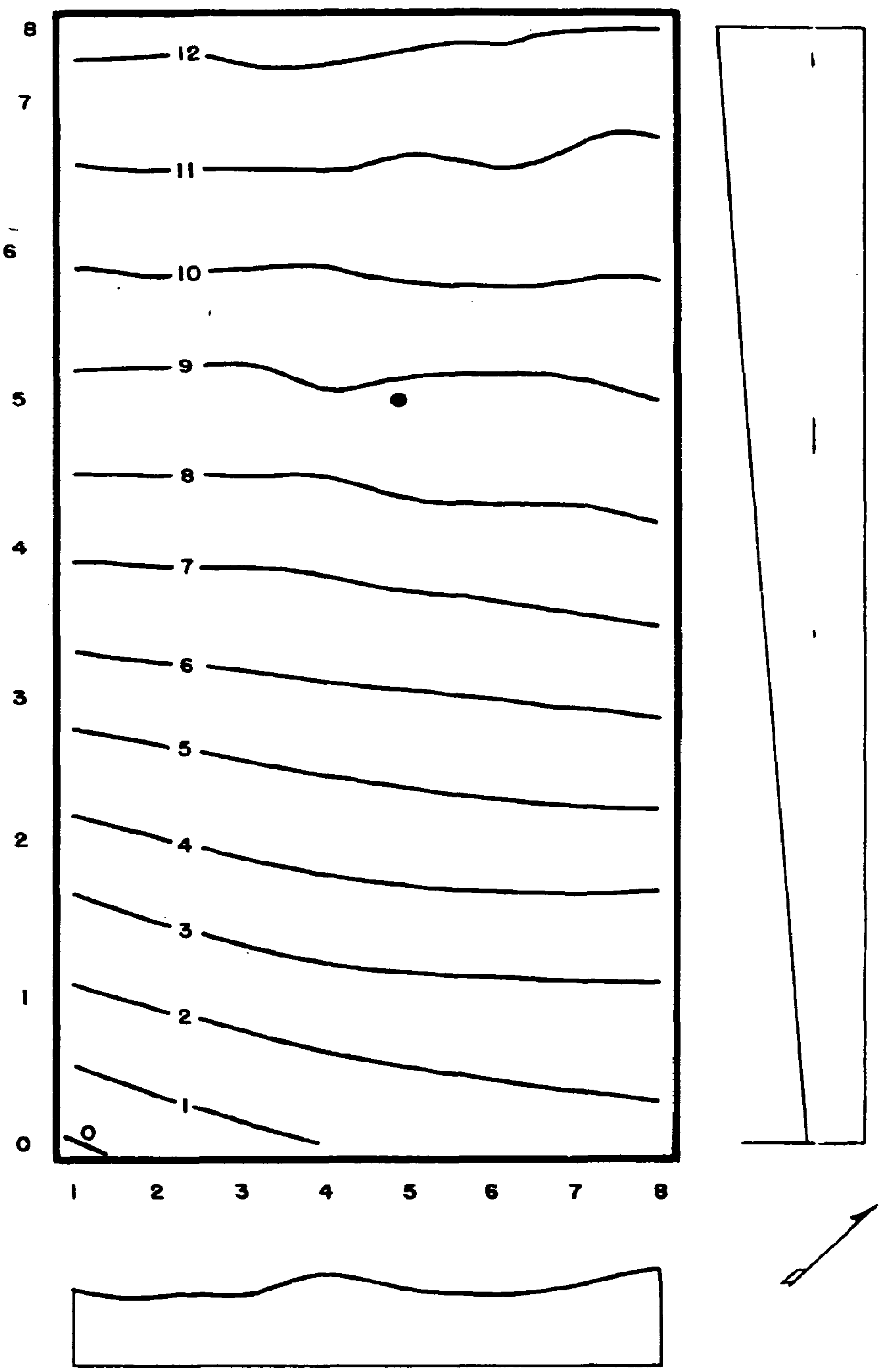

Fig. 64. Topographic map and cross sections of the Dover, Ohio, area around the crashed plane The point of deposition is the black spot near contour line 9. Contour interval is 1 ft. Zero elevation is approximately 1150 ft above sea level. The dimensions of the plot are 35 x 75 ft.

level, and carpenter's rule. The land had a 16.5% slope.

GM tube readings taken on the leaf litter at grid intersections is shown in Fig. 65A. These observations were made with a thin end-window probe held directly on leaves from 0 to 0.5 in. from their surfaces. As in previous measurements the finger was held along side and extending beyond the window to act as a bumper to prevent puncture of the window.

Soil core samples were taken with a 2.54 cm (1 in.) diameter King tube along the grid. Activity of the top two 6 in. samples, and subsequent 1 ft long samples down to 7 ft were taken, placed in numbered plastic bags and returned to the lab where they were sifted in a 1 mm sieve. Large particles were discarded.

Three methods of extracting malathion and S^{35} from the soil were tested. Three-tenths ml of the 25 May formulation was added to three, 3 g samples of sifted soil from the control area. These samples after oxidization in Pirie's reagent and perchloric acid were filtered to remove the silica and undigested material. After washing with water and ethyl alcohol the filtrate was precipitated as described for biological samples. The Pirie's reagent method, most effective in removing the total radioactivity, was adapted for all subsequent samples. Perchloric acid was 91% as effective as Pirie's reagent. Use of a sodium peroxide bomb calorimeter to measure the S^{35} was not successful.

Mr. Harold Black, S.C.S., Coshocton, Ohio, on 8 August 1962 visited the area and reported the proceeding soil character of the vicinity of the plane crash. The soil was judged comparatively

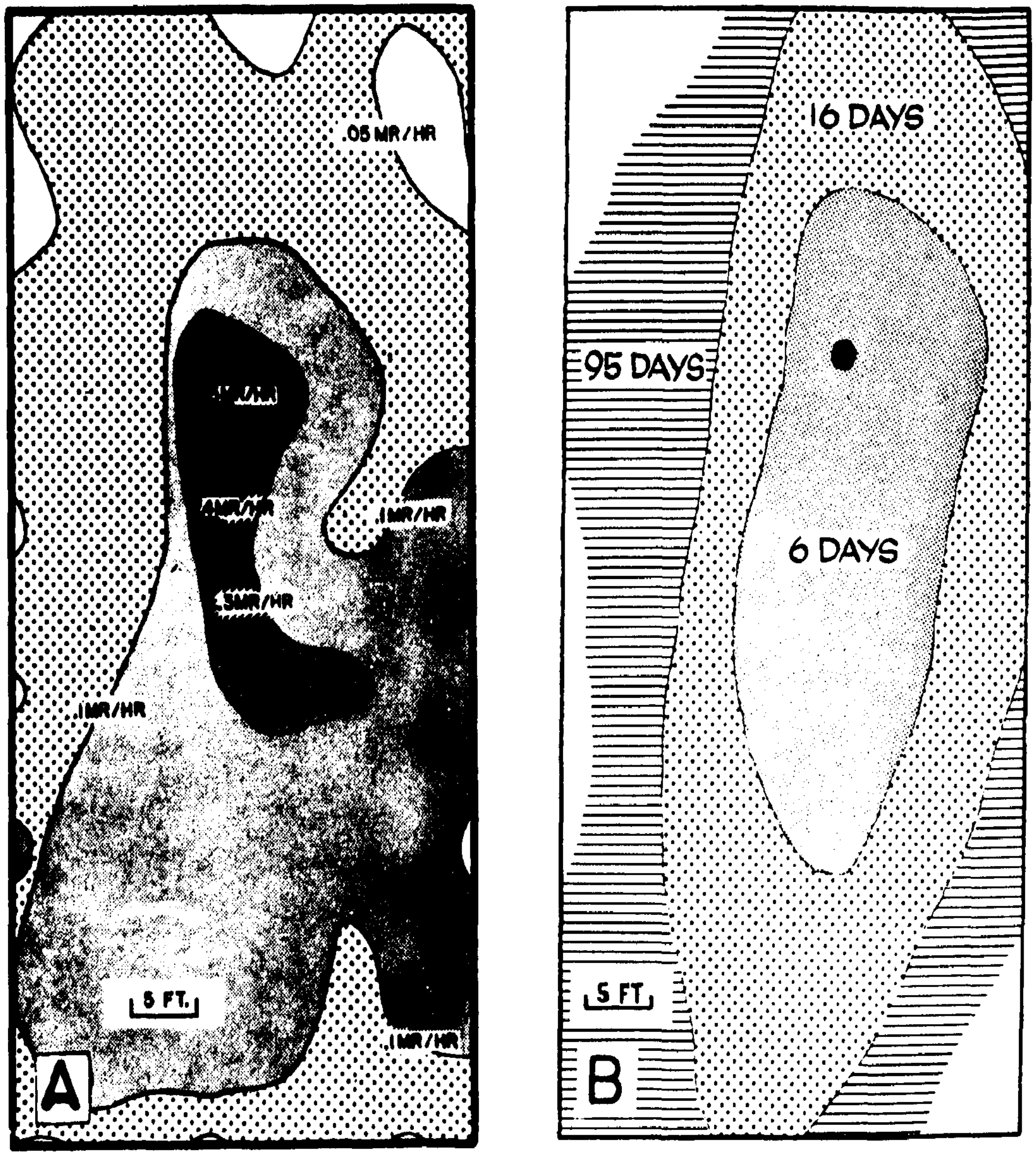

Fig. 65. A. Distribution of insecticide in an intensively studied plot around the crashed plane. The activity at the tank located in the center of the black zone was in excess of 20 mr/hr. The activity around the plot was 0 to 0.05 mr/hr. B. Lateral internal movement (0.9 to 2.1 cpm) of 30 to 40 gal of malathion-S^{35} formulation in Wellston silt loam soil following a spray plane crash, Dover, Ohio, 1962.

shallow and developed from a laminated, platey, micaceous siltstone. The A layer was 2 in., the B layer extended to about 2 ft where parent material, siltstone, occurred. The soil was deeper than the Muskingum and is a Muskingum-Wellston transition.

Mechanical analysis of a sample taken earlier (13 July 1962) from a point NE of the crash 100 ft into the control watershed is reported in Table 87. The O.S.U. Soils Lab would not perform an analysis on the contaminated soil. The soil analyzed and the soil at the crash site are slightly different. On the control area the A_1 horizon was 4 in., the A_2, 4 in. but more dilute in organic matter than the crash site sample. The B layer was developed to 3 ft. The surface A layer was a silt-loam; the B, a silty clay loam. Three to 4 ft below the surface a mottled layer of disintegrated micaceous sandstone occurred. From 4 to 5 ft was weathered shale mottled with gray and reddish brown. There were indications of the presence of iron. The soil was Wellston silt loam developed over thin-bedded micaceous sandstones and shale.

Radioactivity of soil samples as measured with the field GM tube are shown in Table 88. The high counts that occurred on the control area are thought to be due to unexplained natural radioactivity.

Fig. 65B shows above and below ground deposition and movement of the insecticide. Fig. 66 presents the results of soil core analyses showing cross-sectionally, the movement of the insecticide with time. It appears that even in large quantities, on Muskingum-Wellston soils the malathion formulation moves relatively slowly. The majority of the activity of the solution remained at the site of

Table 87. - Percentages of particle sizes in a Wellston silt loam soil sample taken with a King tube 150 ft from the plane crash near 2-13, 2 months post-crash, Dover, Ohio, 1962.

	Particle size distribution in mm										
Soil Depth	Very Coarse sand 2-1	Coarse sand 1-.5	Medium sand .5-.25	Fine sand .25-.1	Very fine sand .1-.05	Total sands	Silt .05-.002	Clay <002	Fine Clay <.0002	Textural Class	Organic Matter
0-3 in.	4.2	4.4	2.6	5.8	7.9	24.9	60.1	15.0	3.5	Silt	7.5
3-6 in.	2.8	3.6	2.9	6.4	7.5	23.2	66.0	10.8	1.9	Silt	2.5
6-12 in.	2.2	3.7	4.0	10.4	9.8	30.1	57.2	12.7	2.1	Silt	1.1
1-2 ft	3.1	5.1	4.0	10.2	13.0	35.4	47.5	17.1	4.1	Loam	0.3
2-3 ft	2.7	5.0	3.8	9.8	16.7	38.0	46.7	15.3	3.8	Loam	0.4
3-4 ft	2.8	6.7	6.2	16.6	20.7	53.0	36.5	10.5	2.4	Sandy Loam	0.4
4-5 ft	4.9	6.9	4.5	11.2	16.9	44.4	44.3	11.3	2.5	Loam	0.4

Table 88. - Radioactivity (corrected for decay) of soil cores from the plane crash site at plot points 5-5 on 13 July and 8-5 on 8 August 1962 and at a similar area 100 ft away in watershed no. 2. Activity was measured with a portable GM tube. Mechanical analyses of sample No. 2 are reported in Table 87.

	mr/hr			
	Crash Site Sample date		Adjoining Area Sample date	
Sample depth	7-13	8-8	7-13	8-8
0-3 in.	0.27	0.08	0.05	0.08
3-6 in.	0.14	0.07	0.06	0.06
6-12 in.	0.14	0.05	0.07	0.07
1-2 ft	0.16	0.06	0.07	0.06
2-3 ft	0.10	0.06	0.12	0.14
3-4 ft	0.14	0.10	0.18	0.10
4-5 ft	0.08	0.08	0.20	0.12

deposition as evidenced by low counts delineating the movement boundaries.

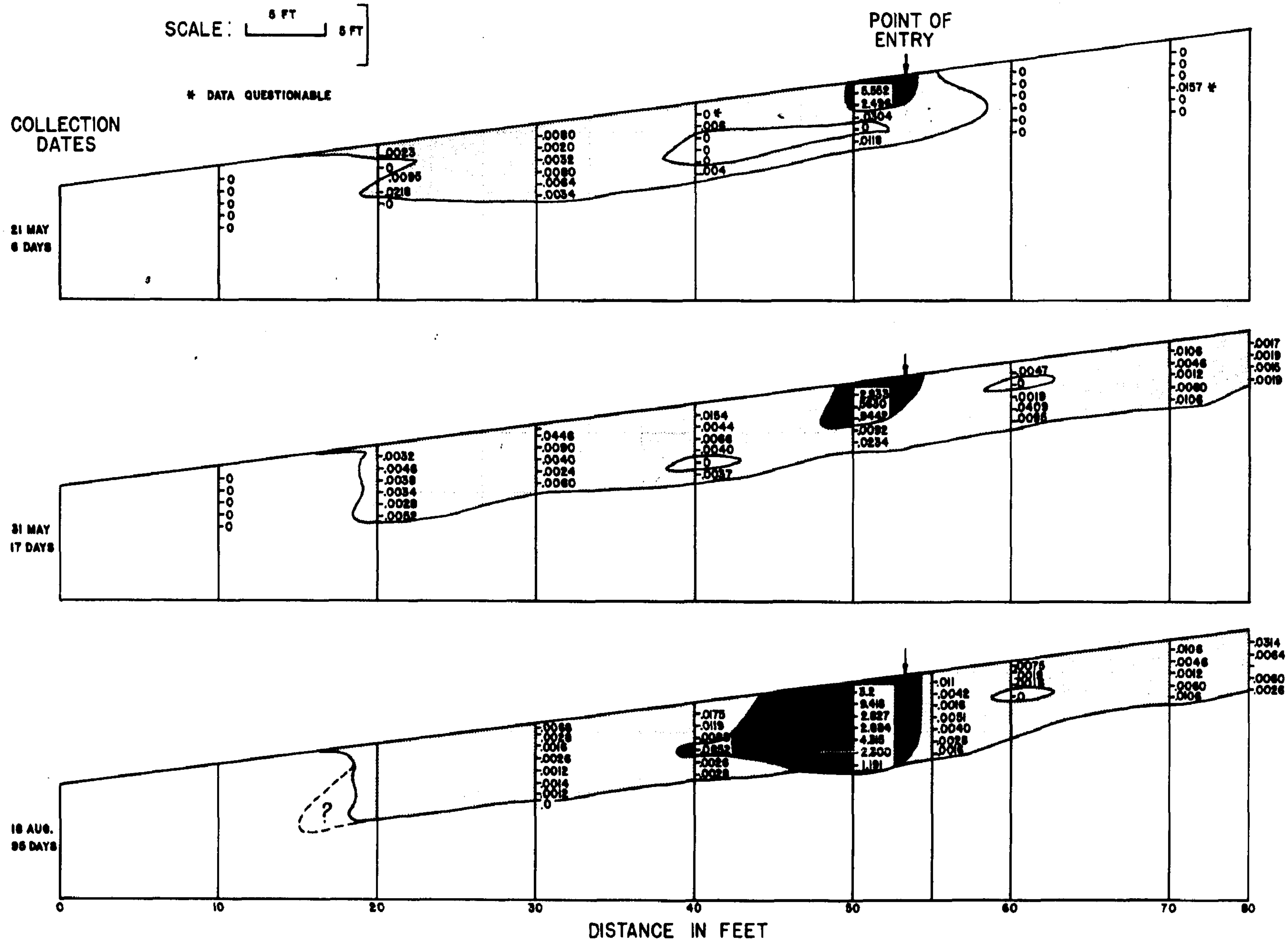

Fig. 66. Cross section of the soil below the plane crash site on the Dover, Ohio, study area in 1962 showing the distribution of S^{35} as measured from King tube samples taken at 1 ft intervals. Samples were taken only where numbers appear.

DISSCUSSION

The essential problem of ecology is how to synthesize the observations of an environment into a statement of understanding. Ecologists are still roughing-in in pencil when it comes to painting the true picture of the forest ecosystem. As has been made painfully obvious, even the initial descriptive phases of an ecological study are extremely difficult and many are yet beyond our present abilities. Advanced studies of interaction when the interactants cannot be defined is not usually fruitful but must be attempted. By such studies descriptive statements can be limited and more rapid progress be made in discovering the nature of interactions.

The natural community, alone, is bewildering in its complexity. To add an unnatural influence such as an insecticide, compounds complexity. Yet, a promising new concept is that of an "ecoectomy" or removal in order to study function. Rapid strides in endocrinology, for example, were made not from studying the endocrine glands per se, but by studying the functions of the body after the removal or suppression of the organ. Thus, in ecology, the study of an ecosystem without the influence of an organism may yield more knowledge about the role of that organism within the system, than much study of the organism itself within its natural environments. The broad spectrum insecticides offer limited possibilities but newer, selective insecticides and rodenticides, hold great promise for progress in ecological understanding.

The subtle effects of an insecticide are far more disquieting to the ecologist than the death of thousands of life forms. Interdependence of species is recognized and when bold change occurs, some species will suffer. Rarely considered, however, is that such bold change occurs constantly, naturally. The giant fallen tree changes radically the environment of at least 1 acre of forest. Millions of forms perish, habitat niches are destroyed, and some successional phenomena begin, others cease. The changes that occur are good or bad depending on one's point of reference. For example, a wild goose close to a small child is an ominous creature; to an average adult, worthy of note; to the pilot, a speck on the bay; to the astronaut, invisible. In each case the goose is equally real. The combined factors of human development and perspective govern the significance of the observation. The field of insect ecology is a direct parallel, for insecticide effect depends on interpretation based on our intellectual, cultural, and spiritual state of development. The statement of "yes, there were effects" must be logically followed by: "what was their significance?" The fact that an organism disappears, even though unnoticed, makes the dissappearance no less real. The frustration of the ecologist is that he "knows" that there are insecticide effects but he cannot become sensitively enough attuned to nature through his own intellectual efforts, observations, or instruments to measure them or even perceive them in some instances. Nor is he prophet enough to confidently interpret for the poorly lit future the observations he does make. Such was the case with this study. The effects were apparent but recovery of populations was rapid and apparent normalcy attained. Ecologists, nevertheless, must approach the issue objectively to enable sound decisions with basic value judgements.

Certain broad recommendations or goals have become evident. With increasing spraying of insecticides there is need for the development of therapeutics for faulty applications. Low residual, low vertebrate toxicity compounds need to be encouraged. Insecticides should be used to bring pest populations to levels at which natural agents may control them. All available knowledge should be brought to bear on development of efficient, effective forest pest control operations which include use of formulations and insecticides compatible with all forest uses. Critical points in ecosystems, e.g., primary consumers, should be studied to allow insecticide use at the least critical time of the year that is compatible with control.

Man, examining the insecticide problem, sits as an audience viewing a strange and unreal drama, one coming to a close and not having provided an inkling of whether it be comedy or tragedy. The drama is one in which all the characters have not been presented by the last act, one of deep plot perhaps only partially recognized, and one that some reviewers predict "success", others "failure."

SUMMARY

The faunal ecology of two, deciduous-forested, 20 acre watersheds near Dover, Ohio, was studied intensively for one year. The second year, one of the watersheds was aerially sprayed with 2 lb/acre of malathion labeled with S^{35}. Effects of the insecticide were studied by sampling all faunal segments from the microbiota of the soil to raccoons. The insecticide and its metabolites were followed from the airplane through the air, the forest vegetation, and in soil and water.

A literature review would not allow correct prediction of the effects of a well known insecticide on the forest fauna.

No effects from the insecticide were observed on bacteria or fungi. Soil microarthropods were affected for a short time. Earthworms and snails were unaffected. Fish and crayfish, sensitive to malathion in tests, were unaffected in the stream environment. Arthropod numbers were greatly reduced, but, generally, rapid recovery was made. Reptiles and amphibians were unaffected. Birds reacted to the insecticide for 2 days but no lasting effects were noted. Mammal populations of mice and chipmunks were reduced from normal on the treated area. Shrews and larger mammals were apparently unaffected.

S^{35} allowed critical movement and concentration studies of a one-swath spray flight and a total aerial treatment. Amounts of malathion and its metabolites in plants, animals, air, soil, and water were determined radiologically. Little insecticide was lost above the forest; major portions are intercepted within the vegetation. Malathion remained in the upper 0.5 ft of soil. Its presence in

water was thought to be due to stream channel interception and to stream-side surface run-off.

Malathion appears to be an effective insecticide that, although harmful to some forms, is of short residue and permits rapid recovery of decimated or altered populations.

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APPENDIX

Appendix Table 1. - List of common and scientific names of trees and plants not listed in Table 4 or elsewhere (based on Fernald, 1950).

Yellow or Tulip Poplar	*Liriodendron tulipifera*
White Oak	*Quercus alba*
Red Oak	*Quercus rubra*
Black Oak	*Quercus velutina*
Yellow Oak (perhaps Black Oak)	*Quercus velutina*
Pin Oak	*Quercus palustris*
Red Maple	*Acer rubrum*
Sugar Maple	*Acer saccharum*
Beech	*Fagus grandifolia*
Hop Hornbeam	*Ostrya virginiana*
Ironwood	*Carpinus caroliniana*
Sassafras	*Sassafras albidum*
Cherry	*Prunus serotina*
Elm	*Ulmus americana*
Chestnut	*Castanea dentata*
Buckeye or Horse Chestnut	*Aesculus glabra*
Hickory	*Carya* spp.
Black Walnut	*Juglans nigra*
White Walnut	*Juglans cinerea*
Honey Locust	*Gleditsia triacanthos*
Ash	*Fraxinus* sp.
Spicebush	*Lindera benzoin*
Hazelnut	*Corylus* sp.
Pawpaw	*Asimina* sp.
Quaking or Trembling Aspen	*Populus tremuloides*
Dogwood	*Cornus florida*
Huckleberries	*Gaylussacia* sp.
Raspberries	*Rubus* sp.
Blackberries	*Rubus* sp.
Dewberries	*Rubus* sp.
Cranberries	*Vaccinium* spp.
Ginseng	*Aralia* sp.
Nettles	Urticacea (?)

Appendix Table 2. - List of common and scientific names of vertebrates referred to in the text and not listed elsewhere (after Hall and Kelson, 1959).

Mammals	
White-tailed deer	Dama virginianus
Black bear	Ursus americanus
Elk (Wapiti)	Cervus canadensis
Beaver	Castor canadensis
Buffalo	Bison bison
Panther	Felis concolor
Bobcat	Lynx rufus
Wolf	Canis sp.
Otter	Lutra canadensis
Muskrat	Ondatra zibethicus
Striped skunk	Mephitis mephitis
Opossum	Didelphis marsupialis virginiana
Fox - Red	Vulpes fulva
Gray	Urocyon cinereoargenteus
Raccoon	Procyon lotor
Woodchuck or groundhog	Marmota m. monax
Fox squirrel	Sciurus niger
Gray squirrel	Sciurus carolinensis
Red squirrel	Tamiasciurus hudsonicus
Flying squirrel	Glaucomys volans
Eastern chipmunk	Tamias striatus fisheri
Cottontail rabbit	Sylvilagus floridanus
White-footed mouse	Peromyscus leucopus noveboracensis
House mouse	Mus musculus
Woodland jumping mouse	Napaeozapus insignis
Short-tailed shrew	Blarina brevicauda
Smoky shrew	Sorex fumeus
Birds	
Wild turkey	Meleagris gallopavo
Ruffed grouse	Bonasa umbellus
Bob White or quail	Colinus virginianus
Crow	Corvus brachyrhynchos
Blackbirds (probable grackles and red-wing)	Quiscalus sp. Agelaius phoeniceus
Bluebirds	Sialia sialis
Turtle dove	Zenaidura macroura
Cranes (probably herons and egrets)	Ardea herodias and others
Woodpeckers	(See checklist under "Birds")
Pigeons (passenger)	Ectopistes migratorius

Appendix Table 3. - Correlation cuefficient, r, matrix for some characteristics of the 46 grid intersections or sampling points of the Dover, Ohio, watersheds, 1961.

	Column number corresponds to row number at left of table								
	1	2	3	4	5	6	7	8	9
1. Elevation	1.00	-0.40	0.49	0.32	0.43	0.94	0.04	-0.22	0.02
2. p^H		1.00	-0.32	-0.46	-0.86	-0.35	-0.11	0.01	0.14
3. Organic Matter			1.00	0.48	0.49	0.39	-0.11	-0.11	-0.12
4. Phosphorus				1.00	0.53	0.24	-0.06	0.14	-0.16
5. Potassium					1.00	0.36	-0.03	-0.09	-0.13
6. Distance from water						1.00	0.08	-0.17	0.04
7. Cover Rating: Tree							1.00	-0.09	-0.11
8. Shrub								1.00	-0.01
9. Herb									1.00

Appendix Table 4. - Checklist of the arthropods of the Dover, Ohio, watersheds, common name, and methods used for the collection of each.

Order and Family or Superfamily	Common Name	Light Trap	Sticky Boards	Sweep Net	Molasses Trap	Catch Trays	Miscellaneous	Berlese Funnel	Tree Bands	Strained Stream	Drift Net
										Collection Methods	
<u>Protura</u>	Proturans (Telson tails)							x			
<u>Thysanura</u>	Bristletails					x		x			
<u>Collembola</u>	Springtails		x		x	x	x	x		x	x
Ephemeroptera (adults)	Mayflies	x		x		x				x	x
(nymphs)										x	x
<u>Odonata</u>	Dragonflies										
Aeshnidae	Darners						x				
<u>Orthoptera</u>											
Blattidae	Roaches	x			x		x				
Tettigoniidae	Long-horned grasshoppers	x		x			x				
Gryllacrididae	Cave and camel crickets				x	x	x				
Gryllidae	Crickets	x			x	x	x	x			
Phasmatidae	Walking sticks			x			x				
Acrididae	Grasshopper			x							
<u>Plecoptera</u> (adults)	Stoneflies	x	x		x	x	x			x	
<u>Psocoptera</u>	Booklice and Psocids	x	x	x	x	x		x	x	x	
<u>Thysanoptera</u>	Thrips	x	x		x	x		x			
<u>Hemiptera</u>											
Corixidae	Water boatmen	x					x			x	
Miridae	Plant bugs	x	x	x	x	x				x	x
Reduviidae	Assassin bugs	x		x		x	x				
Lygaeidae	Lygaeid bugs	x		x	x	x	x			x	
Aradidae	Flat bugs	x	x								
Pentatomidae	Stink bugs	x		x	x		x				
Ploiariidae		x									
Tingidae	Lace bugs	x	x	x	x	x					
(nymphs)						x					
Belostomatidae	Giant Water bugs						x				
Coreidae	Coreid or Leaf-footed bugs		x				x				
(nymphs)					x		x				
Nabidae	Damsel bugs			x							

Appendix Table 4. - Continued.

Order and Family or Superfamily	Common Name	Light Trap	Sticky Boards	Sweep Net	Molasses Trap	Catch Trays	Miscellaneous	Berlese Funnel	Tree Bands	Strained Stream	Drift Net
		Collection Methods									
<u>Hemiptera</u> (cont.)											
Dipsocoridae								x			
Reduviidae (nymphs)				x							
Neididae	Stilt bugs			x							
Anthocoridae	Flower bugs, minute pirate bugs		x								
<u>Homoptera</u>											
Cercopidae	Spittlebugs	x	x	x	x						
Cicadellidae	Leafhoppers	x	x	x	x	x	x		x	x	x
Fulgoridae	Fulgorid Planthoppers	x	x	x	x	x	x		x	x	x
Membracidae	Treehoppers	x	x			x				x	x
Psyllidae	Jumping plantlice	x	x	x		x					
Aphididae	Aphids or plantlice	x	x	x	x	x		x			
(nymphs)							x				
Phylloxeridae	Phylloxerans		x								
Aleyrodidae	Whiteflies		x								
Coccidae	Soft scales		x								
<u>Neuroptera</u>											
Corydellidae	Dobsonflies	x									
Chrysopidae	Green lacewings	x	x	x	x	x					
Sialidae	Alderflies	x									
Hemerobiidae	Brown lacewings	x	x	x		x					
(nymphs)										x	
Chrysopidae (larvae)						x					
<u>Coleoptera</u>	Beetles										
Carabidae	Ground beetles	x	x	x	x	x	x	x			
Haliplidae	Crawling water beetles	x									
Dytiscidae	Predaceous diving beetles	x			x		x				
Gyrinidae	Whirligig beetles	x									
Hydrophilidae	Water scavenger beetles	x			x		x			x	x
Leoididae		x			x		x				
Silphidae	Carrion beetles	x			x		x				
Staphylinidae	Rove beetles	x	x	x	x	x	x	x	x	x	x

Appendix Table 4. - Continued.

Order and Family or Superfamily	Common Name	Collection Methods: Light Trap	Sticky Boards	Sweep Net	Molasses Trap	Catch Trays	Miscellaneous	Berlese Funnel	Tree Bands	Strained Stream	Drift Net
Coleoptera (cont.)											
Orthoperidae		x	x	x	x						
Cantharidae	Soldier beetles	x	x	x	x	x	x			x	x
Lampyridae	Fireflies	x	x	x	x		x			x	
Dermestidae	Dermestid beetles	x	x								
Cisidae		x	x						x		
Elateridae	Click beetle	x	x	x	x	x	x			x	x
Ptilodactylidae		x	x		x		x				
Heteroceridae	Variegated mud-loving beetles	x					x				
Erotylidae	Pleasing fungus beetles	x	x		x		x				
Phalacridae	Shining fungus beetles	x	x	x	x					x	
Coccinellidae	Ladybird beetles	x	x	x		x	x				
Mycetophagidae	Hairy fungus beetles	x	x	x	x	x					
Colydiidae	Cylindrical bark beetles	x									
Pyrochroidae	Fire colored beetles	x			x						
Alleculidae	Comb clawed beetles	x	x	x	x						
Tenebrionidae	Darkling beetles	x	x		x						
Melandryidae	Melandryid bark beetles	x	x	x	x	x					
Ptinidae	Spider beetles	x									
Anobiidae	Drugstore beetle	x	x	x		x					
Bostrichidae	False powder post beetles	x									
Trogidae	Skin beetles	x									
Scarabaeidae	Scarab beetles	x			x	x	x	x			
Cerambycidae	Long-horned wood boring beetles	x	x	x	x	x	x				
Chrysomelidae	Leaf beetles	x	x	x	x	x				x	x
Curculionidae	Weevils or snout beetles	x	x	x	x	x	x	x		x	
Nitidulidae	Sap feeding beetles	x			x		x				
Dascillidae		x	x								
Lycidae	Net-winged beetles	x	x	x		x	x				
Lathridiidae	Minute brown scavenger beetles	x	x	x	x						
Scolytidae	Bark beetles	x	x		x	x				x	

Appendix Table 4. - Continued.

Order and Family or Superfamily	Common Name	Light Trap	Sticky Boards	Sweep Net	Molasses Trap	Catch Trays	Miscellaneous	Berelese Funnel	Tree Bands	Strained Stream	Drift Net
		Collection Methods									
Coleoptera (cont.)											
Histeridae	Hister beetles					x	x				
Oedemeridae	Oedemerid beetles					x					
Cleridae	Checkered beetles		x			x				x	x
Buprestidae	Flatheaded or Metallic wood borers		x			x				x	x
Mordellidae	Tumbling flower beetles	x	x	x		x				x	x
Ptiliidae	Feather-winged beetles		x		x		x	x			
Scaphidiidae	Shining fungus beetle				x			x			
Pselaphidae	Ant-loving beetles		x				x	x			
(larvae)								x			
Cicindellidae	Tiger beetles						x				
Endomychidae	Handsome fungus beetles						x				
Meloidae	Blister beetles						x				
(larvae)							x				
Geotrupidae	Geotrupid dungbeetles						x				
Cucujidae	Flat bark beetles		x		x	x					
Anthribidae	Fungus weevils			x	x						
Dryopidae	Long-footed water beetles	x									
Euglenidae			x	x							
Melyridae	Soft-winged flower beetles									x	x
Cryptophagidae	Cryptophagid or silken fungus beetles		x								
Eucnemidae			x								
Throcidae	Pseudo clickbeetles		x								
Anthricidae	Ant-like flower beetles		x								
Monotomidae					x						
Lucanidae	Pinching beetles				x						
Mecoptera	Scorpionflies										
Bittacidae	Hangingflies	x		x		x					
Panorpidae	Common scorpionflies		x	x	x	x					
Trichoptera	Caddisflies	x			x	x	x			x	
Hydropsychidae	Netspinning caddisflies	x									

Appendix Table 4. - Continued.

Order and Family or Superfamily	Common Name	Light Trap	Sticky Boards	Sweep Net	Molasses Trap	Catch Trays	Miscellaneous	Berlese Funnel	Tree Bands	Strained Stream	Drift Net
		Collection Methods									
Trichoptera (cont.)											
Limnephilidae	Northern caddisflies										
Phloeothripidae				x							
(nymphs)										x	
Lepidoptera	Butterflies, moths, skippers										
Sphingidae	Sphinx moths	x			x						
Saturniidae	Giant silkworm moths	x					x				
Citheroniidae	Royal moths	x									
Arctiidae	Tiger moths	x					x				
Phalaenidae	Noctuid moths	x					x				
Notodontidae	Prominents	x									
Lasiocampidae	Tent caterpillars	x									
Geometridae	Geometrid moths and Measuring worms	x			x		x				
Pyralidae	Pyralid moths	x									
Microlepidoptera		x	x	x	x	x	x		x	x	x
Undetermined		x									
Caterpillars						x	x	x			
Papilionidae	Swallowtail butterflies						x				
Nymphalidae	Brush-footed butterflies						x				
Agaristidae	Forester moths						x			x	
Thyatiridae							x				
(larvae)	Caterpillars		x		x		x				
Lycaenidae	Blues, Coppers, Hairstreaks						x				
Macrolepidoptera				x	x						
Diptera											
Muscidae	House flies *et al*.	x									
Anthomyiidae		x									
Sarcophagidae	Flesh flies	x		x							
Tipulidae	Crane flies	x	x	x	x	x	x		x	x	x
Drosophilidae	Vinegar flies	x		x	x	x					
Chironomidae	Midges	x	x	x	x	x	x		x	x	x
Ceratopogonidae	Biting midges	x	x	x	x	x			x	x	

Appendix Table 4. - Continued.

Order and Family or Superfamily	Common Name	Collection Methods: Light Trap	Sticky Boards	Sweep Net	Molasses Trap	Catch Trays	Miscellaneous	Berlese Funnel	Tree Bands	Strained Stream	Drift Net
Diptera (cont.)											
Culicidae	Mosquitoes	x	x	x		x					
Bibionidae	March flies	x	x	x	x	x			x	x	
Mycetophilidae	Fungus gnats	x	x	x	x	x	x			x	x
Sciaridae	Dark-winged fungus gnats	x	x	x	x	x			x	x	x
Cecidomyiidae	Gall midges	x	x	x	x	x	x	x	x	x	x
Psychodidae	Moth flies	x	x	x	x	x				x	x
Simuliidae	Black flies	x		x		x					
Rachiceridae		x									
Stratiomyidae	Soldier flies	x	x	x	x	x			x	x	x
Rhagionidae	Snipeflies	x	x	x	x						
Empididae	Dance flies	x	x	x	x	x			x	x	x
Dolichopodidae	Long-legged flies	x	x	x	x	x			x	x	x
Pipunculidae	Big headed flies	x	x	x		x				x	
Phoridae	Humpbacked flies	x	x	x	x	x	x		x	x	
Calypterates		x	x	x	x	x	x		x	x	x
Acalypterates		x	x	x	x	x			x	x	x
Platypezidae	Flat-footed flies	x		x							
Lauxaniidae	Picture-winged flies	x	x	x	x	x					
Syrphidae	Flower flies	x	x		x	x	x			x	x
Tabanidae	Horse, deer flies		x			x				x	
Tachinidae	Tachina flies					x	x				
(larvae)					x		x	x			
Bombyliidae	Bee flies						x				
Rhyphidae	Woodgnats				x				x	x	
Helomyzidae	Helomyzidflies				x						
Sciomyzidae	Marsh flies		x	x	x					x	x
Asilidae	Robber flies		x								
Sphaeroceridae	Small dung flies			x							
Mycomyinae		x									
Sepsidae	Black scavenger flies			x							
Clusiidae				x							
Siphonaptera	Fleas					x	x				
Hymenoptera											
Cynipidae	Gall wasps	x	x	x	x	x				x	x

Appendix Table 4. - Continued.

Order and Family or Superfamily	Common Name	Light Trap	Sticky Boards	Sweep Net	Molasses Trap	Catch Trays	Miscellaneous	Berlese Funnel	Tree Bands	Strained Stream	Drift Net
Hymenoptera (cont.)											
Ichneumonidae	Ichneumons	x	x	x	x	x	x			x	
Braconidae	Braconids	x	x	x	x	x			x	x	x
Chalcidoidea	Chalcids	x	x	x	x	x		x	x	x	x
Formicidae	Ants	x	x	x	x	x	x	x	x	x	x
Apidae	Bees	x			x	x	x			x	
Tenthredinidae	Typical sawflies	x		x	x	x				x	x
Eulophidae		x		x							
Sphecidae	Sphecid wasps		x	x		x				x	x
Siricidae	Horntails						x				
Vespidae	Potter wasps, hornets, yellow jackets			x	x		x			x	
Halictidae	Halictid bees, Sweat bees						x				
Platygasteridae		x	x	x	x						
Proctotrupidae			x	x	x						
Diapriidae		x	x	x	x	x					
Mymaridae	Fairyflies		x								
Pergidae				x							
Encyrtidae	Encyritids			x							
Eupelmidae				x							
Trigonalidae				x							
Colletidae	Colletid (yellow-faced) bees			x							
Pompilidae	Spider wasps		x			x					
Chrysididae	Cuckoo wasps										
Isopoda	Sow bugs						x				
Diplopoda	Millipedes					x	x	x			
Chilopoda	Centipedes						x	x			
Pauropoda	Pauropods							x			
Symphyla	Symphylans						x	x			
Chelonethida	Pseudoscorpions				x			x			
Phalangida	Daddylonglegs	x	x	x	x	x	x	x	x	x	x
Arachnida								x			
Acarina			x		x						
Orbatid mites							x	x			

Appendix Table 4. - Continued.

Order and Family or Superfamily	Common Name	Collection Methods: Light Trap	Sticky Boards	Sweep Net	Molasses Trap	Catch Trays	Miscellaneous	Berlese Funnel	Tree Bands	Strained Stream	Drift Net
Acarina (cont.)											
Non-orbatid mites							x	x			
Araneida	Spiders		x	x	x	x	x	x	x	x	x

Appendix Table 5. - Phenologically comparable dates, (month-day) 1961 and 1962, Dover, Ohio, watersheds.

1961	1962	1961	1962	1961	1962
4-25	4-29	6-1	5-18	7-7	6-22
4-26	4-30	6-2	5-19	7-8	6-23
4-27	5-1	6-3	5-20	7-8	6-24
4-28	5-2	6-4	5-21	7-9	6-25
4-29	5-3	6-5	5-22	7-10	6-26
4-30	5-4	6-6	5-23	7-11	6-27
5-1	5-5	6-7	5-24	7-12	6-28
5-2	5-6	6-8	5-25	7-13	6-29
5-3	5-7	6-9	5-26	7-14	6-30
5-4	5-8	6-10	5-27	7-15	7-1
5-5	5-8	6-11	5-27	7-16	7-2
5-6	5-8	6-12	5-28	7-17	7-3
5-7	5-9	6-13	5-29	7-18	7-4
5-8	5-9	6-14	5-30	7-19	7-5
5-9	5-9	6-15	6-1	7-20	7-6
5-10	5-9	6-16	6-1	7-21	7-7
5-11	5-9	6-17	6-2	7-22	7-8
5-12	5-9	6-18	6-3	7-22	7-9
5-13	5-10	6-19	6-4	7-23	7-10
5-14	5-10	6-20	6-5	7-24	7-11
5-15	5-10	6-21	6-6	7-25	7-12
5-16	5-10	6-22	6-7	7-25	7-13
5-17	5-10	6-23	6-8	7-26	7-14
5-18	5-11	6-24	6-9	7-27	7-15
5-19	5-11	6-25	6-10	7-28	7-16
5-20	5-12	6-26	6-11	7-29	7-17
5-21	5-12	6-27	6-12	7-30	7-18
5-22	5-12	6-28	6-12	7-31	7-19
5-23	5-13	6-29	6-13	8-1	7-20
5-24	5-13	6-30	6-14	8-2	7-21
5-25	5-14	7-1	6-15	8-2	7-22
5-26	5-14	7-1	6-16	8-3	7-23
5-27	5-15	7-2	6-17	8-4	7-24
5-28	5-15	7-3	6-18	8-5	7-25
5-29	5-16	7-4	6-19	8-6	7-26
5-30	5-16	7-5	6-20		
5-31	5-17	7-6	6-21		

Appendix Table 6. - Oribatid mites collected by Berlese funnel extractions from nylon net bags filled with leaf litter, Dover, Ohio, 1962.

Treated Area

Date: Month-Day	1-19	1-20	1-21	1-14	1-15	1-16	1-8	1-9	1-10	1-5	Total
5-1	43	11	72		5	15	14	2	2	29	193
5-10	13	6	66	42	30	115	74	26	26	61	459
5-23	12	3	14	31	20	5	15	5	9	14	128
5-29	6	8	1	8	3	3	1	6	4	6	46
6-11	39	19	35	22	8	6	27	51	6	11	224
7-4	44	4	13	2	7	2		29	10	1	112
7-19	25				44			12	18	7	106
7-30	19	5	7	7	1	2	16	5	4	3	69
8-16	8	6	1	6	4	6	18	3	18	7	77
Total	209	62	209	118	122	154	165	139	97	139	1414

Untreated Area

Date: Month-Day	2-20	2-21	2-22	2-15	2-16	2-17	2-9	2-10	2-11	2-7	Total
5-1	3	4	4	12	14	8		6	8	16	75
5-10	58	30	15	11	37	9		17	17	2	196
5-23	14	5	3	11	12	1		2	9		57
5-29	8		6					24	6	3	47
6-11	28	24	45	5	17	1		26	17	36	199
7-4	23	2	2	2	24			15	7	18	93
7-19		23	40	21				13		14	111
7-30	5	40	15	12	6	5		3	2	18	106
8-16	8	11	16		11			2	10	34	92
Total	147	139	117	74	121	24		108	76	141	976

Appendix Table 7. - Non-oribatid mites collected by Berlese funnel extractions from nylon net bags filled with leaf litter, Dover, Ohio, 1962.

Treated Area

Date: Month-Day	1-19	1-20	1-21	1-14	1-15	1-16	1-8	1-9	1-10	1-5	Total
5-1	17	23	62	9	58	47	42	13	54	54	379
5-10	22	33	33	55	113	44	71	36	53	31	491
5-23	15	6	22	20	32	7	7	16	6	5	136
5-29	11	6	8	7	7	8	4	3	1	4	59
6-11	39	19	35	22	8	6	27	51	6	11	224
7-4	128	27	38	11	38	40		44	30	35	391
7-19	36				22			15	19	25	117
7-30	48	42	14	14	1	11	72	35	12	63	312
8-16	27	52	17	34	38	42	52	30	30	20	342
Total	343	208	229	172	317	205	275	243	211	248	2451

Untreated Area

Date: Month-Day	2-20	2-21	2-22	2-15	2-16	2-17	2-9	2-10	2-11	2-7	Total
5-1	8	5	15	39	13	47		32	21	22	212
5-10	44	39	7	13	23	62		10	15	12	225
5-23	37	13	5	11	21	4		13	14	4	122
5-29	39	2	21	6	3	1		16	2	8	98
6-11	28	24	45	5	17	1		26	17	36	199
7-4	52	30	53	19	78			28	27	22	309
7-19		16	27	13				13		17	116
7-30	19	31	91	32	28	22		12	23	27	285
8-16	26	10	31		15	4		9	25	22	142
Total	253	170	295	138	198	141		159	144	170	1698

Appendix Table 8. - Collembola collected by Berlese funnel extractions from nylon net bags filled with leaf litter, Dover, Ohio, 1962.

Treated Area

Date: Month-Day	1-19	1-20	1-21	1-14	1-15	1-16	1-8	1-9	1-10	1-5	Total
5-1	27	14	28	13	27	18	118	28	61	152	486
5-10	22	35	13	225	164	42	74	53	84	134	846
5-23	12	10	12	62	41	8	36	27	5	32	245
5-29	6	2	8	7	6	4	2	4		1	40
6-11	16	31	11	26	9	17	31	21	14	14	190
7-4	63	4	12	7	16	13		15	18	74	222
7-19	6				32				34	7	79
7-30	29	6	9	17	1	3	31	10	12	18	136
8-16	13	19	7	19	24	37	34	16	15	28	212
Total	194	121	100	376	320	142	326	174	243	460	2456

Untreated Area

Date: Month-Day	2-20	2-21	2-22	2-15	2-16	2-17	2-9	2-10	2-11	2-7	Total
5-1	88	16	24	23	11	39		21	33	404	659
5-10	82	82	12	14	64	22		28	57	27	388
5-23	47	33	16	17	48	4		10	31	14	220
5-29	28	6	25	4	1			9	8	5	86
6-11	6	22	65	13	56	4		6	52	34	258
7-4	42	14	34	26	48			40	27		231
7-19		44	45	38				16		52	195
7-30	9	43	45	57	30	22		15	43	18	282
8-16	13	21	24		12	7		18	37	28	160
Total	315	281	246	192	270	98		163	288	582	2479

APPENDIX 9

PREPARATION OF MALATHION-S^{35}

Mr. J. R. Ogle, Inorganic Department, The Radiochemical Centre, Amersham, Buckinghamshire, Great Britain, (pers. correspondence, 9 August 1962) outlined the procedure used for the formulation of malathion-S^{35} purchased from them for this research.

Elemental sulfur, S^{35}, in slight excess, is heated to 400° with red phosphorus to give phosphorus pentasulphide-S^{35} which is then allowed to react with an excess of methanol in refluxing benzene to give dimethyl phosphorodithioate-S^{35}, together with hydrogen sulphide. The dithioate (b.p. 0.04, 40-40.5° C) is purified by fractional distillation, then allowed to react without solvent with a slight excess of diethyl maleate for 68 hours at 40° C. Acidic impurities are removed by partitioning the mixture between ether and sodium hydrogen carbonate; after evaporation of the ether, unreacted diethyl maleate is washed out in light petroleum (b.p. 40-60° C) and finally the product is heated at 100° C at 10 $^{-2}$mm pressure to remove all volatile impurities. The malathion was not distilled since at higher temperature there appears to be the possibility of isomerisation and decomposition.

The reaction sequence is represented below:

$$P_2S_5 + 4CH_3OH \rightarrow 2(CH_3O)_2 \cdot PSSH + H_2S$$

$$(CH_3O)_2 \cdot PSSH + C_2H_5OOC.CH{:}CH.COOC_2H_5 \rightarrow (CH_3O)_2PSS.\underset{\displaystyle CH_2.COOC_2H_5}{\underset{|}{CH}}.COOC_2H_5$$

The overall yield based on red phosphorus is better than 60%. The radiochemical yield is about 45% due to the use of excess sulphur in the preparation of the pentasulphide and loss of hydrogen sulphide at the second state.

The radiochemical purity of the product was checked by paper chromatography in the following five solvent systems:

1. Ethanol : acetone : water (1:1:2) on paraffin treated paper.
2. Ethanol : choloroform : water (10:10:5) on silicone treated paper.
3. Methanol : water : toluene : light petroleum (70:30:50:50).
4. Acetonitrile : water : 0.88 ammonia (40:10:1).
5. Methanol : hexane (1:2).

In the first four solvents only one radioactive component was detected while in the fifth eluent a minor impurity, totaling 4% of the activity, was detected. The S^{35} assay on the material indicated a specific activity of 17.5 mc/mM on 13 May 1962 and the four ampoules contained the following quantities of malathion: (1) 4.98 g, 263 mc; (2) 4.98 g, 263 mc; (3) 4.92 g, 260 mc; and (4) 5.02 g, 265 mc.

AUTOBIOGRAPHY

I, Robert Hayes Giles, Jr., was born in Lynchburg, Virginia, 25 May 1933. I received my secondary-school education largely in the public schools of Lynchburg, Virginia. I was granted the Bachelor of Science in Forestry degree from Virginia Polytechnic Institute in 1955. I was also commissioned as a second lieutenant in the U. S. Army Infantry in June, 1955. I was granted a Master of Science degree with a major in wildlife management from the same institute in June, 1958. While working toward the Master's degree, I was a Graduate Fellow with the Virginia Cooperative Wildlife Research Unit studying under the supervision of Dr. James S. Lindzey, Unit Leader. I was granted a fellowship by the Wildlife Management Institute for research leading to the preparation of my thesis: Conservation Knowledge of Virginia School Pupils.

After leaving V.P.I., I successfully completed Basic Infantry Officer's School and U. S. Army Ranger School at Fort Benning, Georgia.

I was employed by the Virginia Commission of Game and Inland Fisheries from December, 1957 to March, 1960 as a District Game Biologist working in forest game management on the George Washington National Forest.

In March, 1960, I joined the Ohio Cooperative Wildlife Research Unit, The Ohio State University, on a Unit fellowship which I held until completion of the Ph.D. degree.