DISTRIBUTION OF AERIALLY APPLIED MALATHION-S^{35} IN A FOREST ECOSYSTEM*

R. H. GILES, JR. AND T. J. PETERLE
OHIO CO-OPERATIVE WILDLIFE RESEARCH UNIT,
COLUMBUS, OHIO

Abstract — Résumé — Аннотация — Resumen

DISTRIBUTION OF AERIALLY APPLIED MALATHION-S^{35} IN A FOREST ECOSYSTEM. The distribution of malathion (0, 0-dimethyl dithiophosphate of diethyl mercaptosuccinate) in a forested area of east-central Ohio was studied during the summer of 1962. This broad-spectrum insecticide was selected for study on the basis of its increased use in the control of many important forest insect pests in deciduous and coniferous forests of the United States. The need for greater knowledge of the ecology of a forested area, coupled with the need for a more complete understanding of the effects of an insecticide on the fauna, provided the unique possibility of studying the problems simultaneously through the utilization of an isotope-labelled insecticide. S^{35} was selected because of its low beta energy (0.167 MeV) and the adequate half-life (87.1 d). Preliminary one-tenth-acre plot studies in the summer of 1961 provided us with potential application rates in terms of total radiation and also allowed the development of sample preparation technique. A faunal survey of two 20-acre watersheds was conducted during the summer of 1961. In May of 1962, one of the watersheds was treated with an application of 2 lb technical-grade malathion per acre in a formulation of xylene, triton X-155 emulsifier and water. The malathion was synthesized with S^{35} by the Radiochemical Centre, Amersham, England. 1 c of activity was aerially applied to one of the 20-acre forested areas on 15 May and 25 May 1962. The specific activity of the synthesized malathion was 17.5 mc/mM.

The distribution of components of the aerial spray within the forest was measured. Electrically-operated air samplers provided estimates of drift off the area; helium-filled balloons bearing frosted-glass discs measured above-canopy application; glass discs suspended vertically as well as bark samples measured quantities settling out at different layers in the canopy; glass discs and spotting-enamel paper not only allowed a measure of horizontal distribution but a check of a standard spray-distribution detection device. Soil samples and monitoring of marked stakes allowed sub-surface distribution studies.

Samples of water from the intermittent streams, insects, mammals, reptiles and birds indicated the initial and subsequent distribution of the insecticide and its metabolites in the ecosystem. Population studies of the faunal system continued throughout the summers of 1961-62 and a limited amount of survey data will be collected in the summer of 1963. Preliminary results indicate that the insect populations returned to normal in about three weeks and there was no detectable effect on the densities of the vertebrate animals on the sprayed area.

DISTRIBUTION DU MALATHION-^{35}S PULVERISÉ PAR AVION DANS UN ENSEMBLE ÉCOLOGIQUE FORESTIER. La distribution de malathion (0, 0-diméthyl dithiophosphate de diéthyl mercaptosuccinate) dans une zone forestière du centre-est de l'Ohio a été étudiée au cours de l'été 1962. Cet insecticide à gamme d'utilisations étendue a été choisi comme sujet d'étude à cause de son emploi croissant dans la lutte contre de nombreux insectes nuisibles aux forêts de feuillus et de conifères des Etats-Unis. La concomitance du besoin d'une connaissance plus complète de l'écologie d'une zone forestière et de la nécessité d'une compréhension plus approfondie des effets d'un insecticide sur la faune a donné une occasion unique d'étudier les problèmes simultanément grâce à l'utilisation d'un insecticide marqué avec un radioisotope. On a choisi ^{35}S à cause de sa faible énergie bêta (0,167 MeV) et de sa période (87,1 j). Des études préliminaires portant sur des parcelles de quatre ares, au cours de l'été 1961, ont permis aux auteurs de fixer des doses d'application en fonction du rayonnement total et ont permis aussi la mise au point de techniques de préparation d'échantillons. Une étude

* This project was supported by The United States Atomic Energy Commission, Contract No. AT(11-1) 967 through The Ohio State University Research Foundation, and The Ohio Cooperative Wildlife Research Unit. The Unit is supported by The Ohio State University, The United States Fish and Wildlife Service, The Ohio Division of Wildlife, and the Wildlife Management Institute.

de la faune de deux secteurs de huit hectares, dans deux bassins différents, a été faite au cours de l'été 1961. En mai 1962, un de ces secteurs a été traité à raison de 2,25 kg par hectare de malathion commercial dans une formule comprenant du xylène, du triton X-155 comme émulsifiant et de l'eau. Le malathion avait été marqué avec ^{35}S par le Radiochemical Centre d'Amersham (Royaume-Uni). On a pulvérisé par avion une quantité correspondant à une activité de 1 c dans l'un des deux secteurs forestiers de huit hectares, les 15 et 25 mai 1962. L'activité spécifique du malathion marqué était de 17,5 mc/mM.

On a mesuré la distribution des composants de la pulvérisation aérienne dans la forêt. Des échantillonneurs d'air électriques ont permis d'estimer les quantités emportées hors du secteur d'expérience; des ballons d'hélium portant des disques de verre dépoli ont permis de mesurer la quantité en suspension au-dessus de la frondaison; des disques de verre suspendus verticalement et des échantillons d'écorces ont permis de mesurer les quantités à diverses hauteurs de la frondaison; des disques de verre et du papier pour analyse à la touche ont permis, non seulement de mesurer la distribution horizontale, mais aussi de contrôler un appareil courant de détection de la distribution des produits pulvérisés. Des échantillons du sol et des pieux marqués ont permis d'étudier la distribution au-dessous de la surface.

Des échantillons d'eau provenant de cours d'eau intermittents, des insectes, des mammifères, des reptiles et des oiseaux ont indiqué la distribution initiale et ultérieure de l'insecticide et de ses métabolites dans l'ensemble écologique. Des études de la faune ont continué pendant les étés 1961 et 1962 et certaines observations seront encore faites au cours de l'été 1963. Les résultats préliminaires indiquent que les populations d'insectes sont revenues à la normale au bout d'environ trois semaines et qu'il n'y a pas eu d'effets décelables sur la densité des vertébrés dans la surface traitée.

РАСПРЕДЕЛЕНИЕ МАЛАТИОНА, МЕЧЕННОГО S^{35} И РАСПЫЛЯЕМОГО С ВОЗДУХА ДЛЯ ИЗУЧЕНИЯ ЭКОЛОГИЧЕСКОЙ СИСТЕМЫ ЛЕСНЫХ МАССИВОВ. Распределение малатиона (0,0-диметилдитиофосфат диэтилмеркаптоянтарной кислоты) в лесном массиве восточной части Центрального Огайо, США, изучалось весной 1962 года. Этот инсектицид широкого действия был выбран для изучения в связи с его возрастающей ролью в борьбе с многими важными насекомыми-вредителями лиственных и хвойных лесов Соединенных Штатов. Для расширения знаний по экологии лесных массивов, а также более полного понимания влияния инсектицидов на фауну была использована своеобразная возможность изучения этих проблем одновременно посредством применения меченных изотопами инсектицидов. S^{35} была выбрана ввиду низкой энергии бета-излучения (0,167 Мэв) и достаточно короткого периода полураспада (87,1 дня). Предварительное исследование участка земли 0,1 акра летом 1961 года показало степень возможного применения в условиях общей радиации, а также возможность разработать подготовительные методы для взятия проб. Летом 1961 года был проведен обзор фауны двух бассейнов реки площадью в 20 акров каждый. В мае 1962 года один из них был обработан технически-чистым малатионом в количестве 2 фунтов на 1 акр в смеси с ксилолом, тритоном X-155 (эмульгатор) и водой. Малатион, меченный S^{35}, был синтезирован в Великобритании в Радиохимическом центре Эмершем. Малатион общей активностью 1 кюри был распылен на лесном массиве 20 акров 15 и 20 мая 1962 года. Специфическая активность синтезированного малатиона была 17,5 милликюри на миллимоль.

Измерялось распределение компонентов при воздушном распылении в пределах лесного массива. С помощью электрически управляемых пробоотборников воздуха производилась оценка границ зоны распыления; с помощью наполненных гелием баллонов с охлаждаемыми стеклянными дисками измерялось осаждение над кроной; с помощью вертикально установленных стеклянных дисков, а также образцов коры измерялись количества осаждающегося малатиона в различных горизонтальных слоях леса; с помощью стеклянных дисков и специальной бумаги с эмалиевым вкраплением можно было измерять горизонтальное распределение и проверять работу стандартного устройства для изучения распределения при распылении. Измерение образцов почв и маркированных вех позволяло изучить подповерхностное распределение.

По образцам воды, взятым из ручьев, насекомым, млекопитающим, пресмыкающимся и птицам можно было судить о первоначальном и последующем распределении инсектицида и его продуктов обмена в экологической системе. Изучение обитателей леса проводилось в летнее время на протяжении 1961 - 1962 годов, и некоторое количество обзорных данных будет получено летом 1963 года. Предварительные результаты указывают, что количество насекомых восстановится до нормального уровня примерно через три недели и что не наблюдается заметного воздействия на количество позвоночных животных, обитающих в зоне распыления.

DISTRIBUCIÓN EN UN SISTEMA ECOLÓGICO FORESTAL DE MALATIÓN- ^{35}S PULVERIZADO DESDE EL AIRE. Durante el verano de 1962 se estudió la distribución del malatión (0,0-dimetil ditiofosfato del mercaptosuccinato de dietilo) en una zona forestal de la región centro-oriental de Ohío (Estados Unidos). Se eligió este insecticida de espectro tan amplio su empleo se generaliza cada vez más para combatir muchas plagas de insectos importantes en los bosques de coníferas y de árboles de hoja caediza de los Estados Unidos. El empleo de un insecticida marcado con un isótopo radiactivo permite estudiar simultáneamente la ecología de una zona forestal y los efectos del insecticida sobre la fauna. Los autores de la memoria eligieron el ^{35}S debido a su reducida energía beta (0,167 MeV) y a que su período de semidesintegración (87,1 d) resulta conveniente. Los estudios preliminares realizados en verano de 1961 en parcelas de cuatro áreas permitieron determinar las dosis de aplicación posibles en función de la irradiación total, y establecer una técnica de preparación de las muestras. En el mismo verano se estudió la fauna en dos vertientes de ocho hectáreas. En mayo de 1962 una de ellas fue tratada con cinco libras de malatión de calidad comercial por hectárea, disuelto en xilol y emulsionado en agua con Tritón X-155. El malatión marcado con ^{35}S fue sintetizado en el Radio Chemical Centre de Amersham (Gran Bretaña). El 15 y el 25 de mayo de 1962 se aplicó desde el aire un curie de actividad a una de las vertientes de ocho hectáreas. La actividad específica del malatión era de 17,5 mc/mM.

Los autores midieron la distribución de los componentes del insecticida pulverizado sobre el bosque. Aparatos eléctricos de toma de muestras de aire permitieron calcular la parte del insecticida que pasó a las zonas colindantes; el aplicado por encima del nivel de las copas de los árboles se midió con globos de helio portadores de discos de vidrio esmerilado; las cantidades depositadas a diversos niveles del follaje se midieron con discos de vidrio colgados verticalmente y con muestras de corteza; la distribución horizontal se determinó con discos de vidrio y papel especial, que sirvieron también para ensayar un dispositivo destinado a determinar la distribución del insecticida pulverizado. Por toma de muestras de tierra y recuento de zonas delimitadas se estudió la distribución bajo la superficie del suelo.

Las muestras de agua tomadas en arroyos intermitentes y los ensayos realizados con insectos, mamíferos, reptiles y aves indicaron la distribución inicial y subsiguiente del insecticida y sus metabolitos en el sistema ecológico. Los estudios de la población animal siguieron durante los veranos de 1961 y 1962; en el verano de 1963 se reunirán todavía algunos datos. Los resultados preliminares indican que la población de insectos vuelve a la normalidad unas tres semanas después del tratamiento; no se observó efecto alguno en los animales vertebrados de la zona tratada.

INTRODUCTION

A greater understanding of the complexities of man's total environment is necessary for the maintenance and improvement of our standard of living and for our survival. One aspect of our environment, the forest, making up about one third of the vegetative cover of the land, has profound influences on man, topography, climate, and use of other natural resources.

The need for a greater ecological understanding of forested areas has become evident to biologists as man intensifies his use of such lands for wood products, watersheds, wildlife habitat, and recreation. Parallel needs for an understanding of pesticidal effects on forests have become obvious to many people following widespread use and misuse of these chemicals.

Insecticides play an integral part in the management of productive land with approximately 92 million acres treated annually in the United States, 22% of which is aerially treated. Millions of acres are sprayed world-wide for protection of trees and control of disease vectors. True multiple use of forested and agricultural areas can only be accomplished with an appreciation for the entire biotic community, including man. Our study presented an unusual opportunity to study the problems of insecticides and forest ecology simultaneously for mutual benefit.

The need for more knowledge in these complex fields of investigation is evidenced by our meeting here to discuss mutual problems and exchange ideas.

THE INSECTICIDE MALATHION

Malathion, 0, 0-dimethyl dithiophosphate of diethyl mercapto-succinate ($C_{10}H_{19}O_6PS_2$) is a broad-spectrum, general-purpose, residual organophosphate insecticide and acaricide. Extensive reviews of the use and characteristics of malathion are readily available in the literature [1, 2, 3], that of SPILLER [4] being very comprehensive. Malathion ranks second only to Parathion in world production of organo-phosphates, over 5000 tons being produced annually [5].

Since we are primarily concerned in this report with an ecological problem, a complete review of the mode of action and metabolism of malathion does not seem warranted. The insecticide is converted to the oxygen analogue, malaoxon, which reacts in vivo as an anticholinesterase [6]. One of the most important properties of the insecticide and a justifiable reason for its widespread use is its low mammalian toxicity. O'BRIEN [6] describes this metabolism or detoxification performed primarily by the liver, which makes it unavailable to bird and mammal blood-stream cholinesterases.

We selected malathion for this study because it has great potential use in forest insect control, has been extensively studied, has a low mammalian toxicity and has a short residual action. Malathion has been successfully labelled with P^{32} and S^{35}. We selected the latter isotope primarily because of its longer half-life. The Radiochemical Centre, Amersham, Bucks, England, synthesized a small quantity of the material for us in 1961 and produced 1 c of S^{35}-labelled malathion for delivery in May 1962. The radiochemical purity of the synthesized insecticide was checked by paper chromatography. The material was tested 96% pure and was assayed on 13 May 1962 at a specific activity of 17.5 mc/mM. The malathion was delivered to our project area in four ampoules containing a total of 1051 mc of activity. The radioactive insecticide was formulated at the project area with xylene solvent (30%), Triton X-155 emulsifier (8%), and water at the rate of 2 lb technical-grade malathion (62%) to each acre treated. The one-swath application on 15 May 1962 was made at the rate of 10 US gal of formulated material per acre, while the 25 May application was made at the rate of 5 gal per acre. Each application was at 2 lb per acre technical-grade malathion. The earlier one-swath application was made with a Piper J-3 aircraft and the latter on 25 May was applied with a Piper P-18 aircraft. The aerial application was under the supervision of The Ohio State University School of Aviation.

DESCRIPTION OF STUDY AREA

The two forested watersheds under study total 41.5 acres and are located in Tuscarawas County in the unglaciated eastern portion of Ohio, United

Fig. 1

An aerial photograph of the two watersheds studied (Dover, Ohio 1962): the southern (lower) watershed was treated, the northern served as a control.

States of America (Fig. 1). The gradient of the wooded slopes of the watersheds ranges from 5 to 40% and is covered by a second-growth dry mixed mesophytic hardwood forest, composed largely of yellow poplar (Liriodendron tulipifera), white oak (Quercus alba), red oak (Quercus borealis), red maple (Acer rubrum), and black oak (Quercus velutina). Timber stocking is about 70 ft^2 of basal area per acre, and approximately 6500 board feet per acre. The shrub layer consists largely of sassafras (Sassafras albidum), spice bush (Lindera benzoin), maple-leaf viburnum (Viburnum acerifolium), and the saplings of the various tree species (Fig. 2).

The study area was commercially clear cut about 1900 and, except for a stand-improvement cut made in 1948 and a few commercial trees taken from the ridges in 1949, it has remained in a natural state. Ground fires apparently burned over the watersheds during the early 1930's. The area

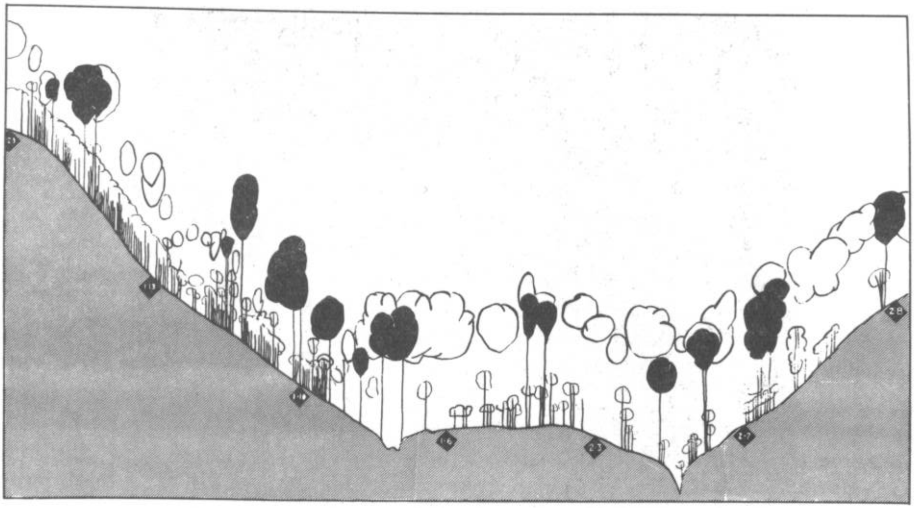

Fig. 2

A vegetative transect diagonally across both watersheds showing all woody stems 10 or more feet tall (Dover, Ohio 1962)

Trees within the transect 35 or more feet tall are shown with black canopies; canopies intercepted by the transect are shown in white.

The horizontal to vertical ratio is 4:1; there are 290 ft between numbered squares.

is currently part of a 160-acre tract leased to the United States Forest Service for watershed research purposes.

METHODS OF STUDY

Preliminary project planning began in the summer of 1960 and actual field observations were initiated in March 1961. The two watersheds, one of 23.4 acres and the other containing 18.1 acres, were surveyed and marked with a grid of 200 ft. Each grid intersection acted as a central sampling point for the various segments of the faunal system.

The problem of designing an adequate sampling scheme for the entire faunal system of the watersheds under study was recognized and the work programme was adjusted to provide the most satisfactory data within the limits of funds and manpower available. Table I lists the methods used to sample the various segments of the animal populations occuring on the study areas. Some of the methods proved successful and provided adequate information for comparing differences in populations. Major problems occurred in sampling reptiles, amphibians and the invertebrates found in the small intermittent streams. Any stream-bottom sampling method described in the limnological literature so altered the limited habitat available that these methods were not acceptable. Drift fences and traps designed to capture reptiles moving across the study units were not successful.

Sampling in 1961 and 1962 took place between April and September. Limited samples of certain species of insects will be taken during the

TABLE I

SUMMARY OF SAMPLING AND OBSERVATIONAL TECHNIQUES USED WITH THE FAUNAL SEGMENTS DURING THE STUDY
Dover, Ohio 1961-62

Soil micro-and macro-fauna	Soil CO_2 output
	Plate counts
	Berlese funnel extractions from standard-size nylon net bags
	Cryptozoan oak drop boards
Insects and invertebrates	Light trap
	Sweep net
	Earthworm extractions
	Molasses trap
	Sticky boards suspended
	Stream drift nets
	0.5-m^2 quadrats
	Stream bottom sample
	Random collecting
	Cryptozoan oak drop boards
	Tree-trunk sticky bands
Molluscs	Random collection
	Quadrat counts
Amphibians	Roofing-felt quadrats
	Random observations
	Streamside counts
	Sherman-trap counts
Reptiles	Random capture and numbering of turtles
	Snake and turtle drift-fence traps
	Random capturing and marking of snakes
	Tar-paper quadrats
Birds	Early-morning transect lines
	Breeding-bird observations
	Random observations

TABLE I (cont.)

Mammals	Large mammal live traps
	Sherman live traps
	Transect lines for mammal holes
	Mammal holes under drop boards
	Plastic tile dropping boards
	Feeding activity along hole transect lines
	Large-mammal burrow counts
Fish	Stream cages and buckets on plots

summer of 1963 in addition to S^{35} residue analyses in both vertebrates and invertebrates.

Table II presents data on the formulation and treatments applied to the watersheds in 1962.

RADIOACTIVE SAMPLE PREPARATION TECHNIQUES

All specimens collected for radioassay of S^{35} were either air-dried, placed in preservative, frozen, or brought to the laboratory in plastic bags and processed within 24 h. Information on each specimen included month, day, number of collection for each day, location, description, date received by the taxonomist, date received in the laboratory, date of the radioactive assay, and the final disposal.

Techniques described by JEFFAY et al. [7] were used with modifications to determine the level of S^{35} in each sample. The final $BaSO_4$ precipitate on a Millipore filter * in an aluminium planchet was assayed in a thin-window gas-flow counter. Analysis of the resultant data were made on the basis of statistical tests of sampling errors as described by JARRETT [8].

Spray deposition and assessment throughout the study was made on 1-in (2.54 cm) diam. circular glass discs, frosted on one side. After air-drying, these were placed on planchets and counted as described for the filtered samples.

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 simple fall-out nuclides such as Sr^{90} or Cs^{137} are extremely

* Type HAWPO 2500

** Nuclear Chicago Model C-115

TABLE II

SUMMARY OF SPRAY FORMULATION DATA FOR TWO APPLICATIONS OF MALATHION-S^{35}
Dover, Ohio 1962

Crash flight	
Date	15 May 1962
Area treated	1 spray swath (approx. 4 acres)
Rate malathion	unknown, (2 lb/acre planned)
Volume of formulation per acre	10 gal/acre
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
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	10-20 gal
Estimated amount entering soil at crash site	30-40 gal
Type aircraft	Piper J-3
Final flight	
Date	25 May 1962
Area treated	23.4 acres
Rate malathion	2 lb/acre
Volume of formulation per acre	5 gal
Loads	3
Total volume of each load	39 gal
Total volume to be applied	117 gal
Malathion per load	15.6 lb
Xylene per load	1.86 lb
Triton X-155 emulsifier per load	0.5 lb

TABLE II (cont.)

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
Amount malathion S^{35} per acre	0.638 g
Activity per 10-μl formulation 13 May 1962	3220 counts/min
Activity per 10-μl formulation 26 Feb. 1963	323 ± 6.44 counts/min
Type aircraft	Piper P-18

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 WEIDHASS [9] comment that radioactivity in insects may represent the original insecticide, its metabolites, or both. At any specific time an equilibrium may be established between the amount taken up and that excreted. These vary with the insecticide and the insect.

AUERBACH [10] presents some factors needed to evaluate the effects of a radionuclide which 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.

Many of these factors are now being studied in our project. We shall report only on those studies now largely completed, namely, quantity and distribution of the insecticide and insecticide effects.

ISOTOPIC MEASUREMENT OF SPRAY DISTRIBUTION

LADD *et al.* [11] and JENKINS and DAVIS [12] used isotopes for studying the dissemination of airplane sprays. Isotopes have the advantages over dyes of greater accuracy, more rapid assessment, fewer personnel required and ability to measure depositions on vegetation and uneven surfaces without panels or other sampling devices. Additional advantages include: independence of light, temperature, pressure and chemical state of the nuclide; no solubility problems as with dyes; dilution phenomena of isotopes are well known; samples are easy to prepare; and the method is applicable

with volatile chemicals. LADD et al. [11] found that dye and isotope methods agreed within 20%, the isotope tracer giving higher values. They state that the practical limiting sensitivity of the tracer method is more than 100 times that of the dye procedure.

The disadvantages are greater expense, half-life decay and the need for special counting equipment and safety precautions. We agree with LADD et al. [11] that a nuclide with a half-life of 1 to 30 d is most desirable for simple dispersal studies. Where factors of molecular-tagging or other project objectives must be considered these limits do not apply. If the half-life is too short, the isotope deteriorates too rapidly for suitable field work. If the half-life is too long, the area and the equipment may be contaminated too long for desirable subsequent tests.

LADD et al. [11] used 60 mc of Mn^{54} naphthenate in a DDT formulation to test spray dispersal. They used two 6 × 36-in blotter panels and one 11-in 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 the activity. JENKINS and DAVIS [12] used Au^{198}.

Many factors modify the dispersal of aerial spray. 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 behaviour is needed.

Like most insecticide distribution studies, this one was concerned with final spray size and amount of application per unit area. The observed particle size is 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 [13] state 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 [14] 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 it was 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 [15] 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 was in nearby water, although direct spraying of waterways had been avoided. DAVIS et al. [16] list 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 several authors [17,18]. CARLETON et al. [18], for example, state: "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 note that sprays are rotated to the side by the plane propeller. The results of a spray-plane crash on 15 May 1962 in the study area after one swath are shown in Fig. 3. The wide variability of the deposition pattern can only generally be explained. Wind velocity at ground level was less than 2 mile/h. The above mentioned "aerodynamic forces" offer the only explanations. The high concentration "island" to the west is most difficult to explain. It indicates variability and, practically, points up the unlikelihood of avoiding waterways by paralleling them "a reasonable" distance away.

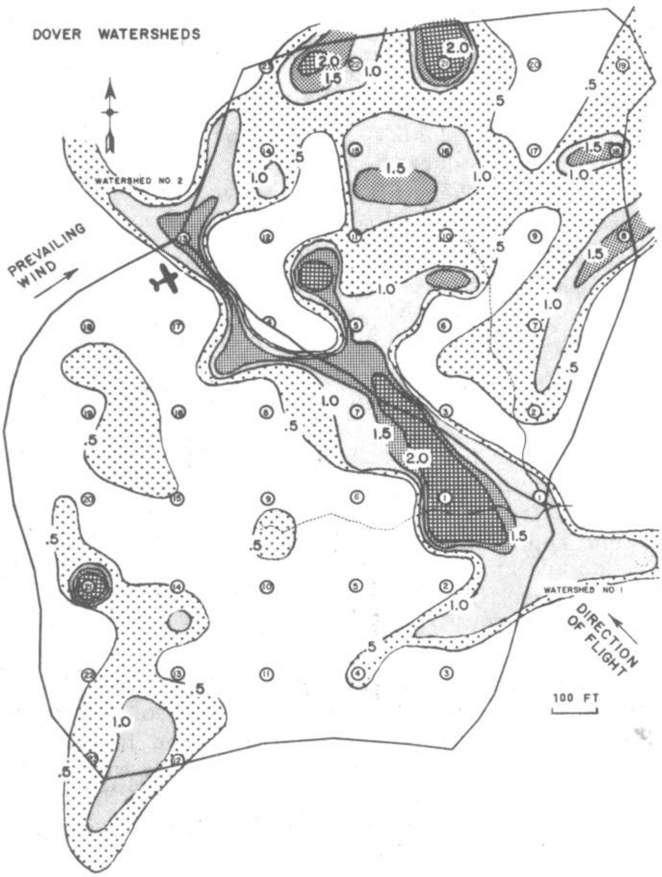

Fig. 3

Distribution of insecticide from one spray swath as measured in mean counts/min from 2 frosted glass discs at 100 ft intervals along the permanent 200 ft grid lines (Dover, Ohio)
The plane crashed where shown near the ridge top on 15 May 1962.

SPRAY ATOMIZATION AND DISPERSAL STUDIES

CARLETON et al. [18] state 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 [19] contends that the ecological hazards of pesticides seem more closely related to the manner of use than to the volume used. Certainly the two are related, for, as CARLETON et al. [18] point 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 and so presents the most complex ecological problems.

In forest aerial spraying, control effectiveness depends largely on two factors, timing and atomization (CARLETON et al. [18] and DAVIS et al. [16]. 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, increased effectiveness and total coverage is difficult to obtain. Failure to obtain such a balance has one alternative – ineffective control. Residue longevity can compensate 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 improve the coverage and materially reduce the load required. Consequently the costs and the effects of weather and other factors on insecticide loss are reduced. Coarse atomization results in less vaporization and loss of spray, but finer atomization allows wider spray swaths and more uniform deposits.

Because the glass-disc method of determining spray distribution was considered highly reliable, attempts were made to correlate disc readings with more standard aerial spray assessment techniques. DAVIS and ELLIOTT [13] have summarized the methods normally used and their disadvantages. The assessment of water-emulsion sprays is more difficult than that of oil sprays,especially when droplet size measurement is desired. Following a suggestion from Dr. J.S. Yuill, United States Forest Service [25], assessment cards of woodbine enamel paper were tested. The Appleton Coated Paper Co., Appleton, Wisconsin, donated thirteen 25× 30-in (63.5× 76.2 cm) sheets of different colour enamel 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 colour, 25 × 38–160 M, BS 80 # bases, was judged best by a panel of three people to provide the most visible and distinct spots. Orange colour, 25 × 38–160 M, BS 80 #, was next best. These two were far more suitable than any of the other 11 samples tested.

For the 15 May flight, two emerald cards, each 4 × 4 in (10.2 × 10.2 cm) were placed in previously installed wire holders which were set up on opposite sides of and 1 ft (30 cm) from each grid stake. The wire holders were those described by MAKSYMIUK [20] and were of 16-gauge wire which, though useable, was too light. The cards were put out less than one hour before the flight and removed 6 h later. Orange cards were used for the second flight. A light rain (unrecorded on the gauge) ruined 40% of the cards, most of them on area 2 following the 25 May 1962 application.

The cards at each location were picked up, numbered and placed in a plastic bag. The radioactivity of the cards was determined in a way perhaps more expedient than statistically sound. In the laboratory a circular disc of 1.25-in (2.98-cm) diam. and an area of 1.23 in^2 (7.93 cm^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 interests of the study in comparing visual spray-deposit assessment methods with "known" radioactivity counts from the same cards or from other sampling devices.

Pirie's reagent (JEFFAY et al. [7] did not satisfactorily digest the paper as it did for preparing "biological samples." The planchet-size paper disc was considered an infinitely thick sample and was counted without further treatment. There are unknown absorption properties of the paper for the spray and the self-absorption of beta activity which influenced the readings.

Counting numbers of droplets and measuring droplet sizes on assessment cards is a tedious, difficult task with much possibility of error. While the comparison of cards with previously prepared "standards" is a desirable method (DAVIS and ELLIOTT [13]), no such standards were available for water emulsion or woodbine enamel paper.

THORNTON and DAVIS [21] recognized the difficulty of direct counts for measuring mass median diameter (MMD) and determined a sampling technique for counting card spots. We used a constant-area sampling intensity (1.23 in^2) which was well over that specified as desirable (0.5 in^2) when droplet sizes exceed 312 μm.

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 (1.25 in) as that removed from each card was drawn with a compass slightly off-centre 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 we measured 20 droplets of malathion formulation suspended on the tip of a finely-drawn glass thread. Half of the droplets were placed on each of the two coloured 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: (see Table III and MAKSYMIUK [20]):

Spread factor = mean spot diam. on card/mean spherical droplet diam.

TABLE III

COMPARISON OF METHODS OF ASSESSING SPRAY DROPLET SIZES AND ACTUAL RATES OF APPLICATION ON ENAMEL-COATED PAPER CARDS
Dover, Ohio 1962

Flight description card type and location	Median spot diameter B	Weighted mean spot diameter C	D_{max} A	Activity of card (counts/min)	Maximum activity of glass discs (counts/min)	Spread factor
Emerald: 15 May						
1-1	83	134	1300	14.0	8.0	
2-4	825	853	1300	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	

TABLE III (cont.)

Flight description card type and location	Median spot diameter B	Weighted mean spot diameter C	D_{max} A	Activity of card (counts/min)	Maximum activity of glass discs (counts/min)	Spread factor
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	981.8	
1-22b	963	914	2900	359.0	281.8	
$\bar{x}$	943	957	2200	137.5	188.3	2.66

The D_{max} reading was obtained from the entire card as described by MAKSYMIUK [22]. The five largest droplet spots were picked by eye, circled with a pencil, measured to the nearest 100 μm, and tabulated in descending order of magnitude. One spot, termed D_{max}, was the largest spot diameter with not more than 200 μm difference between it and the next smaller spot.

The mean spot size was determined by dividing the sum 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 an even numbers of spots, the larger of the two median spots was 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 [22]. A similar manipulation of figures provides almost identical conversion factors of 2.7 and 2.3 when the mean $\overline{D_{max}}$ is divided by the mean of the weighted mean spot diameters. The conversion factor makes use of the measured relationship between diameter and D_{max} to convert subsequent D_{max} observations to the more meaningful MMD:

$$\text{Estimated MMD} = \text{spot } D_{max}/\text{spread factor} \times \text{conversion factor.}$$

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 counts/min yielded a significant correlation coefficient, r, of 0.97 for both spray flights. Either cards or glass discs therefore seem equally suitable for measuring isotope-labelled aerial spray.

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 counts/min is 1:16. Our data indicate that D_{max} overestimates the actual quantity of deposition more frequently than it underestimates it.

ISLER and MAKSYMIUK [23] found that test helicopter flights provided an estimated 144 μm 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 et al. [16] set the bounds of coarse, medium and fine atomization as 452, 291 and 151 μm, 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 [24] found that a MMD of 150 μm provided the most efficient swath pattern for forest spraying. Finer sprays gave a wider, more uniform swath than medium sprays, but more spray was "lost." Coarse sprays resulted in narrow

swaths, reduced uniformity of distribution across the swath and high deposit peaks.

Figure 4 shows the variable pattern of spray distribution on the treated watershed and drift off the area as measured from spray-assessment cards.

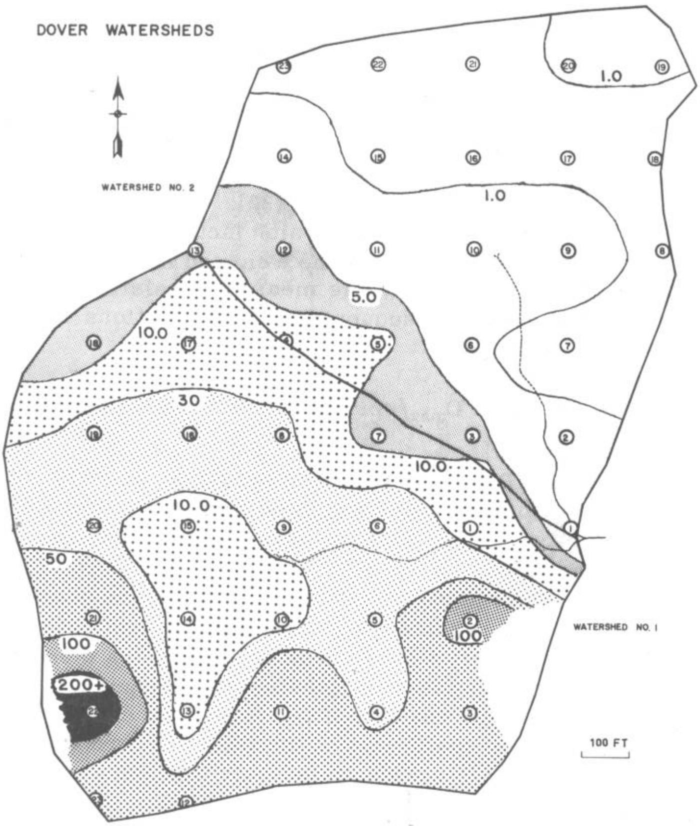

Fig. 4

Distribution of insecticide as measured in mean counts/min from enamel-coated spray assessment cards (25 May 1961 flight) (Dover, Ohio)

Figure 5 shows the amount of insecticide reaching the ground within two days after the application. Glass discs picked up on the date of spray were replaced and picked up two days later. This secondary "application" is of interest in co-ordinating aerial applications with foliation, with insecticide half-life and life histories of insects whose control is sought.

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

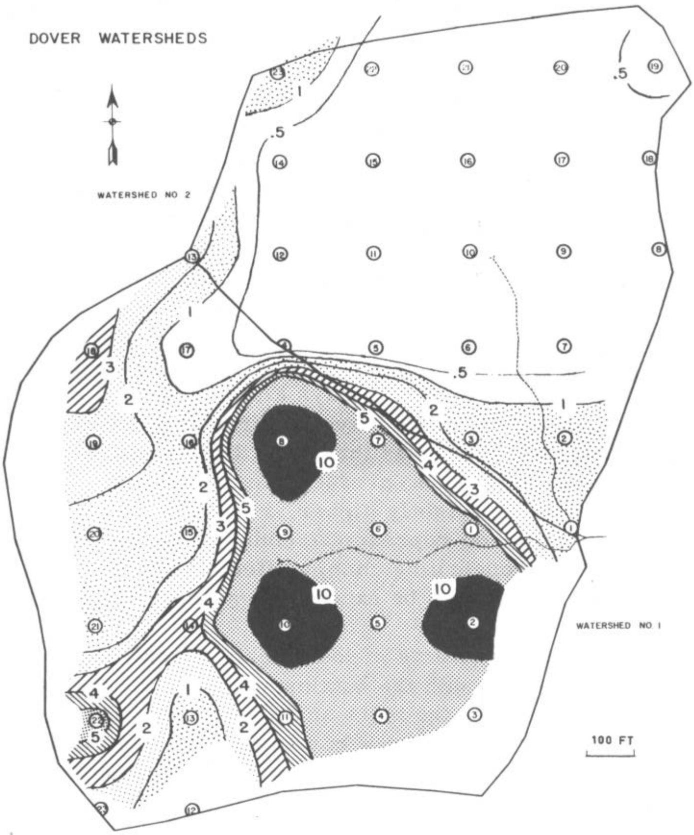

Fig. 5

Mean secondary dispersal of insecticide from rain-out 2 d after the 25 May application (Dover, Ohio) Counts/min from glass discs.

of the amount applied minus all the effects of the plane-to-card environment. The forest is the most complex of such environments and its effects, varying from direct interception to micro-turbulence, greatly influence the measured ground deposition.

An attempt was made to reduce numerically the effects of the forest on the glass-disc deposition readings. Over each of 43 sample points within the watershed, 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.

Within the forest, each layer is not believed to have equivalent effect on spray interception and re-distribution. The open area between strata canopies is variable: tree to shrub, approximately 40 ft; shrub to herbaceous, approximately 4 ft; herbaceous to ground, approximately 1 ft.

We believe that the greater the distance between strata canopies, the move homogeneous will be the unintercepted spray. On the basis of this as-

sumption, each vegetative strata density was multiplied by the following factors: tree, 0.18, shrub, 0.22, and herbaceous, 0.60, to give a truer picture of the influence of the density of each strata on the insecticide finally deposited. Vegetative density observations can range from 1, 1, 1 (all layers heavy) to 4, 4, 4 (all layers absent). The density influence rating (DIR) therefore ranges from 0.33 to 1.33. It is expected that the higher ground disc readings will positively correlate with higher DIR. Lack of correlation is the influence of variable amounts applied above the canopy. When DIR was compared with the maximum disc reading, a significant correlation coefficient of 0.32 was obtained; with mean disc readings, r equalled 0.28, but was not significant. A slight negative correlation r = -0.051 was obtained between DIR and maximum disc readings on the neighbouring control area where drift occurred.

From glass discs left out two days after spraying, a negative correlation would be expected, since leaves intercepting the higher quantities would "apply" the spray. Such was the case. DIR compared with maximum glass-disc readings 2 d after spray produced an r of -0.015. The correlation of DIR with the mean was slightly higher, r = -0.07. Neither correlation was significant, but they do represent trends. This tends to support the previous assumptions concerning canopy effects on atomization, since the post-spray "rain-out" would not be expected to approximate the same pattern as the aerial application.

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. The application within a 1-ft-square area is assumed to be uniform. Spray assessment units on the ground would be expected to receive nearly identical deposits. Such is not the case. At one sampling point in watershed 1, three glass discs within a 1-ft-square area received 217.4, 42.7, and 16.3 counts/min 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 1-ft-square area, and I the amount of spray intercepted by trees, evaporated, etc., then D = A - I. If A is assumed constant for a given area, 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 given area is obtained by:

$$\frac{\text{Maximum sample unit reading per sampling station}}{\text{DIR}} \times \frac{\Sigma\ \text{DIR}}{\text{Number of stations}}$$

PATTERNS OF DISTRIBUTION

1. Above the forest

Helium-filled balloons, on which were glued glass discs, were tethered above the centre of the treated area. Counts recorded were 277.0, 217.6, and 206.1 counts/min. This represents the approximate rate of deposition of 2 lb/acre of malathion on top of the forest canopy. Data from air-sampling devices near the study area have not yet been analysed.

2. Within the tree layer

Glass discs were suspended in pulley-attached ropes within six trees in a transect through the forest. Discs were at intervals of 5 ft. The uppermost 10-15 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 over 50% of the spray. The remaining spray is intercepted irregularly throughout the tree layer (see Fig. 6). There is no apparent correlation between height above the ground and amount of spray (counts/min) delivered.

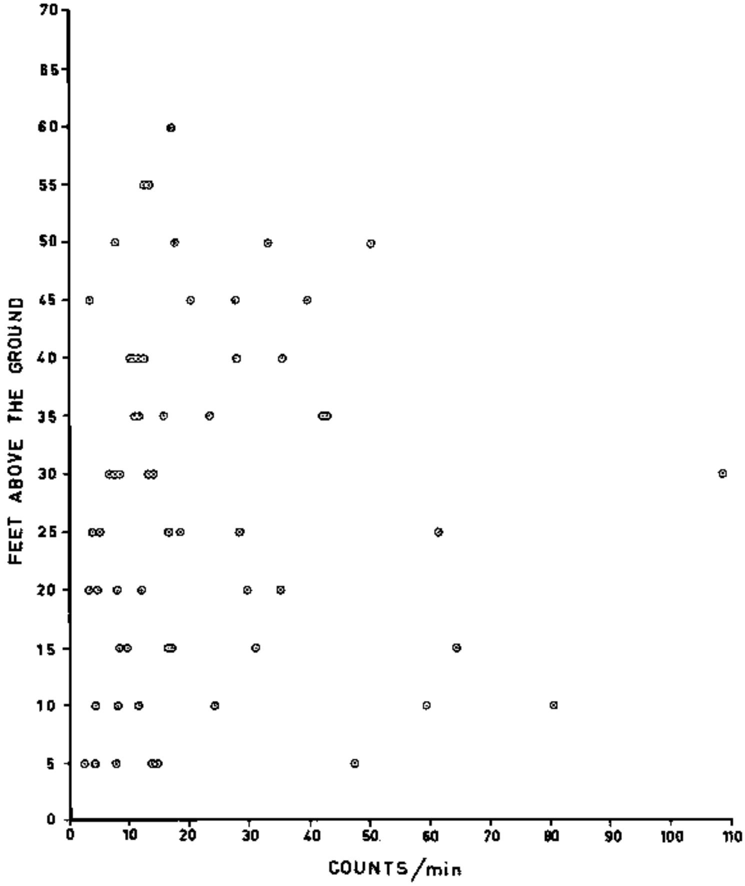

Fig. 6

Scatter diagram of deposition of insecticide (counts/min) on glass discs suspended at 5-ft intervals within 6 tree canopies within the forest (Dover, Ohio 1962)

3. 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

TABLE IV

DISTRIBUTION OF MALATHION IN COUNTS/MIN UNDER SMALL SHRUB OR SAMPLING CANOPIES AS MEASURED FROM GLASS DISCS
Dover, Ohio, 1962

Shrub or sampling species	Distance from ground (in)									
	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

discs were affixed with rubber cement to blocks spaced 6 in (15.24 cm) apart on a 1 in× 3 in× 8 ft pine board set at a 45° angle to the ground. Three of these devices were placed in the centre of watershed No. 1 on 23 May. Each was set up as a tripod under (1) an 8-ft maple coppice, (2) a 6-ft spicebush and (3) a 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 shrub crown radius before the droplets reached the forest floor, where they might be affected by ground winds. In Table IV it can be seen that the irregular pattern of spray dispersal noted within the tree layer prevails. There is a slight tendency for larger amounts to be deposited above 1 m.

4. Within the litter

Field G-M counter readings presented in Table V indicate that radioactivity and thus malathion at 2 lb/acre does not penetrate more than four layers of dead A_1 horizon leaves. The background was about 0.04 mr/h.

TABLE V

PORTABLE FIELD COUNTER READINGS OF RADIOACTIVITY OF LEAF AND SOIL LAYERS
Dover, Ohio 1962*

Description	Plot 2 (mr/h)
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 ¼" below leaf layers	0.01
Soil - 1 in	0.04 - 0.05
Soil - 3 in	0.03 - 0.04
Soil - 4 in	0.01 - 0.02

* Readings taken with a Model P-2612 N.C. thin-window G-M tube, 1.4 mg/cm², under a point near stake 20, plot 2, from 10.0-12.0 a.m. 15/9/1961.

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 G-M readings, are shown in Table VI. The results are variable because of wind blowing the leaves, even though fastened, molestation by animals and continued "rain-out" 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.

TABLE VI

DISTRIBUTION OF MALATHION OR ITS METABOLITES THROUGH VARYING NUMBERS OF LEAVES ON THE FOREST FLOOR
Dover, Ohio, 1962

		27-5-62	6-6-62	15-6-62	13-7-62	7-8-62
Plot 1(1-21)	0	-	3.2	4.4	6.6	-
Number of hickory leaflets	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)	0	7.2	3.8	10.0	8.4	-
Number of beech leaves	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	-
Amount of rainfall since spray		0.09	2.5	3.25	4.7+	4.7+

5. Within the soil

The previous data show that the absorptive properties of the forest litter prevent malathion from entering the soil. This was also borne out by contamination readings on marked stakes driven into the soil for this purpose and glass discs placed in a pit face.

An airplane crash on 15 May provided an unusual opportunity to study the movement of a large (30-40 gal) quantity of radioactive insecticide in a Welston silt loam soil. Figures 7 and 8 show above- and below-ground deposition and movement of the insecticide. Soil core samples were taken with a 1-in diam. King tube in a gridded plot around the crash site. The activity of each one-foot core sample was determined, using a modification of the previously described method. Cross-sectional distribution data are now being studied and will be presented in a later paper. It appears that, even in large quantities, the formulation of malathion moves relatively slowly. The majority of the solution activity remained at the site of deposition as evidenced by low counts delineating the movement boundaries.

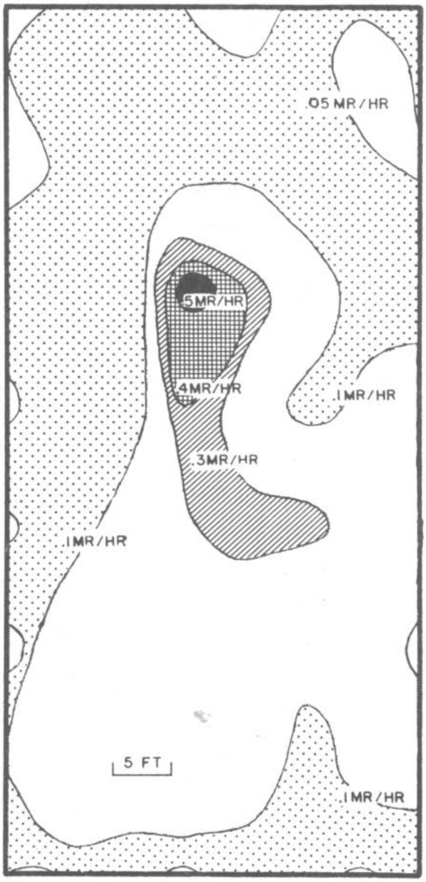

Fig. 7

Surface ground contamination in mr/h in a plot around the crashed Piper J-3 spray plane 10 d post spray. (Dover, Ohio 1962)

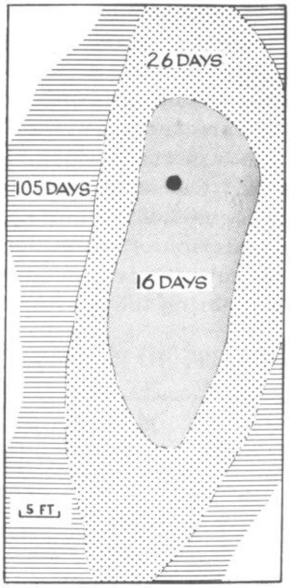

Fig. 8

Internal movement (3-7 ft) of 30-40 gal of radioactive malathion formulation in Welston silt loam soil following a spray plane crash (Dover, Ohio 1962)

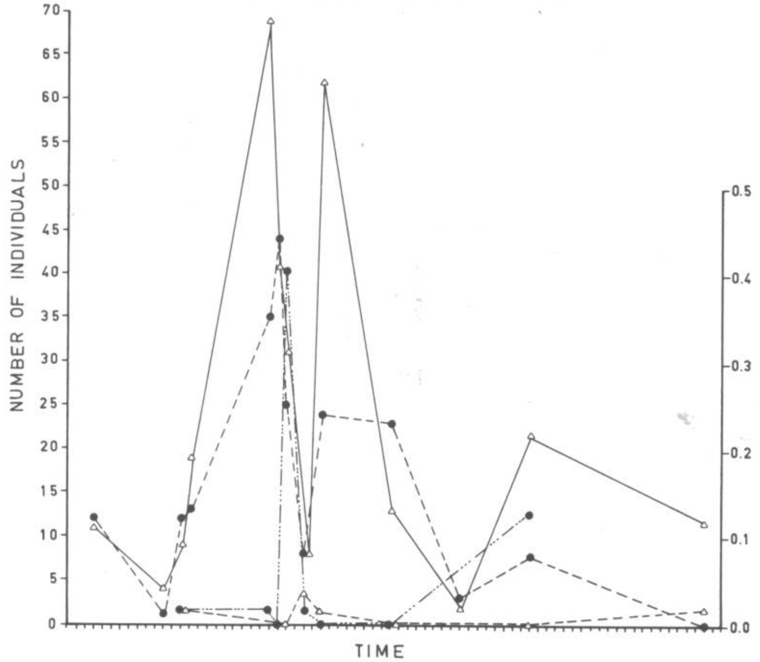

Fig. 9

A comparison of light-trap samples of the adult Geometridae populations with radioactivity of the sample in counts/min mg by days (Dover, Ohio 1962)

FAUNAL EFFECTS

Mammal, reptile and amphibian populations, as suggested by our census indices, were unchanged by the application. One aspect of bird behaviour was altered for three days: they stopped singing in the treated area. Fly-catchers, wood thrushes and warblers continued nesting. Young in nests under observation on the treated area were reared successfully.

Insect population losses were great. Analyses of these data are not complete. Examples of the type of data inspection being made are Fig. 9 of the Geometridae and Fig. 10, the Nitidulidae.

The relationship of the population size to radioactivity in the Nitidulidae sampled with molasses traps is only correlated for about six days, after which there is a decline in activity regardless of population fluctuations. Further studies on life history and feeding habits will be made to attempt to explain this observation.

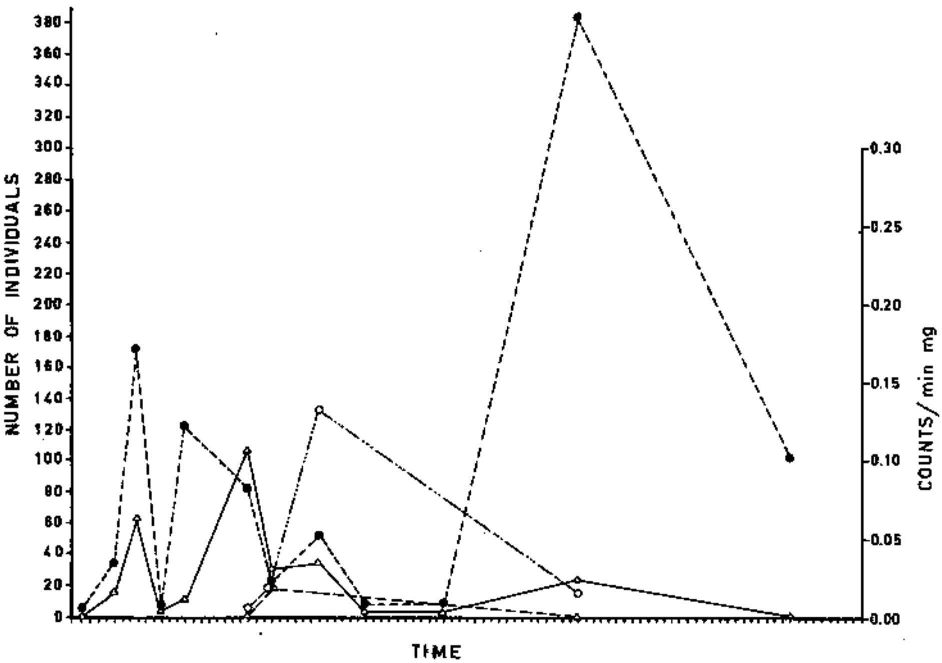

Fig. 10

A comparison of molasses-trapped adult Nitidulidae with radioactivity of the sample in counts/min mg by days (Dover, Ohio 1962)

	Watershed 1	Watershed 2
Number of individuals	-●--●-	-△——△-
Counts/min mg	-O˙˙O-	-△·····△-

In the case of adult Geometridae sampled by light traps, two peaks are evident in the radioactivity measurements. These occur immediately after the application and again following an apparent population decline. Again, analyses of these findings are incomplete.

No effects were observed on soil micro-fauna, cryptozoa or earthworms.

SUMMARY

Malathion-S^{35} was applied to a 23.4-acre Ohio hardwood forest in May 1962. Faunal population changes from previous-year norms were studied, as were the movements of the insecticide within the ecosystem.

Studies of spray distribution are reviewed. Wide variance in lateral, horizontal and vertical distribution of the insecticide formulation in the forest are described and illustrated.

Water emulsion assessment cards were developed and calibrated. The accuracy of the D_{max} system of estimating MMD of atomization was substantiated. The D_{max} system, though mechanically accurate, most often overestimates the actual amount of deposition. A method is presented for computing the best estimate of the actual amount of spray applied to a given area.

Preliminary faunal data inspection indicates no adverse effects of the application except to insects.

REFERENCES

[1] FREAR, D., Chemistry of the Pesticides, 3rd ed., Van Nostrand, New York (1955) 73-94.
[2] METCALF, R.L., Organic Insecticides — Their Chemistry and Mode of Action, Interscience, New York (1955) 392.
[3] NEGHERBON, W.O., Handbook of Toxicology, Vol. III — Insecticides — A Compendium, W.B. Saunders, Philadelphia (1959) 451-464.
[4] SPILLER, D., Advances in Pest Control, Interscience, New York (1961) 249-335.
[5] METCALF, R.L., Bul. Ent. Soc. Amer. 5 1 (1959) 3-15.
[6] O'BRIEN, R.D., Toxic Phosphorus Esters: Chemistry, Metabolism, and Biological Effects, Academic Press, New York (1960) 434.
[7] JEFFAY, H., OLUBAJO, F.O. and JEWELL, W.R., Anal. Chem. 32 (1960) 306-308.
[8] JARRETT, A.A., USAEC Rpt. ORNL-262 (1946) 43.
[9] SCHMIDT, C.H. and WEIDHASS, D.E., J. Eco. Ent. 51 5 (1958) 640-644.
[10] AUERBACH, S.I., Ecology 39 3 (1958) 522-529.
[11] LADD, J.H., MASLEY, P.T. and HARTNETT, J.G., Memorandum Rpt., US Army Chemical Warfare Service, TDMR 1235 (1946).
[12] JENKINS, D.W. and DAVIS, J.M., Amer. Mosquito Control Assoc., Salt Lake City (1952) 24-27.
[13] DAVIS, J.M. and ELLIOTT, K.R., J. Eco. Ent. 46 4 (1953) 696-698.
[14] SAILER, R.I. and ROZEN, J.G., USDA Agricultural Research Service, Special Rpt. ID-2 (administratively confidential) (1958) 12 pp.
[15] COPE, J.B., Circ. 143, US Bureau of Sport Fisheries and Wildlife (1960) 52.
[16] DAVIS, J.M., WATERS, W.E., ISLER, D.A., MARTENEAU, R. and MARSH, J.W., J. Eco. Ent. 49 3 (1956) 338-341.
[17] ISLER, D.A., DAVIS, J.M. and MAKSYMIUK, B., USDA Forest Service, Special Rpt. Br 57-2 (1957) 41 pp.
[18] CARLETON, W.M., LILJEDAHL, L.A., IRONS, F., HEDDEN, O.K. and BRAZEE, R.D., USDA Rpt. ARS 20-9 (1960) 70-84.
[19] HICKEY, J.J., Governor's Special Com. Chemicals and Health Hazards, Madison, Wisc. (1961).
[20] MAKSYMIUK, B., J. Eco. Ent. 52 5 (1959) 1029-1030.
[21] THORNTON, D.G. and DAVIS, J.M., J. Eco. Ent. 49 1 (1956) 80-82.

[22] MAKSYMIUK, B., Beltsville Forest Insect Lab., USDA Forest Service, 3 pp.
[23] ISLER, D. A. and MAKSYMIUK, B., USDA Agricultural Research Service Rpt. (1961) 9.
[24] ISLER, D. A. and THORNTON, D. G., Agric. Engng. 36 9 (1955) 600-601.
[25] YUILL, J. S., personal communication (15 March 1962).

DISCUSSION

W. KLOFT: I think some of the distribution of your labelled insecticide may be due also to secondary sublimation of the insecticide into the air, and to further distribution by air flows. We have done similar studies, on a much smaller scale, with radioactive-labelled organic insecticides such as, for example, Thiodan. This has very high vapour pressure and can be sublimated from the insect, after a rise in body temperature following intoxication, into the air, and also spread by the air.

The insecticide might be secondarily distributed in this way in your case too. The dark surfaces of plants, bark for instance, could be heated by the sun during the day so as to show a secondary sublimation.

T.J. PETERLE: That is possible. The glass discs which we had distributed vertically in the canopy and horizontally in the forested area were picked up approximately seven hours after the aerial application, so some sublimation may have occurred in the seven hours from early morning until we allowed the insecticide to settle out fully and we felt free to go into the area.

J.E. CASIDA: Did you take any precautions to determine whether your S^{35} was as malathion or as degradation products of malathion?

T.J. PETERLE: We did check some of the metabolites of malathion later with the labelled material and found that the sulphur seemed to be distributed very well in the degradation products. Exactly which those are or how many contain actual sulphur I am uncertain, but in doing paper chromatography studies we found the sulphur was fairly well distributed.

J.E. CASIDA: Do you think that the insects which you show in your graphs were picking up malathion?

T.J. PETERLE: Initially yes, because malathion of course has a residual action of about ten days. After that it could have been the metabolites of just S^{35}.

J.E. CASIDA: You indicated that you were going to continue to sample this year? Would you be studying malathion?

T.J. PETERLE: No, I would not expect to find malathion this year. We shall be checking for S^{35} residues.

J.E. CASIDA: Malathion hydrolyses rather quickly at the carboxyl-site when exposed on the surface of plants, and it might be difficult therefore to interpret some of your results.

M. AROYO VARELA: Did you take advantage of the opportunity to study the influence of the insecticides on the insect parasite fauna? I would be interested to know if you did, and if so, what were your conclusions.

T.J. PETERLE: Yes, we used many sampling techniques, and we can sort out the predaceous insects in our data and examine them as the residual quantity builds up for a period of days. The data from specific insect families is available but it has not been analysed from the standpoint of prey-predator relationships. This will be included in the final publication

E.E. TURTLE: Are any of the birds or mammals collected in and around the sprayed site being analysed for S^{35}?

T.J. PETERLE: Yes. We have data on a wide spectrum of the vertebrate fauna. Since this Symposium was entomologically oriented, we have excluded those data from the paper. The vertebrate residue data will be published in the final report.

E.E. TURTLE: These data would be very valuable for assessing the effects of insecticides less readily metabolized than malathion.

NOTE PRÉLIMINAIRE SUR L'UTILISATION DES RADIOISOTOPES DANS L'ÉTUDE DES PARASITES DU COTONNIER EN AFRIQUE

R. DELATTRE
INSTITUT DE RECHERCHES DU COTON ET TEXTILES, PARIS, FRANCE

Abstract — Résumé — Аннотация — Resumen

PRELIMINARY NOTE ON THE USE OF RADIOISOTOPES TO STUDY SOME COTTON-PLANT PESTS IN AFRICA. The larva of Diparopsis watersi Roth is harmful to the cotton-plant, destroying the flower and the boll. This noctuid, which is practically monophagous, passes the quiescent season either in the ground in the chrysalid-at-diapause form (from 10 November onwards) or in continuing generation (polyvoltine) on plants left standing. Laboratory studies have served to elucidate the main mechanisms initiating and terminating diapause.

In the field, the rapid multiplication of the pest during the crop period is due both to univoltine individuals hatching out towards April-May and to non-diapause generations. The respective roles played by these two different sources need to be determined fairly accurately in order to decide which methods of control are appropriate: uprooting of the cotton-plant, destruction of the pupae at diapause in the ground, bringing the crop forward, etc. Radioisotope labelling of larvae coming to maturity at critical periods should make it possible to tackle this problem at the practical level.

Preliminary experiments for determining simple labelling techniques have been carried out at Tikem (Republic of Chad). In a first trial, P^{32} in atomized solution was applied direct to the foliage of young cotton-plants. Despite the effects of rain, absorption by the plant reached about 10% in a few hours. Radioactivity is not retained by various phyllophagous larvae (Sylepta derogata, Prodenia litura), but those feeding on the fruit-bearing parts of the plant (Heliothis armigera, Earias insulana, Diparopsis watersi, etc.) are easily detectable three months after the application.

In a second trial, P^{32} and S^{35} were applied to old cotton-plants immediately before the natural time for Diparopsis to enter diapause. The overall findings give hope of being able to distinguish without too much difficulty, among chrysalid populations taken from the ground, those which fed on the cotton-plant before labelling, and hence which underwent a diapause.

A control experiment will now be required, consisting of labelling, during the season, cotton-plants in a field where cotton was also grown the previous year; this time non-diapause chrysalids will be labelled. Similar observations are also being performed on a number of other cotton-plant pests.

NOTE PRÉLIMINAIRE SUR L'UTILISATION DES RADIOISOTOPES DANS L'ÉTUDE DES PARASITES DU COTONNIER EN AFRIQUE. Le chenille de Diparopsis watersi Roth est nuisible au cotonnier en détruisant fleurs et capsules. Cette noctuelle, pratiquement monophage, passe l'inter-saison, soit dans le sol sous forme de chrysalides en diapause à partir du 10 novembre, soit par des générations continues (polyvoltines) sur les plants non arrachés. Des études de laboratoire ont permis d'élucider les principaux mécanismes déclenchant la diapause et y mettant fin.

Dans la nature, les pullulations en cours de culture s'établissent à partir d'individus univoltins venant à éclosion vers avril-mai aussi bien que des générations sans diapause; la participation respective de ces deux souches différentes devrait être déterminée assez exactement pour connaître les moyens de lutte appropriés: arrachage du cotonnier, destruction des pupes en diapause dans le sol, avancement de la campagne, etc. Le marquage par des radioisotopes des chenilles venant à maturité aux époques critiques devrait permettre de serrer ce problème sur le plan pratique.

Des expériences préliminaires ont eu lieu à Tikem (République de Tchad) en vue de déterminer des techniques de marquage simples. Dans un premier essai, ^{32}P a été appliqué en pulvérisation aqueuse directe sur le feuillage de cotonniers jeunes. Malgré la pluie, l'absorption fut de 10% environ par la plante en quelques heures. Diverses chenilles phyllophages (Sylepta derogata, Prodenia litura) ne retiennent pas de radioactivité, mais les chenilles qui se nourrissent d'organes fructifères (Heliothis armigera, Earias insulana, Diparopsis watersi, etc.) sont facilement détectable trois mois après l'application.

Dans un deuxième essai, ^{32}P et ^{35}S ont été appliqués sur des cotonniers âgés, juste avant la période de mise en diapause naturelle de Diparopsis. Les résultats d'ensemble laissent espérer que l'on pourra sans trop de difficultés distinguer, parmi les populations de chrysalides prélevées dans le sol, celles nourries sur le cotonnier avant qu'il ne soit marqué, donc qui auront subi une diapause.

Une contre-expérience sera ensuite nécessaire; elle consistera à marquer, en cours de saison, les cotonniers d'un champ ayant déjà porté la même culture l'année précédente, les chrysalides sans diapause étant cette fois marquées. Des observations annexes sont également en cours sur divers autres parasites du cotonnier.

ПРЕДВАРИТЕЛЬНЫЕ ЗАМЕЧАНИЯ О ПРИМЕНЕНИИ РАДИОИЗОТОПОВ ДЛЯ ИЗУЧЕНИЯ НЕКОТОРЫХ ВРЕДИТЕЛЕЙ ХЛОПЧАТНИКА В АФРИКЕ. Гусеница Diparopsis watersi (Roth) наносит вред хлопчатнику, уничтожая цвет и коробочку. Эта, практически, монофаговая совка проводит межсезонный период либо в земле в виде куколки в состоянии диапаузы, начиная с 10 ноября, либо в виде новых (поливольтинных) поколений на не выкопанных из земли растениях. Лабораторные исследования позволили выяснить основные механизмы возникновения и прекращения диапаузы.

В природе интенсивное размножение при разведении культур происходит у одних особей с одним поколением в год, вылупляющихся к апрелю-маю, у вторых - появление поколений без диапаузы; относительную роль этих двух различных ветвей особей следовало бы точно определить для выбора метода борьбы: выкорчевка хлопчатника, уничтожение куколок в состоянии диапаузы в земле, сокращение сроков сельскохозяйственной кампании и т.д. Мечение радиоизотопами гусениц, которые в критический период достигают зрелости, должно сузить эту проблему в практическом плане.

В Тикеме (Республика Чад) были проведены предварительные опыты для определения простых методов мечения. В первом опыте был использован P^{32} при прямом водном обрызгивании листвы молодого хлопчатника. Несмотря на дождь, поглощение растением в течение нескольких часов составляло около 10%. Различные филлофаговые гусеницы (Sylepta derogata, Prodenia litura) не накапливают радиоактивности, однако питающиеся плодоносными органами гусеницы (Heliothis armigera, Earias insulana, Diparopsis watersi, и т.д.) легко обнаруживаются спустя три месяца после применения этого метода.

Во втором опыте P^{32} и S^{35} были использованы для взрослых кустов хлопчатника как раз накануне периода естественного перехода Diparopsis в диапаузу. Результаты позволяют надеяться на то, что удастся без излишних трудностей выявить среди изъятых из земли куколок тех, которые питались хлопчатником до его мечения и которые поэтому пройдут диапаузу.

Необходимо проведение обратной проверки, которая состоит в том, чтобы в течение сезона пометить хлопчатник на поле, уже находившемся под этой культурой в прошлом году. В данном случае куколка без диапаузы оказывается меченой. В настоящее время проводятся также дополнительные наблюдения за другими вредителями хлопчатника.

NOTA PRELIMINAR SOBRE EL EMPLEO DE RADIOISÓTOPOS EN EL ESTUDIO DE PARÁSITOS DEL ALGODONERO EN ÁFRICA. La oruga de Diparopsis watersi Roth es perjudicial para el algodonero, pues destruye sus flores y cápsulas. Este noctúido, prácticamente monófago, pasa el período interestacional bien en el suelo en forma de crisálidas en diapausa a partir del 10 de noviembre, bien por generaciones continuas (especies que se reproducen varias veces al año) en las plantas sin arrancar. Los estudios de laboratorio han permitido dilucidar los principales mecanismos que determinan el comienzo y el fin de la diapausa.

En la naturaleza, las pululaciones durante el cultivo provienen de individuos de una sola generación al año, que alcanzan la eclosión hacia abril o mayo, así como de generaciones sin diapausa. Debería averiguarse con suficiente exactitud el papel que desempeñan estas dos cepas diferentes, para determinar los medios de lucha más apropiados: arranque del algodonero, destrucción de las ninfas durante la diapausa en el suelo, anticipación de la campaña, etc. La marcación radioisotópica de las orugas que alcanzan la madurez en las épocas críticas permitiría seguramente abordar este problema en el plano práctico.

En Tikem (República del Chad) se han realizado experimentos preliminares para preparar técnicas sencillas de marcación. En el primer ensayo se aplicó ^{32}P en pulverización acuosa directa sobre el follaje de algodoneros jóvenes. Hay orugas filófagas (Silepta derogata, Prodenia litura) que no retienen la radiactividad, pero las que se alimentan de órganos fructíferos (Heliothis armigera, Earias insulana, Diparopsis watersi, etc.) se pueden detectar fácilmente tres meses después de la pulverización.

En el segundo ensayo se aplicaron ^{32}P y ^{35}S a algodoneros viejos, inmediatamente antes de comenzar el período de diapausa natural de Diparopsis. Los resultados obtenidos hacen pensar que no será muy difícil distinguir, entre las poblaciones de crisálidas recogidas del suelo, las que se han nutrido en el algodonero antes de ser marcado, es decir, las que habrán sufrido una diapausa.

Seguidamente será preciso un experimento comprobatorio, consistente en marcar en plena temporada a los algodoneros de un campo que haya tenido el mismo cultivo que el año precedente, pero limitándose esta vez a las crisálidas sin diapausa. También se están haciendo estudios complementarios sobre otros parásitos del algodonero.

Parmi les nombreux parasites qui infligent chaque année des pertes considérables aux cultures cotonnières en Afrique, la chenille d'une noctuelle, Diparopsis watersi, est particulièrement néfaste dans les zones sahéliennes et subsahéliennes qui s'étendent depuis le Mali jusqu'au Tchad.

Cette chenille a fait l'objet de longues recherches de la part d'auteurs anglais en Nigeria et au Sudan, et la Station IRCT de Tikem, au Tchad, a fait porter son programme sur ce parasite. Les résultats obtenus par Galichet ont complété ou éclairci nombre de points sur la biologie de l'insecte et contribué à une meilleure connaissance des facteurs de la diapause.

En effet, Diparopsis offre deux particularités biologiques importantes. Tout d'abord, c'est un insecte à peu près strictement monophage, admettant les diverses espèces de cotonnier comme nourriture constante, et ne pouvant que très occasionnellement et très temporairement vivre aux dépens de quelques malvacées très proches. Dans la nature par conséquent, le maintien de Diparopsis dépend strictement de la présence d'une espèce ou d'une autre du genre Gossypium.

En second lieu, cette noctuelle possède, comme bien d'autres lépidoptères et bien d'autres arthropodes, un stade de repos plus ou moins prolongé, touchant le stade pupal (chrysalide), auquel le déterminisme précis donne un caractère de véritable diapause. Cette phase a lieu dans le sol, à quelques centimètres seulement de la surface. Toutefois, tous les individus ne sont pas obligatoirement touchés par cette diapause; au début de la saison, les conditions extérieures (température et aussi photopériodisme, hygrométrie, etc.) ne sont pas favorables à la mise en diapause, et seuls quelques individus la subissent. Au début du mois de novembre, la température nocturne s'abaisse en-dessous de 16° tandis que la température diurne reste encore élevée (supérieure à 32°) et en même temps la durée du jour diminue de façon assez rapide; à cette époque une forte majorité de chenilles entre en diapause, pas la totalité cependant.

Galichet a pu émettre l'hypothèse de l'existence de deux races de Diparopsis se trouvant en mélange dans toute la zone tchadienne, l'une insensible à la diapause et évoluant donc sans interruption d'une campagne à l'autre, l'autre pouvant se mettre en diapause à une époque déterminée et attendant jusqu'au printemps sous forme de chrysalide la culture suivante. Au laboratoire, des élevages de souches différentes auraient donné une première confirmation de cette hypothèse.

Mais en se plaçant sur le plan pratique, il est un problème que les études de laboratoire, en milieu artificiellement conditionné, ne sauraient résoudre : c'est le problème de la participation respective des générations venues de diapause et des générations qui au contraire se sont succédé sans interruption depuis la campagne précédente. En effet, la lutte doit s'orienter de manière à briser le cycle au moment le plus favorable; dans le premier cas, c'est la phase chrysalide en diapause dans le sol, et on procédera par des interventions agricoles (labour, scarifiage, etc.) sur le champ venant de porter du coton, ou encore par un assolement tel que les champs de

l'année soient très éloignés des emplacements ayant porté du coton l'année précédente; dans le second cas, ce sont les plants de cotonniers non arrachés en fin de campagne qui hébergent les chenilles pendant la morte saison théorique que l'on devra pourchasser complètement.

Il faudrait donc déterminer dans un champ cultivé en coton deux années de suite, quel est le pourcentage de chrysalides en diapause et celui de chrysalides sans diapause au début de la deuxième campagne. Comme il s'agit de races physiologiques ne pouvant se reconnaître par aucun détail anatomique externe ou interne, on se trouve face à une difficulté pratiquement insurmontable jusqu'à présent.

En faisant intervenir le marquage par les radioisotopes, on a pensé tenir un moyen théoriquement plus simple de résoudre cette question. En effet, si l'on peut marquer la plante pendant un temps donné, les chenilles ayant évolué sur cette plante seront elles aussi marquées, ainsi que les chrysalides qui en résulteront plus tard, tandis que les chenilles ayant donné des chrysalides avant que la plante ne soit marquée ne seront pas actives. On peut donc avoir un datage relatif de ces chenilles - et reconnaître celles appartenant aux générations sans diapause.

Ce datage peut être réalisé dans un autre sens si l'on peut trouver un isotope possédant une vie assez longue : on reconnaîtra alors les chrysalides qui ont vécu la campagne précédente sur des plants marqués.

C'est en vue de déterminer les conditions préliminaires d'une telle recherche que nous avons réalisé les premières expériences de marquage, à la Station de Tikem (Tchad), dans un programme de coopération avec la Section «Biologie» du CENS.

Les questions préalables à résoudre étaient celles-ci :

- Peut-on procéder par pulvérisation directe sur le feuillage afin de diminuer autant que possible la contamination du sol, qui estomperait en les allongeant les limites du marquage?
- Quelles substances employer qui soient absorbées par la plante et assimilées par l'insecte, sans présenter de danger trop grand à l'usage dans les conditions très rustiques où l'on se trouve?
- Quelles durées valables de marquage obtiendra-t-on, et comment le diagnostic sera-t-il obtenu avec le plus de précision?

PREMIER ESSAI PRÉLIMINAIRE

Dans une première expérience on a choisi le ^{32}P pour des raisons évidentes de prix de revient, de facilité d'obtention et de repérage de probabilité d'absorption aisée par le feuillage, etc. Les inconvénients auxquels on s'attendait a priori étaient : vie assez limitée, manipulation en plein air ayant un certain risque, etc.

L'application a été réalisée le 13 août dans les conditions suivantes :

Matériel

- Equipement de protection : scaphandre autonome avec masque respiratoire et appareil générateur d'oxygène «Feuzy», gants et surchausses de caoutchouc;
- Compteur portatif pour le contrôle et la décontamination;

- Réserve d'eau et seaux pour lavages;
- Pulvérisateur type Vermorel (fixé sur le ventre en raison de l'appareil respiratoire fixé sur le dos) et lance de pulvérisation;
- Seringue et récipients pour la manipulation du ^{32}P.

Conditions d'application

Attendre que la rosée ait presque complètement disparu, pour éviter le ruissellement, mais aussi opérer avant que le vent ne se lève, ce qui aurait présenté un accroissement de danger pour l'opérateur et diffusé la substance nors des limites tracées. Bien qu'on se trouva à cette époque en pleine saison des pluies, pluies après l'exécution, la chance a permis que l'application ne soit lessivée que trois jours après.

Commencés à 9 h 30, les préparatifs ultimes et le traitement étaient terminés à 9 h 50.

4 billons de 20 m avaient reçu 8 l de solution contenant environ 9 mc. (Le 7 août, jour du contrôle de l'activité au Laboratoire de Saclay, l'échantillon envoyé donnait 13 mc.)

Les obstacles rencontrés au cours de cette application furent :
- la gène apportée par les hauts billons dans la marche rendue déjà pénible par le scaphandre et l'appareil respiratoire;
- l'encombrement du pulvérisateur de grand volume porté en position très incommode sur le ventre;
- la température et surtout l'hygrométrie très élevées s'établissant rapidement à l'intérieur du scaphandre et rendant particulièrement pénible le travail dans un soleil déjà chaud.

Malgré celà, l'opération eut lieu jusqu'au bout sans incident. Une équipe dirigée par un européen procéda ensuite aux premières décontaminations sur place. La décontamination fut complétée ensuite à l'aide du compteur gammamètre; les instruments et accessoires ne pouvant être décontaminés furent immergés dans le vaste lac de Tikem ou jetés dans les galeries d'une termitière profonde.

On peut suggérer quelques améliorations de détail à ce modus operandi. En particulier nous pensons qu'un système de pulvérisation comportant une bouteille de Freon comme source de pression allégerait notablement le travail physique de l'opérateur.

Ramassages de chenilles

On « enrichissait » la parcelle en y apportant les chenilles de tous âges rencontrées au cours des autres recherches entomologiques faites par la Section. Puis on ramassait ces chenilles aux dates suivantes : 12 septembre, 24 septembre, 1er octobre et 8 octobre. Elles avaient donc passé, sur les plants traités, des temps variables, mais au moins égaux à 24 h.

Ces chenilles mises en alcool furent expédiées en quatre lots au Laboratoire d'applications agronomiques du CENS où, grâce à l'obligeance des collaborateurs, leur activité fut déterminée les 22-23-24 et 25 octobre. Pour cela le tégument est coupé longitudinalement et la chenille est ouverte, les viscères sont dilacérées avec des épingles, et le tout est passé à l'étuve

jusqu'à dessication. On a vérifié que l'alcool qui a servi au transport ne retenait aucune activité appréciable.

Les exsiccats individuels sont ensuite pesés, et le tableau des résultats bruts est ainsi obtenu.

Pour utiliser ces résultats, il convient d'abord d'établir des corrections puisque l'activité a décru notablement entre la date de récolte et la date du comptage.

On obtient la valeur de l'activité le jour de la récolte en multipliant les chiffres de comptage respectivement par 6,6 4 3,10 et 2.

Le tableau I donne des valeurs moyennes, établies par classes, de 30 en 30 mmg.

TABLEAU I

VALEURS MOYENNES DE L'ACTIVITÉ

	0 - 30	30 - 60	> 60
1er lot (12.9)			
Nombre chenilles	16	3	3
Poids moyen	12	42	72
Activité/larve	5	10,1	10,2
" corrigée	33	66,6	67,3
Activité/mmg	0,44	0,24	0,19
" corrigée	2,9	1,58	1,18
2e lot (24.9)			
Nombre chenilles	4	4	10
Poids moyen	5	50,1	83,2
Activité/larve	9,7	10,7	5,88
" corrigée	38,8	42,8	23,5
Activité/mmg	1,86	0,20	0,07
" corrigée	7,4	0,8	0,3
3e lot (1.10)			
Nombre chenilles	14	5	1
Poids moyen	12	37	70
Activité/larve	1,3	3,7	3
" corrigée	4	11,47	-
Activitée/mmg	0,11	0,07	-
" corrigée	0,34	0,22	-
4e lot (8.10)			
Nombre chenilles	9	5	13
Poids moyen	11,4	47,2	86
Activité/larve	4,5	27,4	11,2
" corrigée	9	55	22,4
Activité/mmg	0,4	0,58	0,14
" corrigée	0,8	1,16	0,28

Discussion

Le nombre de chenilles ramassées et leur répartition en catégorie sont très variables, suivant l'évolution de l'espèce en plein champ. Les nombres obtenus par l'exploitation de ce tableau ne peuvent pas être interprétés dans un sens strict.

Cependant, on notera que l'activité par larve semble maximale pour la catégorie moyenne.

Si l'on se rapporte à l'activité au mmg de matière sèche, on constate que ce sont les larves les plus jeunes qui présentent généralement le maximum: elles sont dans une phase active de croissance et assimilent rapidement le phosphore, tandisque les larves dans leur phase finale accumulent surtout des lipides peu riches en phosphore.

En fonction de l'éloignement du traitement, on peut constater que le dernier lot donne encore des chiffres très nets permettant une distinction moyenne sans ambiguïté. Il est probable que le temps moyen d'alimentation sur les cotonniers marqués a été plus long que pour le lot n° 3, d'où souvent des chiffres plus favorables que pour celui-ci.

Dans l'expérience finale projetée, où les larves se nourriront toute leur vie sur cotonniers marqués, on peut donc espérer un diagnostic valable trois à quatre mois après, dans le cas du ^{32}P.

Quant aux chenilles n'ayant montré aucune activité, leur nombre diminue avec leur âge, et il reste des chenilles âgées non marquées simplement parce qu'elles ont été placées sur la parcelle peu de temps avant le prélèvement (tableau II).

TABLEAU II

CHENILLES N'AYANT MONTRÉ AUCUNE ACTIVITÉ

	0 - 30	30 - 60	> 60
Nombre chenilles	13	2	3
% total	30%	11%	11%

Résultats avec d'autres chenilles

En même temps que Diparopsis - mais en nombre plus limité puisque ce n'était pas l'objet principal de l'essai - on a récolté des insectes variés : Sauterelles, Dysdercus etc. Ce sont les chenilles de Prodenia, de Sylepta et de Cosmophila et Heliothis qui ont donné lieu à la remarque générale suivante :

Une bonne partie des échantillons d'Heliothis montraient une activité en gros comparable à la catégorie de Diparopsis correspondante. Mais les chenilles strictement phyllophages (Cosmophila et Sylepta) n'ont montré aucune activité.

Aussi donc, l'emploi du ^{32}P pour ce genre de travaux conduit à une limitation dont il faut rechercher la cause dans le métabolisme même de

cet élément dans la plante. En effet, bien que l'absorption ait eu lieu pour une large part directement à travers le limbe foliaire, une partie de la pulvérisation ayant atteint le sol, le transport du phosphore vers les extrémités en croissance active et surtout vers les méristemes «nobles» devant donner des organes florifères se fait très rapidement. Les chenilles devorant seulement le limbe des feuilles ne sont donc pas marquées, tandis que Heliothis et Diparopsis qui se nourrissent presque exclusivement de boutons floraux, de fleurs et de jeunes capsules, sont le plus souvent bien marqués.

Il ne faut donc pas s'attendre, avec cette méthode de pulvérisation directe, à des résultats positifs pour n'importe quel insecte.

DEUXIÈME ESSAI PRÉLIMINAIRE

Nous avions donc vérifié l'existence d'une possibilité d'utilisation du ^{32}P en certaines conditions : saison des pluies, croissance encore active du cotonnier, etc. Après cet encouragement, il importait de se placer dans un cadre à la fois élargi et plus proche des conditions normales des recherches biologiques qui constituent le but réel.

La saison chaude s'est prolongée anormalement en 1962, et ce n'est qu'à la fin novembre que les températures nocturnes s'abaissèrent en dessous de 16°.

Le 4 décembre on procéda à une deuxième application dans des conditions voisines de la première, mais comportant cette fois du ^{32}P et du ^{35}S.

L'application eut lieu par temps sec et calme, le matin sur des cotonniers âgés mais ayant reçu des irrigations afin de susciter un nouveau départ de végétation en contresaison.

A l'expédition le 13 novembre les activités totales étaient 16 mc pour le ^{32}P et 2,7 cm^3 pour le ^{35}S. Au moment de la pulvérisation, il restait donc respectivement 6 mc et 1,7 cm^3.

La plus longue période du ^{35}S permettra peut-être d'allonger la durée et la précision des observations. Des expériences classiques ont en effet montré que le soufre joue un rôle capital dans l'alimentation du cotonnier, qui en réclame des quantités du même ordre de grandeur que celles de phosphore et d'azote. Il reste à savoir si la migration du soufre à partir de la feuille se fait de façon différente de celle du phosphore et si le métabolisme du soufre chez les insectes variés permettra le repérage par ce traceur.

Les observations se poursuivent actuellement, et les résultats ne sont pas encore connus pour l'instant.

DISCUSSION

C.H. SCHMIDT: In Table I it is not clear to me what units you are using in relation to the activity of the larvae. Would you elucidate?

R. DELATTRE: The figures given in Table I are the average figures for total counts per five minutes in an ordinary G-M counter. We do not wish to attach any precise quantitative meaning to these figures, but simply to show that the positive or negative response provides reasonable certainty, despite the fact that counting is carried out away from the actual site of the experiment.

USE OF ISOTOPES FOR INVESTIGATING THE BEHAVIOUR AND ECOLOGY OF INSECT PESTS IN SOME RECENT STUDIES

M. SAYEED QURAISHI
CENTO INSTITUTE OF NUCLEAR SCIENCE, TEHERAN, IRAN

Abstract — Résumé — Аннотация — Resumen

USE OF ISOTOPES FOR INVESTIGATING THE BEHAVIOUR AND ECOLOGY OF INSECT PESTS IN SOME RECENT STUDIES. Investigations into the ecology, behaviour, dispersal and longevity of insects have always provided challenges to the entomologist. The use of isotopes is an effective tool and the following is a report on some interesting problems solved by their application.

Anopheles stephensi is the main vector of malaria in southern Iran. On P^{32}-labelled mosquitoes, the dispersal, behaviour, digestion of blood meal, maturation of ovaries and length of gonotrophic cycles were successfully worked out. It was found that in about 80% of the cases the mosquito needed two blood meals for the completion of the first cycle. The first cycle itself was completed in 4-5 d depending upon the temperature.

Labelled mosquitoes which had emerged overnight were released in an isolated village. The ratio of active mosquitoes to total catch was worked out every day and thus, on the assumption that the natural population remained constant, the mortality rate, which was found to be exponential for the first six days, was worked out.

The mating behaviour of the female was also studied by using normal females which had mated once with P^{32}-labelled males. It was found that the female mates more than once and that after mating with an active male, the spermatheca became active. Counts of up to twice the background (12 counts/min) were obtained by using males giving about 15 000 counts/min.

Studies of the injection of saliva in glucose solution during feeding were also made on P^{32}-labelled mosquitoes.

Eurygaster integriceps is a serious pest of wheat in Iran, Pakistan and the Middle East. Using P^{32}-activated wheat plants, the feeding behaviour of the first-instar nymph was investigated. Other foods, radioactively labelled, were also studied and it was found that feeding was essential for the first moult, even if the food consisted of water absorbed in a filter paper.

EMPLOI DES RADIOISOTOPES DANS DES RECHERCHES RÉCENTES SUR LE COMPORTEMENT ET L'ÉCOLOGIE DES INSECTES NUISIBLES. Les recherches sur l'écologie, le comportement, la dispersion et la longévité des insectes ont toujours posé des problèmes intéressants aux entomologistes. Les radioisotopes constituent un outil efficace, et l'auteur expose les résultats auxquels il est parvenu grâce à leur emploi dans l'étude de certains de ces problèmes.

L'Anopheles stephensi est le principal vecteur du paludisme en Iran méridional. En marquant des moustiques avec ^{32}P, on a pu déterminer la dispersion, le comportement, la digestion du sang, le développement des ovaires et la durée des cycles gonotrophiques. On a constaté que, dans environ 80% des cas, le moustique a besoin de deux repas de sang pour réaliser le premier cycle. La durée de ce cycle a été de 4-5 j selon la température.

Les moustiques marqués qui étaient parvenus à l'état adulte pendant la nuit ont été lâchés sur un village isolé. On a calculé tous les jours le pourcentage de moustiques irradiés par rapport à la prise totale; en partant de l'hypothèse que la population naturelle demeurait constante, on a déterminé le taux de mortalité et constaté que c'était une fonction exponentielle pour les six premiers jours.

On a également étudié le comportement sexuel de la femelle à l'aide de femelles normales qui s'étaient accouplées une fois avec des mâles marqués avec ^{32}P. On a pu établir que la femelle s'accouple plus d'une fois et que la spermatothèque devient radioactive après accouplement avec un mâle irradié. On a enregisté un nombre de coups pouvant atteindre jusqu'à deux fois le bruit de fond (12 cpm) en utilisant des mâles donnant environ 15 000 cpm.

Avec des moustiques marqués avec ^{32}P, on a également étudié l'injection de salive dans une solution de glucose pendant l'absorption de nourriture.

L'Eurygaster integriceps est un insecte très nuisible au blé en Iran, au Pakistan et au Moyen-Orient. Au moyen de grains marqués par ^{32}P, on a étudié le comportement alimentaire au premier stade nymphal. On a également étudié d'autres nourritures marquées et on a constaté que la nymphe devait se nourrir, ne serait-ce que d'eau absorbée par un filtre en papier, pour franchir le premier stade de son évolution.

ИСПОЛЬЗОВАНИЕ РАДИОИЗОТОПОВ ДЛЯ ИССЛЕДОВАНИЯ ПОВЕДЕНИЯ И ЭКОЛОГИИ НАСЕКОМЫХ-ВРЕДИТЕЛЕЙ В ИРАНЕ. Исследования экологии, поведения, распространения и срока жизни насекомых всегда привлекали энтомологов. Использование радиоизотопов является эффективным орудием исследования; ниже дается сообщение относительно нескольких интересных проблем, разрешенных с их помощью.

Anopheles Stephensi является главным переносчиком малярии в южном Иране.

Благодаря использованию меченных P^{32} комаров были получены сведения относительно их распространения, поведения, усвоения ими крови, созревания яичников и продолжительности гонотрофических циклов. Было установлено, что приблизительно в 80% случаев для завершения первого цикла комарам требовалось дважды кровяное питание. Сам первый цикл завершался в зависимости от температуры за четыре или пять суток.

Выплодившиеся за ночь меченые комары выпускались на свободу в изолированной деревне. Соотношение между активными комарами и общим их уловом устанавливалось каждый день и, при условии неизменности природной популяции, определялась смертность, причем оказалось, что она в течение первых шести суток являлась экспонентной.

Было изучено также брачное поведение самок путем использования нормальных самок, спаривавшихся в прошлом один раз с меченными P^{32} самцами. Было установлено, что самка спаривается более одного раза и что после спаривания с активным самцом сперматека становится активной. Используя самцов, дававших 15 000 отсчетов в минуту, были получены счеты, вдвое превышавшие фоновые (12 отсчетов в минуту).

При помощи использования меченных P^{32} комаров были проведены также исследования по инъекции слюны в раствор глюкозы во время питания.

Eurygaster intergriceps является опасным вредителем пшеницы в Иране, Пакистане и на Среднем Востоке.

С помощью пшеницы, меченой P^{32}, был изучен режим питания куколки на ее первовозрастной стадии. Были также изучены другие виды пищи, меченной радиоактивными веществами, и обнаружено, что питание необходимо в период первой линьки, если даже пища состоит из воды, впитанной фильтровальной бумагой.

EMPLEO DE ISÓTOPOS EN ESTUDIOS RECIENTES SOBRE EL COMPORTAMIENTO Y LA ECOLOGÍA DE LAS PLAGAS INSECTILES. Las investigaciones sobre la ecología, el comportamiento, la distribución y la longevidad de los insectos suelen ofrecer a los entomólogos problemas de difícil solución. Los isótopos han resultado auxiliares muy eficaces para estas investigaciones; en la memoria se informa sobre algunos problemas interesantes que se han podido resolver gracias a su empleo.

El Anopheles stephensi es el principal vector palúdico en el sur del Irán. Empleando mosquitos marcados con ^{32}P se pudo determinar su distribución, su comportamiento, la digestión de la sangre, la maduración de los ovarios y la longitud de los ciclos gonotróficos. Se comprobó que en un 80 por ciento de los casos el mosquito necesita alimentarse dos veces con sangre para completar el primer ciclo, cuya duración es de 4 a 5 días según la temperatura.

A los mosquitos marcados que surgían de la ninfa durante la noche se los liberaba en una aldea aislada. Diariamente se calculaba el porcentaje de anofeles capturados que estaban marcados y así, suponiendo que el número de mosquitos de la zona permanecía constante, se pudo determinar el índice de mortalidad, que resultó ser exponencial durante los seis primeros días.

También se estudió la copulación de las hembras utilizando hembras normales que habían copulado una vez con machos marcados con ^{32}P. Se pudo observar que las hembras copulan más de una vez y después de hacerlo con un macho activado sus espermatotecas se hacían también radiactivas. Con machos que daban hasta 15 000 impulsos/min se obtuvieron cifras de recuento de hasta el doble del fondo parasitario (12 impulsos/min).

Con mosquitos marcados con ^{32}P se hicieron estudios sobre la inyección de saliva en una solución de glucosa durante el proceso de alimentación.

El Eurygaster integriceps es una plaga muy peligrosa del trigo en el Irán, el Pakistán y el Oriente Medio. El autor investigó el modo de alimentarse de las ninfas del primer estadio utilizando plantas de trigo activadas con ^{32}P. También se estudiaron otros alimentos marcados radiactivamente y se encontró que la alimentación es esencial para la primera muda, aunque consiste solamente en agua embebida en un papel de filtro.

INTRODUCTION

Investigations into the ecology, behaviour, dispersal and longevity of insects have always provided challenges to the scientist. From the early days the research worker has used a variety of ingenious aids in the pursuit of his knowledge, but more often than not his attempts have been thwarted because no matter how intricate the aids used, they failed to match the complexities of the situation involved.

Studies of the movements of small larvae underground, or the frequency of the mating of a eurygamous species in nature, or the food and feeding habits of a predator, apparently seem to be simple, but the solutions of problems of that kind have defied the ingenuity of the scientist for a long time. Only since isotopes were made available has the research worker found a potent weapon of attack.

In this paper I intend to give an account of the use of isotopes in the solution of a few intricate problems related to pests of agricultural and public health importance. In fact, the health of the rural population is directly related to agricultural production, and control of a disease like malaria has been shown by the World Health Organization team working in Pakistan to result in a substantial increase in food production [1].

USE OF P^{32} IN THE STUDY OF THE BIOLOGY OF ANOPHELES STEPHENSI

Malaria has since time immemorial plagued the tropical countries, sapping the energy of the inhabitants, reducing their productivity and seriously affecting food production. The high hopes for malaria eradication placed in residual insecticides were soon belied by the discovery of resistance in vector mosquitoes. This discovery has once again emphasized the value of the study of the biology and ecology of insects affecting the economy of a country.

With the aid of P^{32} we have successfully studied the following aspects of the biology of Anopheles stephensi:

(i) Dispersal;
(ii) Digestion of blood meal, simultaneous development of ovaries and length of gonotrophic cycle in nature;
(iii) Mortality rate; and
(iv) Mating behaviour.

This mosquito is one of the most serious vectors of malaria in the region and has developed a very high degree of resistance to the insecticides used for malaria eradication.

Mosquitoes were labelled by rearing IVth-instar larvae in water containing 10 μc of P^{32} per litre per 500 larvae. Radioactivity measurements of about 4000 counts/min in the case of males and about 5000 counts/min

in the case of females, as detected in the top shelf of an Ericsson scaling unit type 1221C, were obtained [2].

Several thousands of mosquitoes which had emerged overnight were released at a predetermined point in the evening. Starting next morning, regular collections were made in neighbouring villages. All collections were monitored to study the dispersal of the labelled mosquitoes. Radioactive A. stephensi were captured up to 4.5 km from the point of release [3].

All radioactive females were dissected to study the digestion of the blood meal (Sella stage) and the development of ovaries (Christopher's stage). Our studies showed that in the majority of cases the first cycle was completed within five days (about 110 h) after release when the mean daily temperature during the period of trial varied between 28.3°C and 29.6°C and the mean relative humidity between 21.5% and 33.2% [4].

Radioactive A. stephensi which had emerged overnight were released in isolated villages and the ratio of radioactive mosquitoes to the total catch was worked out daily. On the assumption that the natural population was constant, the daily fall in this ratio gave us an idea of the daily mortality of the labelled mosquitoes. This mortality rate was found to be exponential [3].

Since the successful eradication of screw-worm fly, the study of the mating behaviour of insects has assumed importance. Using radioactive male A. stephensi we studied the mating behaviour of this species. When normal females were mated with radioactive males (7000 - 13 000 counts/min) detectable radioactivity was acquired by the spermathecae (17.5 - 25 counts/min). The background in this case was 12 counts/min. It was thus proved that the female of A. stephensi mates more than once. (QURAISHI and ARTHUR [5]).

Before releasing labelled mosquitoes we wanted to find out the amount of radioactivity a female would transfer to an animal being bitten. Even when several hundred radioactive females (about 5000 counts/min) were allowed to feed on a guinea-pig there was no detectable radioactivity transferred to its blood. We therefore tried to find out the radioactivity transferred to sugar solution when radioactive A. stephensi fed on it. Diluted honey absorbed in a thin layer of cotton was counted for its radioactivity and kept overnight in a cage of freshly emerged mosquitoes. Next morning the radioactivity of the honey solution was determined and the mosquitoes which had fed on it were collected and their radioactivity was determined separately. Several experiments thus conducted showed that while feeding the mosquitoes transferred about 0.1% of their total radioactivity to the honey on which they fed. (QURAISHI, unpublished data.)

USE OF ISOTOPES IN THE STUDY OF THE ECOLOGY OF EURYGASTER INTEGRICEPS

Eurygaster integriceps Put., known locally as Senn pest, is a serious pest of wheat and to a lesser extent of barley in the Middle East, Turkey and Southern USSR. Though some literature is available on the feeding habits and behaviour of the various stages of this insect, descriptions are sometimes conflicting and at times lack conclusive evidence. FEDOTOV [6]

states that the main reserves of food are built up in larval stages, whereas VODJDANI [7] is of the opinion that in the first instar the insect does not feed. This interested us in the feeding habits of the first-instar nymph. Wheat plants were made radioactive by growing them in Hoagland's water culture medium containing P^{32}. Freshly emerged first-instar nymphs were then released on the plants in specially prepared plastic containers. After 24 h the nymphs were removed, cleaned by a brush dipped in phosphorus carrier to remove any external contamination, and counted. Considerable radioactivity was recorded in the nymphs, proving conclusively that they did feed on the plants. P^{32}-activated sugar solutions and water of known activity were absorbed in filter papers and tried as an alternative food for first-instar nymphs of known weight. It was found that the radioactivity of the fed nymph was nearly equal to the radioactivity of the weight of liquid imbibed [8].

By using a label of tantalum-182 $0.05 \times 0.16 \times 0.46$ mm in size (specific activity 8 μc) glued to the scutellum of the insect, the horizontal and vertical movements and daily feeding habits of six young adults were studied in open (42 wheat stems per 0.25 m^2) and closed (82 wheat stems per 0.25 m^2) wheat fields in Northern Iran, for three to four days at the beginning and end of the adult feeding period.

It was found that on an average the labelled insects changed their position four times a day and moved about 60 - 70 cm each day. Those in the open field moved more often and covered somewhat longer distances.

The labelled insects in closed wheat spent nearly the whole time above ground but the bugs in the open wheat spent a considerable time in cracks in the soil.

Feeding occurred chiefly in daytime. It increased in frequency from sunrise to a maximum at 8 a.m., declined to a minimum at midday and rose to another peak in the afternoon. Feeding almost ceased at sunset.

At the end of the feeding period, just before their flight to the mountains, the insects spent most of the time in cracks in the soil and were rarely seen feeding [9].

Microphanurus sp. is being reared on a large scale for the biological control of Senn pest in Iran and in order to plan effective releases of this parasite for that purpose it is important to find out its effective flight range. Attempts were made to label the parasite by rearing it in radioactive eggs of E. integriceps. To obtain radioactive eggs, female Senn pest were either fed on wheat soaked in P^{32} or injected with 10 μc of P^{32} isotonic solution. An activity of up to 100 counts/min was measured in the eggs and parasites reared in them became radioactive. However, the activity was not sufficient for our purpose and we are trying other methods of obtaining radioactive parasites [8].

I have in this short paper endeavoured to discuss in broad outline the uses to which we have put isotopes in our studies. Most of this work would have been extremely difficult to carry out without their help; besides, the use of other labels like dusts, powders or paints would have involved unnecessary handling or anaesthetizing of the insects, thereby adding a factor of uncertainty to the validity of our results.

ACKNOWLEDGEMENTS

I wish to thank Dr. H.A.C. McKay, former Director, and Dr. M.L. Smith, present Director of the Institute, for their encouragement and help during the progress of the various projects. Thanks are also due to the members of the staffs of the Department of Parasitology and Malariology, University of Teheran, of the Ministry of Agriculture and the Department of Meteorology for their help during the various phases of these studies.

REFERENCES

[1] WORLD HEALTH ORGANIZATION, Final Comprehensive Report, Pakistan - East Bengal Malaria Control Demonstration Team, Pakistan J. Health 2 (2) (1952) 61-91.

[2] QURAISHI, M. Sayeed, LAMSACHI, R. Farvar, ERGUL, C., Laboratory Studies on Tagging of Anopheles stephensi (submitted for publication in J. Econ. Entomol.).

[3] QURAISHI, M.S, et al., Flight Range, Length of Gonotrophic Cycles and Mortality in Nature in Anopheles stephensi with the aid of P^{32} (in preparation).

[4] QURAISHI, M. Sayeed, MOFIDI, Ch.M.H., and MOTABAR, M., Flight Range and Length of Gonotrophic cycles in Nature in Anopheles stephensi with the Aid of P^{32} (to be published).

[5] QURAISHI, M. Sayeed and ARTHUR, Mervyn, Nature, 197 4864 (1963) 312-312.

[6] FEDOTOV, D.M., Inst. A.N. Severtzov de Morph. E., Vol.I, 35-80, Vol.II, 3-16 and 49-66 (1947) Vol. III (1955) 272 pp.

[7] VODJDANI, S., Annales des Epiphyties No.2 (1954) 105-160.

[8] QURAISHI, M. Sayeed, Water and Food Relationship of the Eggs and First-Instar Nymph of Eurygaster integriceps Put. with the aid of P^{32} (submitted for publication in J. Econ. Entomol.).

DISCUSSION

W. KLOFT: Some of your results are very interesting for me, such as, for example, the ejection of radioactive saliva by mosquitoes. We did similar work with plant-sucking insects, but I think this is the first time that the ejection of labelled saliva has been demonstrated in a blood-sucking insect.

You have also tested how often the Anopheles female mates during her life, by making the sexual organs radioactive. From experience with transfer of labelled substances in the case of social Hymenoptera, we have found that after mating by the male, sometimes only the radioactive secretion of the accessory glands has been transferred. The sperm is not always transferred but only the accompanying radioactive secretion. You have referred to the amount of mating, not of transference, I believe.

M.S. QURAISHI: That is very interesting, Dr. Kloft. As regards the mating habits of the Anopheles female, we only studied the activity of the spermatheca of the female. Subsequently these results were confirmed by field observations where we collected copulating females from swarms and dissected them. We were able to find females which showed one dilatation and two dilatations in their ovarioles, thereby proving conclusively that females which had laid one or two batches of eggs were coming again to mate in these swarms.

It would be very difficult to prove the transfer of sperms conclusively in subsequent matings, because the spermatheca would already have a store of spermatozoa from previous mating. Autoradiography may help.

C.H. SCHMIDT: I was interested in what you said in your oral presentation with regard to honey solution and hot mosquitoes. Is there any possibility that instead of being just regurgitations, you could have defecation by the mosquitoes? We have done some work with Aëdes aegypti and the cages after a while get highly contaminated through the hot excretion.

M.S. QURAISHI: Actually these mosquitoes were captured the very next day. They were only kept overnight with the solution of honey, so I do not think there would be very much contamination due to excretion. Also, when female mosquitoes feed on honey solution you can easily see the distended abdomen, with the solution inside.

C.H. SCHMIDT: You showed that they went about 4.5 km. Was there any prevailing wind during your experiment? Or was there no wind?

M.S. QURAISHI: We kept a good record of wind velocity. It was usually between 2 and 5 knots. Of course there were one or two cases when it was higher than that. Though it is not statistically significant, the first catches of mosquitoes, which flew overnight, were found in a direction almost opposite to the direction of the wind.

C.C. HASSETT: May I say in relation to wind transport of mosquitoes, that I found in field experiments in Canada that A. aegypti were not affected by wind because at wind velocities of over 5 mile/h the insects took cover in vegetation.

ESSAI PRÉLIMINAIRE AVEC ^{32}P SUR LA DISPERSION DES ADULTES DU DACUS OLEAE GMEL

P.S. ORPHANIDIS, C.D. SOULTANOPOULOS ET M.G. KARANDEINOS
LABORATOIRE BIOLOGIQUE, INSTITUT PHYTOPATHOLOGIQUE BENAKI, ATHÈNES, GRÈCE

Abstract — Résumé — Аннотация — Resumen

PRELIMINARY STUDY WITH P^{32} ON THE DISPERSION OF ADULTS OF THE OLIVE FLY (DACUS OLEAE GMEL). The lack of numerical data on the movement of the olive fly led to a preliminary trial with 2500 adults reared in cages and labelled before release with a sugar solution containing 20μc P^{32}/cm^3.

Subsequent measurements showed dispersion of the labelled adults up to a distance of 2 km from the point of release during a calm sunny period in winter.

ESSAI PRÉLIMINAIRE AVEC ^{32}P SUR LA DISPERSION DES ADULTES DU DACUS OLEAE GMEL. Le manque de données numériques sur la dispersion de la mouche de l'olive a amené à faire un essai préliminaire avec 2500 adultes élevés dans des cages et marqués, avant leur lâcher, au moyen d'une solution sucrée contenant 20 μc de ^{32}P par centimètre cube

Les mesures ultérieures ont montré une dispersion des adultes marqués jusqu'à une distance de 2 km du point de lâcher pendant les jours ensoleillés d'hiver, dits alcyoniens.

ПРЕДВАРИТЕЛЬНЫЕ ОПЫТЫ С P^{32} ДЛЯ ИЗУЧЕНИЯ РАССЕЯНИЯ ВЗРОСЛЫХ ОСОБЕЙ (DACUS OLEAE GMEL). Отсутствие количественных данных относительно рассеяния оливковых мух обусловило проведение предварительного опыта с 2500 взрослыми особями, выращенными в ящиках и помеченными перед выпуском с помощью сладкого раствора, содержащего 20 мкк на 1 $см^3$ P^{32}.

Относительные измерения показали удаление меченых взрослых особей до 2 км от места выпуска в период солнечных зимних дней, называемых алкионидными.

ESTUDIO PRELIMINAR CON ^{32}P DE LA DISPERSIÓN DE LOS ADULTOS DEL DACUS OLEAE GMEL. La falta de datos numéricos sobre la dispersión de la mosca del olivo ha inducido a efectuar un estudio preliminar con 2 500 adultos criados en cajas y marcados antes de su suelta con una solución azucarada que contenía 20 μc de $^{32}P/cm^3$.

Las mediciones han puesto de manifiesto una dispersión de los adultos marcados hasta una distancia de dos kilómetros del punto en que fueron soltados durante los días soleados de invierno llamados alciόneos.

Diverses opinions ont été formulées depuis longtemps concernant la dispersion et l'immigration des adultes de la mouche de l'olive. Les essais que nous avons faits pendant les années 1957-1961 avec divers produits attractifs, c'est-à-dire avec des hydrolysats de protéines, ont démontré que le tropisme des adultes du Dacus d'un point donné vers un autre dépend considérablement de la différence de l'humidité relative qui existe entre ces deux points [2].

Ces résultats, qui étaient d'ailleurs en accord avec les données d'ACREE et al. [1] sur la Musca domestica, montraient qu'il était probablement possible d'avoir un mouvement d'adultes des endroits à faible humidité relative vers des points ayant une humidité relative élevée. Ces observations étaient, en plus, en conformité avec l'opinion répandue parmi les agronomes et oléiculteurs de notre pays, selon laquelle la mouche de l'olive attaque précocement les arbres situés dans des endroits humides, d'où la notion des «foyers» du Dacus. La manque des données nu-

mériques sur la dispersion de la mouche de l'olive nous a amenés à exécuter un essai péliminaire avec des mouches de l'olive marquées au ^{32}P élevées dans des cages, d'après la méthode appliquée par SACANTANIS [3], et provenant des olives infestées.

Les olives infestées restaient pendant 25 à 30 j approximativement sur des tamis permettant le passage des larves et chaque jour on collectait les larves du troisième stade ainsi que les pupes produites.

Les adultes apparus ensuite, après l'éclosion des pupes, étaient introduits dans des cages d'insectes et étaient nourris d'une part avec des morceaux de fruits (figues, bananes) et d'autre part avec une solution aqueuse contenant une partie de levure de bière, une partie de protéine hydrolysée partiellement – de la maison «Nutritional Biochemicals» des Etats Unis – et huit parties de sucre.

Les adultes restaient ensuite à jeun et sans eau pendant 24 h; 24 h avant leur lâcher on leur donnait une solution sucrée contenant 20 $\mu c/cm^3$ de phosphore marqué sous la forme d'acide phosphorique (neutralisé au moyen de $NaHCO_3$) et provenant d'un produit concentré (10 mc/ml) de l'«Atomic Energy of Canada Limited».

La détermination de la concentration en ^{32}P était basée d'une part sur les observations de VIEL et CHANCOGNE [4], d'après lesquelles les adultes d'une espèce voisine, les Ceratites, pouvaient absorber per os une quantité de 1 mm^3 d'eau, et d'autre part sur les données de YATES et LINDQUIST [5] d'après lesquelles une quantité de 0,02 μc était suffisante pour le marquage des espèces Musca domestica et Phormia regina.

La solution sucrée à ^{32}P était mise dans des flacons suspendus sur le plafond des cages et contenant du coton en forme cylindrique, dont l'un des bouts se trouvait au fond du flacon et l'autre sortait du goulot.

La manque d'infestation précoce dans la région des expériences ne nous a pas permis d'avoir un nombre suffisant de mouches pendant l'automne. C'est pourquoi le lâcher des adultes a été fait pendant les jours ensoleillés de janvier dits «Alcyonides».

Nous avons utilisé environ 2500 mouches de l'olive ainsi que 62 pièges contenant 300 cm^3 d'une solution 2% de sulfate d'ammonium. Les pièges étaient suspendus sur des branches des arbres de l'oliveraie et placés sur les circonférences de cercles ou arcs de cercles dont les rayons étaient de 100, 400, 1000 et 2000 m approximativement. La distance entre les pièges sur les circonférences ou les arcs était d'environ entre 40 et 150 m.

L'étendue relativement faible de la surface de l'oliveraie expérimentale dans laquelle nos essais sur la lutte de la mouche de l'olive ont été exécutés pendant 1961 ne nous a pas permis de mettre des pièges à une distance supérieure à deux kilomètres. Mais nous avions l'opinion que même cette distance pouvait donner une image sur la dispersion de la mouche de l'olive, dispersion au sujet de laquelle rien n'était établi positivement jusqu'à cette année.

On peut voir des détails sur la disposition des pièges dans l'oliveraie expérimentale dans le diagramme schématique de la figure 1. Le dénombrement des mouches capturées dans des pièges a été fait quatre fois, pendant une période de neuf jours, après le lâcher des adultes marqués.

L'estimation de l'irradiation des mouches a été faite à l'aide d'un appareil G-M (type N. 529 C. Scaler) de la maison «ECKO Electronics Ltd.»,

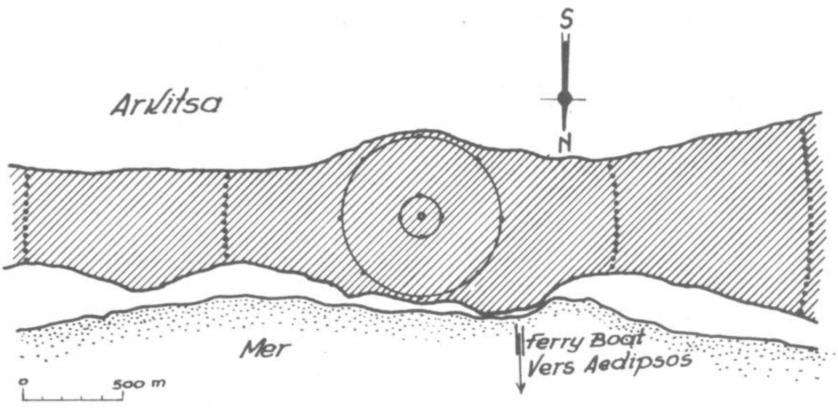

Figure 1

Disposition des pièges dans l'oliveraie expérimentale
• Pièges
O Point de lâcher des adultes marqués

Angleterre. Ces mesures ont mis en évidence une dispersion des adultes marqués dans les oliveraies jusqu'à une distance de deux kilomètres, au moins, du point de lâcher pendant les jours ensoleillés de janvier dits « Alcyonides ».

Les adultes marqués présentaient une irradiation jusqu'à 1350 cpm; leur activité ne surpassait donc pas celle observée par YATES et LINDQUIST [5] pendant les essais sur Musca domestica et Phormia regina.

Il faudrait signaler à titre de comparaison que le bruit de fond était en même temps de 9 à 15 cpm approximativement.

RÉFÉRENCES

[1] ACREE, F. et al., "Nature of the attractant in sucrose fed on house flies", J. econ. Ent. 52, 5 (1959) 981-985.

[2] ORPHANIDIS, P. S., DANIELIDOU-PHYTIZA, R. E. et SOULTANOPOULOS, C. D., « Contribution à l'étude de l'influence de l'eau sur l'intensité du phénomène de l'attraction d'adultes du Dacus oleae Gmel. par diverses substances attractives », Annales de l'Institut phytopathologique, Benaki, N. S., 1, 4 (1962) 39-51.

[3] SACANTANIS, K.B., « Facteurs déterminant le comportement de Dacus oleae Gmel. vis-à-vis des variétés d'oliviers » Rev. Pathol. Végétale Entomol. Agr. France 32 (1953) 51-57.

[4] VIEL, G. et CHANCOGNE, M. M. « Toxicité d'ingestion de quelques insecticides pour Ceratitis capitata Wied », Phytiatr. Phytopharm. 6, 4 (1957) 217-221.

[5] YATES, W. W. et LINDQUIST, A. W., "Further studies of dispersion of flies tagged with radioactive phosphoric acid", J. econ. Ent. 45, 3 (1952) 547-548.

DISCUSSION

J. LECOMTE: What proportion of tagged flies did you capture out of the 2500 released?

P.S. ORPHANIDIS: We trapped about 1.2% of the adults released.

J. LECOMTE: After measuring the activity of the captured flies, did you release them or destroy them?

P.S. ORPHANIDIS: After measurement of their activity the flies were kept in the laboratory.

J. LECOMTE: The number of tagged flies was thus decreasing with time?

P.S. ORPHANIDIS: Yes.

C.H. SCHMIDT: What was the total number of flies, both tagged and non-tagged, recovered in your traps?

P.S. ORPHANIDIS: We captured 1950 flies.

M. FERON: What was the lowest activity measured amongst the captured flies?

P.S. ORPHANIDIS: The minimum was 3 to 4 times the background.

G. SILVA: Could you tell us something about the temperature in January? It is quite remarkable that you found flies kilometres away in that month.

P.S. ORPHANIDIS: Naturally, during the cold months there cannot be a satisfactory dispersal of insects. Our intention was to release the flies during the autumn months, but owing to the late infestation the number of infested olives was not enough to produce a sufficiently high number of adult flies. If the tests had been carried out in the autumn I think a much greater dispersal would have been found. In Greece, however, there is a period of approximately 15 d in January during which the temperature is relatively high, lying between 12 and 16°C during the day and 7 and 10°C at night. Release and capture took place during this period.

C.T. LEWIS: I have been concerned with some work on the migration of Orthotylus virescens bugs in broom plantations. We found quite an interesting phenomenon when we released bugs in the centre of the plantation, marked with P^{32} and others along the boundary of the plantation marked with S^{35}. The bugs released at the centre did not move about very much, but the bugs that were released at the edge of the plantation migrated quite freely. If we had marked all the bugs with the same isotope, we would have got a very small percentage migrating, but in fact, as I say, along the boundary quite a high percentage of bugs migrated and this quite altered the picture we had of the movements of the insects. I wonder whether this difference in behaviour between the centre of the plantation and the boundary might also exist with Dacus oleae.

P.S. ORPHANIDIS: In our case the labelled adults were all released at a point in the centre of the olive grove.

M.S. QURAISHI: I have three question. How soon after emergence does mating take place in the case of Dacus oleae? How soon after mating are eggs laid? And is there any difference in the flight range before mating and after mating?

P.S. ORPHANIDIS: This was just a preliminary study and we did not intend to investigate anything but the dispersal of a given insect population in relation to the age of the insects. It appears, however, from our observations, that egg-laying takes place 8 – 12 d after emergence.

PRELIMINARY STUDIES OF THE FIELD MOVEMENT OF THE OLIVE FRUIT FLY (DACUS OLEAE GMEL.) BY LABELLING A NATURAL POPULATION WITH RADIOACTIVE PHOSPHORUS (P^{32})

CONSTANTINE E. D. PELEKASSIS, P. A. MOURIKOS AND D. N. BANTZIOS
ECONOMIC ENTOMOLOGY LABORATORY,
BENAKI PHYTOPATHOLOGICAL INSTITUTE, ATHENS, GREECE

Abstract — Résumé — Аннотация — Resumen

PRELIMINARY STUDIES OF THE FIELD MOVEMENT OF THE OLIVE FRUIT FLY (DACUS OLEAE GMEL.) BY LABELLING A NATURAL POPULATION WITH RADIOACTIVE PHOSPHORUS (P^{32}). Preliminary trials were conducted to obtain the first data on the field movement of the olive fruit fly, Dacus oleae Gmel. These studies were carried out in the olive-growing area at Rovies, Evvia, Greece, during the autumn of 1961, when the maximum adult fly activity usually occurs.

A mixture of radioactive P^{32} of $H_3P^{32}O_4$ in HCl and hydrolysate protein, Staley No. 7, was prepared for labelling the naturally occurring adult fly population. Twigs of 30 olive trees which were selected in a semi-mountainous olive orchard at Rovies were treated with the P^{32} bait solution on 14 October 1961.

Five hundred McPhail traps, containing 3% of diammonium phosphate as a lure, were used for the collection of flies. Traps were distributed in the olive groves of the entire area at Rovies and also in the adjacent areas (forest or olive groves) up to a distance of 10 km from the treated trees.

A total of 350 tagged flies of both sexes were collected in 48 traps during 15 counts of traps made between 15 October and 18 November. Labelled flies which were caught from 1 to 35 days after treatment represented 2.8% of the total number of flies collected in 48 traps, or 0.15% of the total number of flies caught in 298 traps in the entire area at Rovies. A proportion of 73% of the tagged flies were trapped within the first ten-day period following treatment. Labelled flies were found as far as 4300 m (maximum of dispersal) from the labelling station. Radioactivity of tagged flies ranged between 258 and 9549 counts/min per fly (background radiation 8-21 counts/min).

These preliminary trials have confirmed a more or less continuous local movement (dispersal) of flies from the semi-mountainous grove to the adjacent plains or to the coastal olive groves at Rovies from the north to south. Long-distance movement (migration) of Dacus flies to other areas has not been observed. Pine woods appear to act as a barrier to the movement of Dacus oleae adults.

ÉTUDES PRÉLIMINAIRES SUR LES DÉPLACEMENTS DE LA MOUCHE DE L'OLIVE (DACUS OLEAE GMEL.) PAR MARQUAGE D'UNE POPULATION NATURELLE AU RADIOPHOSPHORE (^{32}P). Les auteurs ont procédé à des expériences préliminaires pour recueillir des données de base sur les déplacements de la mouche de l'olive (Dacus oleae Gmel.). Ces études ont été menées dans les oliveraies de Rovies, Evvia, en Grèce, à l'automne 1961, à l'époque où les mouches adultes sont particulièrement actives.

Les auteurs ont préparé un mélange de H_3-^{32}P-O_4 dans HCl et d'hydrolysat de protéine, Staley n° 7, pour marquer une population naturelle de mouches adultes. Une nourriture alléchante à base de la solution marquée à été appliquée le 14 octobre 1961 sur les branches de 30 oliviers choisis dans une oliveraie semi-montagneuse, à Rovies.

Les mouches ont été capturées à l'aide de 500 pièges McPhail, contenant 3% de phosphate diammonique qui servait d'appât. Les pièges avaient été répartis dans les oliveraies de Rovies ainsi que dans les zones adjacentes (forêts ou oliveraies) dans un rayon de 10 km autour des arbres traités.

Au total, 350 mouches marquées des deux sexes ont été recueillies dans 48 pièges au cours de 15 relevés effectués entre le 15 octobre et le 18 novembre. Les mouches marquées, qui ont été capturées de 1 à 35 jours après le traitement, représentaient 2,8% du nombre total des mouches recueillies dans les 48 pièges ou 0,15% du nombre total des mouches capturées dans 298 pièges dans toute la zone de Rovies; 73% des mouches marquées ont été capturées dans les 10 premiers jours après le traitement. On a trouvé des mouches marquées jusqu'à 4300 m des arbres portant la solution marquée (maximum de dispersion). La radioactivité des mouches marquées était comprise entre 258 et 9549 cpm par mouche (rayonnement ambiant, 8 à 21 cpm).

Ces expériences préliminaires ont confirmé l'existence d'un déplacement local plus ou moins continu (dispersion) des mouches entre l'oliveraie semi-montagneuse et les plaines adjacentes ou les oliveraies côtières de Rovies, suivant une direction nord-sud. On n'a pas observé de déplacement à grande distance (migration) des mouches vers d'autres régions. Il semble que les bois de pins opposent un barrage aux déplacements des adultes de la Dacus oleae.

ПРЕДВАРИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ПОЛЕВОГО ПЕРЕМЕЩЕНИЯ ОЛИВКОВОЙ ФРУКТОВОЙ МУХИ (DACUS OLEAE GMEL.) ПОСРЕДСТВОМ МЕЧЕНИЯ ЕСТЕСТВЕННОЙ ПОПУЛЯЦИИ РАДИОАКТИВНЫМ ФОСФОРОМ (P^{32}). Проведены предварительные эксперименты для получения первых данных о полевом перемещении оливковой фруктовой мухи Dacus oleae (Gmel.). Эксперименты были проведены в районе произрастания олив в Ровиес, Эвина, Греция, в течение осени 1961 года, когда обычно наблюдается максимальная активность взрослых особей мухи.

Для мечения естественных популяций взрослых особей был приготовлен состав Стэли № 7, состоящий из смеси P^{32} в виде $H_3P^{32}O_4$ в соляной кислоте и в гидролизате белка. 14 октября 1961 года ветви 30 оливковых деревьев, выбранных на полугорной оливковой плантации в Ровиес, были обработаны раствором приманки, меченной P^{32}.

Для отлова мух использовались 500 ловушек Макфэйла с 3% диаммонийфосфата в качестве приманки. Ловушки были расставлены в оливковых рощах всего района Ровиес, а также в соседних районах (в лесах и в оливковых рощах) на расстоянии до 10 км от меченых деревьев.

Было собрано 350 меченых мух обоих полов в 48 ловушках во время 15 проверок ловушек в период между 15 октября и 18 ноября. Меченые мухи, пойманные с 1 по 35 день после обработки деревьев индикатором, составляли 2% общего количества мух, отловленных в 48 ловушках или 0,15% общего количества мух, отловленных в 298 ловушках во всем районе Ровиес. 73% меченых мух попали в ловушки в течение первых 10 дней после обработки деревьев индикатором. Меченые мухи были обнаружены на расстоянии 44 300 м от места, где находились меченые деревья (максимальное расстояние). Радиоактивность меченых мух составляла от 258 до 9549 отсч/мин (фоновое излучение составляет 8 - 21 отсч/мин).

Эти предварительные эксперименты подтвердили существование более или менее постоянного местного перемещения (рассеивания) мух от полугорной рощи к близлежащим равнинам или к прибрежным оливковым рощам в районе Ровиес с севера на юг. Дальнее перемещение (миграция) мух Dacus в другие районы не было обнаружено. По-видимому, сосновые рощи являются барьером, препятствующим перемещению взрослых особей Dacus oleae.

ESTUDIO PRELIMINAR DE LA DISPERSIÓN DE MOSCAS DEL OLIVO (DACUS OLEAE GMEL.) MEDIANTE LA MARCACIÓN DE POBLACIONES NATURALES CON ^{32}P. Los autores de la memoria han realizado algunos ensayos preliminares para obtener datos sobre la dispersión de las moscas del olivo Dacus oleae Gmel. Estos ensayos se llevaron a cabo en los olivares de Rovies (Isla de Evia, Grecia) en otoño de 1961, época en que las moscas adultas abundan particularmente.

Para marcar la población natural se utilizó el ^{32}P de una mezcla de $H_3{}^{32}PO_4$ en HCl y proteína hidrolizada Staley No. 7. El 14 de octubre de 1961 se trataron con esta sustancia las ramas de 30 olivos de un olivar semimontañoso situado cerca de Rovies.

Las moscas se cazaron con 500 trampas McPhail en las que se emplearon como cebo fosfato diamónico al 3%. Las trampas se colocaron en los olivares de la región de Rovies y en zonas adyacentes (de bosque o de olivares) hasta una distancia de 10 km de los árboles tratados.

Durante los 15 recuentos efectuados entre el 15 de octubre y el 18 de noviembre se recogieron en 48 trampas un total de 350 moscas marcadas. Estas moscas, capturadas entre 1 y 35 días después del tratamiento, representaban el 2,8% del total de las moscas recogidas en las 48 trampas y el 0,15% del total de las moscas capturadas en 298 trampas colocadas en la zona de Rovies. El 73% de las moscas marcadas se capturaron durante los diez primeros días siguientes al tratamiento. Se encontraron hasta una distancia de 4300 m (dispersión máxima) de la zona donde se marcaron. Su radiactividad oscilaba entre 258 y 9549 impulsos/min y por mosca (actividad de fondo: 8 a 21 impulsos/min).

Estos ensayos preliminares han confirmado un movimiento local más o menos continuo (dispersión) desde el olivar semimontañoso hasta las llanuras adyacentes y los olivares de la costa (norte-sur). No se ha observado una migración de la mosca Dacus a otras regiones. Al parecer, los bosques de pinos actúan de barrera para los adultos de la Dacus oleae.

INTRODUCTION

It has long been known that many species of the family Tephritidae, which includes the olive fruit fly (Dacus oleae Gmel.), are capable of rapid wide-range flight. The Mexican fruit fly, Anastrepha ludens Loew, serves as an outstanding example in this connection. According to studies carried out in Texas in the United States of America, individuals of this species were trapped as far as 160 and 175 miles from the place of their breeding [3].

So far, the flight habits and migration of the olive fruit fly are poorly understood, although many research workers have dealt with this project for years. This lack of satisfactory results is attributed mainly to the inadequate experimental methods and techniques applied in the past. The somewhat arbitrary conclusions of most workers, so far from clearing up the whole matter, have rather led scientists to contradictory assumptions and theories.

The Italian entomologist LUPO, V. [5] carried out extensive investigations on the olive fruit fly migration and concluded that this species makes two seasonal migrations. The first migration, which he calls "summer migration", occurs during June and July, when flies move from mountainous regions (altitude 400 m and more above sea level) to the plains or to the coastal areas; the second or "autumn migration" occurs during September and October, when flies migrate from the coastal areas to the mountains. Both movements were related to the availability of fruits and the weather conditions.

Other research workers, such as BUA, G. [2], MELIS, A. [7], MARTIN, H. [6], SACANTANIS, K. [2] and ISAAKIDES, C.A. [4], recognize a seasonal migration of Dacus but disagree as far as the real causes of the movement are concerned. Some of these investigators attribute this movement to climatic conditions, principally temperature, precipitation or winds, while others ascribe it to the need of the insect to locate suitable fruits in which to lay eggs or its need of shelters for overwintering.

The lack of a method for artificially rearing the olive fruit fly for use in field tests has delayed high-level investigation of the subject in question. Because of this it was impractical for earlier researchers working in this field to use various materials and techniques for tagging the flies, such as marking with dyes.

The commercial production of radioactive isotopes, which have been recently introduced into applied biology, offers to the modern entomologist and biologist a valuable tool for investigation of the flight habits and the dispersal of fruit flies. Trials recently conducted in the United States with radioactive isotopes on species related to Dacus oleae Gmel., such as Dacus dorsalis Hendel, Ceratitis capitata Wied., and Rhagoletis completa Cresson, indicate that the use of isotopes with beta radiation, such as radioactive phosphorus (P^{32}), furnishes a standard technique for labelling a natural population of tephritid flies [1, 3, 11].

Besides having the advantages of accurate results and ease of application, these materials are not likely to cause adverse effects on the behaviour and habits of fruit flies.

An investigation of the flight habits and dispersal of the olive fruit fly by using radioactive P^{32} was the object of the preliminary trials reported

here. These were the first tests of this nature carried out in Greece with Dacus oleae.

These investigations, besides supplementing data already available concerning the biology and the habits of the olive fruit fly, were also considered to offer particular promise in pointing the way to more effective and economical control measures.

The subjects of investigation were (1) the flight-range of the olive fruit fly; and (2) the type and direction of movement. In respect of (2), a special effort was made to ascertain whether individuals of a natural Dacus population occurring in a certain olive-growing area exhibit dispersal (local movement of individuals within the limits of their habitat) or migration (a movement of the population outside the bounds of its habitat to another area and in a definite direction).

MATERIAL AND METHODS

Radioactive phosphorus (P^{32}) of $H_3P^{32}O_4$ in HCl was selected from among the internationally available radioisotopes and was used to label a natural population of Dacus existing in a semi-mountainous olive grove at Rovies, Evvia (Euboea). The use of the naturally occurring population was preferred over the field release of laboratory-reared and labelled individuals for the following reasons:

(1) The flight range of the native Dacus population which has already been adapted to the local environmental conditions may be more typical;

(2) The behaviour and the migration tendency of a reared or caged population may differ from that of the natural population; and

(3) It is difficult to obtain, under laboratory conditions, the large number of Dacus individuals required for studies of this kind.

The experimental area

A semi-mountainous olive grove (altitude 300 m above sea level) near the site known as "Chlio Nero" of Balanos' grove at Rovies, Evvia was selected for the application of the radioactive material used for labelling or tagging the natural fly population. This grove is surrounded on the north and north-east by the foothills of Mount Telethrion, which is largely covered by pine trees. This constitutes a natural barrier for the free movement of the olive fruit fly beyond. In all the other directions this grove is adjacent to Mr. Papadopoulos' olive grove, which extends on to the seashore (Fig. 1).

The selection of Balanos' field for carrying out these studies was decided for the following reasons:

(1) Data obtained from Dacus control experiments conducted in Papadopoulos' orchard during recent years [10] showed that a rather large and rapid increase of adult fly population occurred in this orchard by the end of September, even though the fruit infestation was low or very low. In such circumstances it might be supposed that new Dacus individuals came from Balanos' grove or other adjacent orchards that were by that time heavily infested.

(2) No chemical control had been applied on Balanos' grove during the previous three years. At the time these trials were carried out (October 1961) a heavy fruit infestation (85%) and a dense fly population (65-82 individuals per trap) occurred in Balanos' grove.

(3) The olive fruit of Balanos' orchard had prematurely ripened and was therefore unsuitable for ovipositions by females of the autumn brood, which thus would have to disperse in search of suitable fruit in which to lay their eggs. Furthermore, weather conditions, such as temperature, which drops substantially in the area under study in late autumn and in winter, and the northern and north-eastern winds prevailing there, might also favour the movement of flies from the semi-mountainous area (Balanos' orchard) to the coastal one (Papadopoulos' orchard).

Technique

A quantity of 40 mc of fresh radioactive P^{32} in the chemical form of PO_4 ion in HCl at a concentration of 5 mc per ml HCl, was imported from Canada*. The material (of radio-chemical purity 99.5% carrier-free) was shipped, as requested by us, in 4 bottles each containing 10 mc P^{32} (volume 2.00 ml). Shortly before its use the material was neutralized with normal $NaHCO_3$ until the solution became alkaline (pH 7.4). For this purpose approximately 5 ml of $NaHCO_3$ were used per ml of radioactive solution.

For labelling the natural adult fly population** occurring in the experimental field, the P^{32} solution was used in combination with Staley No. 7 protein hydrolysate. The flies feed upon this material and thus become radioactive. This protein has proved to have a satisfactory olfactory and gustatory attraction for Dacus flies [8, 9, 10]. This mixture of attractant and P^{32} solution was successfully used by BARNES, M. M. [1] to label a naturally occurring population of the walnut-husk fly, Rhagoletis completa Cresson. The senior author participated in the field trials carried out by Barnes in southern California during the fall of 1957.

The following formula was used in preparing the P^{32} bait solution:

1.	Staley No.7 hydrolysate protein	20.0 ml
2.	Radioactive P^{32} solution	12.5 ml
3.	Water	167.5 ml
		200.0 ml

A quantity of 600 ml of this preparation, containing a total of 30 mc radioactive P^{32}, was prepared.

Thirty olive trees were selected in the labelling station for the application of the P^{32} bait solution. On each tree, two twigs about 80 - 100 cm in length and equally developed were selected, one on the south-western and the other on the south-eastern side of the tree. They were treated on the morning of 14 October 1961. Care was taken in the application to insure that approximately 10 ml of solution was delivered per twig. The spray was applied with a small, laboratory air-pressure (pressure limit 200 lb)

* Atomic Energy of Canada Limited.

** Before these trials were carried out in the field, some tests were made in the laboratory with P^{32} bait solution at various concentrations to determine how Dacus adults responded to the beta radiation.

sprayer * . During the treatment favourable weather conditions prevailed in the field.

Precautions were taken during the handling of the radioactive material and the application of the spray, which was performed by the two first-named authors. Special attention was also paid to keeping persons and domestic animals away from the labelling operation.

For the collection of labelled flies and the determination of the Dacus flight range and movement, about 500 McPhail traps were hung on olive trees in the surrounding orchards and also on pine trees in adjacent areas. Traps were placed as far away as 10 km (in direct line) from the P^{32}-baited trees. A 3% solution of diammonium phosphate was used as a lure in the traps. The liquid was renewed at 5-d intervals. Approximately 380 traps were distributed in the nearby olive groves at Rovies (including semi-mountainous areas), while the remaining 120 traps were divided among more distant orchards of adjacent areas as far as Sipias, Limni. Some of these 120 traps were hung on pine trees of the forest area extending north-east of Rovies as far as the "Xiropigado" olive grove near Chronia (Fig. 1).

The traps were placed in zones at intervals of 200 - 300 m. The traps in each zone were specially marked so that they could easily be located and labelled flies trapped in them could easily be recorded.

Groups of experienced field workers were employed under the supervision of trained personnel to collect Dacus adults caught in the traps.

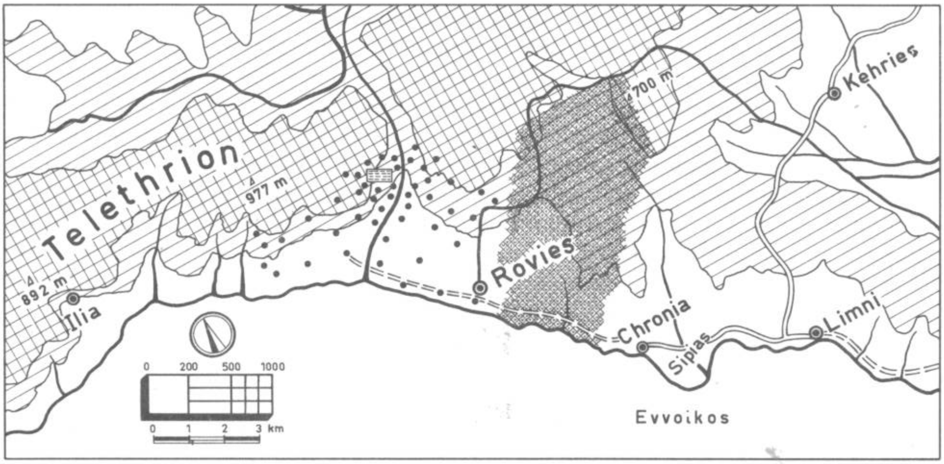

Fig. 1

Map of Rovies, Evvia (Euboea), showing P^{32}-baited trees and the positions of traps for labelled flies

P^{32}-baited trees

Traps for labelled flies

Pine-covered area

---

* "Sure shot", Milwaukee Sprayer Mfg. Co., Milwaukee.

Observations and counts of flies were made every other day. All insects collected in the traps were packed and forwarded to the Economic Entomology Laboratory of the Benaki Phytopathological Institute, Kiphissia, Athens.

RESULTS

To detect tagged flies and to determine their degree of radioactivity, a laboratory Geiger-Müller scaler or monitor (EKCO Electronics Ltd. No. 529) was used. For counts of less accuracy, such as those involving the determination of radioactivity of treated twigs, and for a quick on-the-spot observation of collected flies, a portable Geiger-Müller counter* was used.

Dacus individuals found in each trap were separately checked by the laboratory monitor after they had been carefully washed with distilled water on filter paper. To determine the true radioactivity of each labelled fly, the background radiation was taken into account.

The results obtained are shown in Table I. The data shown in this table together with additional observations permit the following conclusions.

The first labelled flies, 9 individuals collected in 7 traps, were detected 24 hours after treatment. Of these, 8 flies (3♀ and 5♂) were trapped 200 - 400 m away from the treated trees and 1 female fly about 2500 m away in a trap placed in the coastal section of Papadopoulos' orchard. The radioactivity of the first 8 flies ranged between 954 and 2099 counts/min per individual, while that of the ninth fly amounted to 273 counts/min (background radioactivity 8 - 12 counts/min).

A steady increase in the number of labelled flies occurred during the next 2 to 10 days following the treatment. Thereafter the figures declined substantially and the last tagged flies were collected on 18 November, that is 35 days after treatment.

Of a total of 7016 flies collected in 23 traps during the first ten-day period after treatment, 255 flies (3.6%) of both sexes (119♀ and 136♂) were labelled. Of these flies 88.2% were found in traps up to a distance of 1000 m away, 8.3% from 1000 to 2500 m (at Zervou, Kamini and Fytia) and the rest, 3.5%, from 2500 to 4300 m (at Kamini, Fytia and Osios David monastery). Three of the latter individuals were trapped on 24 October at a distance of 4300 m (maximum of flight range) in a trap placed in the Rovies olive grove. The radioactivity of 255 tagged flies ranged between 258 and 9549 counts/min per individual (background radioactivity 9 - 21 counts/min).

During the period under study a large increase in the adult fly population occurred in the whole Rovies area. Catches amounted to 76 914 flies in 293 traps (average 263 flies per trap).

In summary, during the period of observations (15 October - 2 December) 350 labelled flies were collected in 48 traps in which a total of 12 595 flies were trapped in the course of 15 counts made between 15 October and 18 November. Of the tagged flies representing a proportion of 2.8% of the above population, 86% moved up to a distance of 1000 m, 10% between 1000 and 2500 m and the remaining 4% moved up to a distance of 4300 m from

* Geiger-Müller counter TR 56 with window thickness of less than 4.0 mg/cm² (Panax Equipment Ltd.)

TABLE I

NUMBER OF LABELLED FLIES COLLECTED IN TRAPS PLACED AT DIFFERENT DISTANCES FROM THE LABELLING STATION

Date of collection	Time after treatment	Number of labelled and normal flies collected in the same 48 traps									Number of flies collected in 75 traps		
		Up to 1000 m			1000 - 2500 m			2500 - 4300 m			4300 - 10000 m		
		Total collected	Labelled flies		Total collected	Labelled flies		Total collected	Labelled flies		Total collected	Labelled flies	
	(d)		(No.)	(%)		(No.)	(%)		(No.)	(%)		(No.)	(%)
15 October	1	450	8	1.8	-	-	-	169	1	0.6	2130	0	0.0
16 October	2	323	15	4.6	74	2	2.7	218	1	0.5	1535	0	0.0
18 October	4	645	38	6.0	103	6	5.8	-	-	-	4238	0	0.0
20 October	6	1951	73	3.7	517	4	0.8	172	1	0.6	2890	0	0.0
22 October	8	1955	80	4.1	266	4	1.5	320	4	1.3	5106	0	0.0
24 October	10	276	17	6.2	80	5	6.3	116	3*	2.6	3559	0	0.0
28 October	14	43	5	11.6	11	2	18.2	130	3	2.3	1201	0	0.0
30 October	16	19	2	10.5	-	-	-	-	-	-	1211	0	0.0
2 November	19	52	2	3.8	81	5	6.2	-	-	-	2600	0	0.0
4 November	21	905	22	2.4	-	-	-	-	-	-	2007	0	0.0
7 November	24	525	16	3.0	42	2	4.8	-	-	-	2344	0	0.0
10 November	27	71	2	2.8	241	3	1.2	19	1	5.3	1850	0	0.0
13 November	30	300	2	0.8	-	-	-	-	-	-	5005	0	0.0
16 November	33	2089	17	0.7	218	2	0.9	-	-	-	2584	0	0.0
18 November**	35	214	2	0.9	-	-	-	-	-	-	1291	0	0.0
		9818	301	3.0	1633	35	2.14	1144	14	1.2	39551	0	0.0

* Labelled flies trapped at the longest distance (4300 m) from the labelling station.
** Counts of catches continued up to 2 December 1961.

the labelling station. However, the above percentage (2.8%) for labelled flies becomes still lower (0.15%) if the estimate is based on the total flies collected in all traps used for the entire Rovies area. Total catches amounted to 234 373 flies caught in 298 traps during the same period.

The number of labelled flies might have been higher if two coverage sprays applied for the protection of the olive crop of Papadopoulos' orchard on 23 October and 4 November had not decreased the adult fly population.

It was only in the olive groves near Rovies that labelled flies were recovered. During these studies no tagged flies were found in any of the traps placed either in the forest area between Rovies and the olive orchards of Chronia, or between the latter and the olive groves at Sipias, Limni (Fig. 1).

In general, the results obtained from these preliminary studies showed that an unceasing movement or dispersal of Dacus individuals occurred from the semi-mountainous olive groves to the adjacent olive groves in the plain and to the coastal groves at Rovies during the period under study. Long-distance movement or migration of flies was not observed. The farthest distance at which labelled flies were recovered was approximately 4300 m (in direct line). Moving from the north, the flies followed mostly south-easterly and south-westerly directions. The technique used for labelling a naturally occurring Dacus adult fly population proved to be very effective.

REFERENCES

[1] BARNES, M.M., Ann. Entomol. Soc. Amer. 52 (1959) 90-92.
[2] BUA, G., Meteorologia pratica 17 (1936).
[3] CHRISTENSON, L.D. and FOOTE, R.H., Ann. Rev. Entomol. 5 (1960) 187-189.
[4] ISAAKIDÈS, C.A., Un demi-siècle de lutte contre le Dacus, Athènes (1955) 13-14.
[5] LUPO, V., Boll. Lab. Zool. Portici 32 (1943) 137-177.
[6] MARTIN, H., Mitt. schweiz. entomol. Ges. 21 (1948) 370-371.
[7] MELIS, A., Redia 32 (1947) 52.
[8] ORPHANIDIS, P.S., DANIELIDOU, R.K., ALEXOPOULOU, P.S., TSAKMAKIS, A.A. et KARAYANNIS, G.B., Ann. Inst. Phytopath. Benaki, N.S., 1 (1958) 171-198.
[9] ORPHANIDIS, P.S., Report of the Fourth FAO Meeting on the Control of Olive Pests (Tel-Aviv, 1960) FAO, Rome (1961) 8-11.
[10] PELEKASSIS, C.E.D., Report of the Fourth FAO Meeting on the Control of Olive Pests (Tel-Aviv, 1960) FAO, Rome (1961) 13, figs. 2 and 3.
[11] ROAN, C.C., J. Econ. Entomol. 45 (1952) 826-828.
[12] SACANTANIS, K.B., 1ère Réunion FAO sur Dacus oleae (Gmel.) (Florence, 1953) FAO, Rome (1954) 20-21.

DISCUSSION

D.A. CROSSLEY: After the flies have fed on the P^{32} bait solution, how long do they retain detectable amounts of radioactivity? In other words, have you attempted to measure the biological half-life of P^{32} taken in through this bait solution?

C.E.D. PELEKASSIS: In our experiments labelled flies were recovered up to 35 d after treatment. Counts taken on the leaves of trees treated with P^{32} bait solution also indicate a biological half-life of about 30 d.

C.H. SCHMIDT: I was interested to note once again that apparently wind velocity had no effect on the migration of the labelled flies. You mentioned that the prevailing winds are north-east yet you found most of your flies in the south-eastern sector of the treated area.

C.E.D. PELEKASSIS: At the end of the period of observation, in the middle of November, there were some light winds which might have been favourable to movement in a south-easterly direction. But this could not be the sole factor involved. There must be some other explanation for the dispersal of the flies.

D.J. NADEL: I understand that the purpose of the experiments was to collect preliminary data on the movement and migration of Dacus from the test area, especially from the higher altitudes to the coastal regions.

There are several possibilities with regard to bait application methods:

(1) Single treatment with removal or isolation of the treated branches some set time after treatment - say, one day;

(2) Regular treatment twice a week, so as to ensure that an abundant supply of P^{32}-labelled bait is constantly available to the naturally occurring fly population;

(3) The method used in your experiments.

Why did you choose the third method, namely to treat only once and allow the bait to lose its attractiveness for the flies? What advantages do you feel were obtained? It would seem that a continuous series of treatments with the bait constantly available to the flies would have resulted in more complete data regarding fly movement, since there is then no question of bait deterioration.

C.E.D. PELEKASSIS: I do not know why Mr. Nadel has singled out just three methods of application. I think that various other possibilities could be conceived, depending mainly upon the ingenuity of the investigator and the purpose he has in mind.

There were three main reasons why we chose the technique we did. First, we had already had experience with a related technique. Secondly, owing to shortage of funds, import difficulties, etc., it was not possible to obtain more radioactive material at the time. Thirdly, we wanted to check the biological half-life of the radioactive material tested.

ИСПОЛЬЗОВАНИЕ РАДИОИЗОТОПОВ И РАДИАЦИИ В БОРЬБЕ С НАСЕКОМЫМИ-ВРЕДИТЕЛЯМИ РАСТЕНИЙ И ЖИВОТНЫХ

С.В. АНДРЕЕВ, Б.К. МАРТЕНС, В.А. МОЛЧАНОВА, З.И. САМОЙЛОВА
ВСЕСОЮЗНЫЙ ИНСТИТУТ ЗАЩИТЫ РАСТЕНИЙ, ЛЕНИНГРАД
СССР

Abstract — Résumé — Аннотация — Resumen

RADIOISOTOPES AND RADIATION IN ANIMAL AND PLANT INSECT PEST CONTROL. Crop-pest control is of major economic importance and demands the aid of the latest advances in science. Radioisotopes and radiation are being employed to increase the efficiency of existing insect pest control.

They are extremely valuable, since improvements to existing methods depend on having detailed data on the bioecology, toxicology, and so on. Radioactive labelling of insects has been extremely promising in bioecology; the labelling of grain pests (Eurygaster integriceps Put., Hadena sordida Skh.) and grain-pest parasites (Meniscus agnatus Crow, Pseudogonia cinerascens Rond.) has provided information about their areas of migration, habitats, sizes of population and the feeding habits.

The same technique was used to determine the rate of propagation of the Colorado beetle (Leptinotarsa decemlineota Say), which is subject to quarantine controls; subsequently an extermination programme was carried out on the basis of the data obtained.

It also provides a valuable means of studying the extremely complex problems of parasitism and predaceousness, in particular intermediate feeding cycles and chemotaxis. The feeding areas of field rodents have been mapped out with the help of a self-labelling, radioactive-bait technique.

Pesticides synthesized with radioisotopes have been used in conjunction with radiochromatography, fluorimetry and other techniques to study the highly complex biochemical processes caused to toxicants in plants and insects.

It has also been possible to determine the rate of hydrolysis of organic-phosphorus insecticide compounds of the thiphos and metaphos type as a function of the degree of development and the physiological state of plants as well as of environmental conditions. Data have been obtained on the length of time residual quantities of toxicants are retained in agriculture products following different periods of chemical treatment. Radioisotope techniques have yielded information on various metabolic processes exhibiting different degrees of stability when subjected to the action of herbicides.

Pesticides containing trace quantities of isotopes with short half-lives can be used as a means of judging the merits of various spraying systems for treating seeds and agricultural crops with pesticides; this method also provides information about the optimum doses.

Experiments have shown that ionizing radiations can be used to increase the virulence of insect nosophytes (Beauveria vassiana Unill.) and thus improve the efficiency of microbiological insect-pest-control methods. The sterilizing and lethal effects of ionizing radiations are also useful in combatting insect pests. Gamma-ray experiments have made it possible to establish sterilizing doses for a number of insect pests which attack crops and stored products (Calandra granaria L., Acanthoscelides obtectus Say, Pectinophora malvella Hb., Leptinotarsa decemlineota Say, Chloridea obsoleta F.).

The data obtained in these investigations will be used to develop and improve the methods of insect-pest control used in agriculture.

EMPLOI DES RADIOISOTOPES ET DES RAYONNEMENTS DANS LA LUTTE CONTRE LES INSECTES NUISIBLES AUX PLANTES ET AUX ANIMAUX. Le problème de la lutte contre les parasites des cultures présente une grande importance pour l'économie nationale. Il importe, pour le résoudre, de tirer parti des réalisations scientifiques les plus récentes. Pour rationaliser les méthodes actuelles de lutte contre les insectes nuisibles et accroître l'efficacité de ces méthodes, on a fait appel aux radioisotopes et aux rayonnements.

Grâce à l'emploi des radioisotopes et des rayonnements, il est possible de rationaliser dans une large mesure les méthodes utilisées et d'augmenter leur efficacité. A cet effet, il est indispensable d'étudier à fond les problèmes d'écologie, de toxicologie, etc. Dans le domaine de l'écologie, la méthode du radiomarquage des insectes est particulièrement prometteuse. L'emploi de radioisotopes pour le marquage des insectes rava-

geurs des céréales (Eurygaster integriceps Put. et Hadena sordida Skh.) et de leurs parasites (Meniscus agnatus Crow et Pseudogonia cinerascens Rond.) a permis d'établir l'ampleur des migrations, de déterminer l'emplacement des foyers de reproduction et l'importance numérique des populations et d'étudier le mode d'alimentation des parasites.

Par la même méthode, on a établi la dynamique de la propagation du doryphore (Leptinotarsa decemlineota Say), ce qui a permis d'obtenir les données indispensables pour procéder à leur extermination.

En outre, cette méthode permet d'étudier diverses questions extrêmement compliquées ayant trait au parasitisme et au pillage (étude des cycles alimentaires de l'alimentation intérimaire et de la chimiotaxie). Grâce à l'emploi de la méthode d'automarquage des rongeurs des champs à l'aide d'appâts radioactifs, on a pu déterminer les dimensions des zones où ils se procurent leur nourriture.

L'utilisation de parasiticides marqués — conjointement avec les méthodes de radiochromatographie, de fluorométrie, etc. — a permis d'étudier les processus biochimiques les plus complexes qui interviennent dans les plantes et chez les insectes sous l'effet des substances toxiques.

Se fondant sur l'exemple des composés organiques du phosphore du type «tiophosphate» et «métaphosphate», on a déterminé la vitesse de l'hydrolyse de ces insecticides en fonction de la phase de développement de la plante, de son état physiologique et des conditions du milieu ambiant. On a également déterminé la durée de rémanence des résidus de substances toxiques sur les produits agricoles, en fonction de celle des traitements chimiques. Les radioisotopes ont aussi révélé les particularités des processus métaboliques qui s'effectuent chez les plantes qui résistent et celles qui ne résistent pas aux herbicides.

Le marquage des parasiticides à l'aide de radioisotopes de courte période permet d'évaluer l'efficacité du traitement des graines et semences dans différentes conditions de pulvérisation, et quels parasiticides conviennent le mieux pour un traitement donné.

L'action des rayonnements ionisants sur les micro-organismes entomopathogènes (Beauveria bassiana Unill.) a permis de démontrer la possibilité d'accroître leur virulence et, partant, d'améliorer la méthode microbiologique de lutte contre les insectes nuisibles. L'action stérilisante et létale des rayonnements ionisants peut être utilisée comme moyen actif de lutte contre ces insectes. On a déterminé les doses de stérilisation à l'aide des rayons gamma pour un certain nombre d'insectes ravageurs des récoltes stockées (Calandra granaria L., Acanthoscelides obtectus Say, Pectinophota malvella Hb, Leptinotarsa decemlineota Say, Chloridea obsoleta F.) et des semences.

Les données obtenues à la suite des recherches permettront de mettre au point et de perfectionner les méthodes de lutte contre les insectes nuisibles, notamment à l'agriculture.

ИСПОЛЬЗОВАНИЕ РАДИОИЗОТОПОВ И РАДИАЦИИ В БОРЬБЕ С НАСЕКОМЫМИ-ВРЕДИТЕЛЯМИ РАСТЕНИЙ И ЖИВОТНЫХ. Проблема борьбы с вредителями сельскохозяйственных культур имеет крупное народнохозяйственное значение. При разрешении этой проблемы особое значение приобретает использование последних достижений науки. В целях рационализации и повышения эффективности существующих методов борьбы с вредными насекомыми были использованы радиоактивные изотопы и излучения.

Использование радиоактивных изотопов и излучений является могучим средством рационализации и повышения эффективности существующих методов борьбы с вредными насекомыми. Разработка этих методов вызывает необходимость детального изучения вопросов биоэкологии, токсикологии и т.д. В области биоэкологии весьма перспективным является метод радиомаркировки насекомых. Применение радиоизотопов для маркировки насекомых вредителей зерновых культур (Eurygaster integriceps Put, Hadena sordida Skh.) и их паразитов (Meniscus agnatus Crow, Psendogonia cinerascens Rond.) позволило установить размеры миграций их, определить места резерваций, численность популяций и изучить вопросы питания указанных паразитов вредных насекомых.

Этим же методом была установлена динамика распространения карантинного объекта картофельного жука (Leptinotarsa decemlineota Say), что позволило получить необходимые данные для проведения истребительных мероприятий.

Кроме того, метод дает возможность исследовать сложнейший комплекс вопросов паразитизма и хищничества (вопросы изучения пищевых циклов промежуточного питания и хематаксиса). Применение метода самомаркировки полевых грызунов с помощью радиоактивных приманок позволило установить размеры пищевых ареалов.

Использование пестицидов, синтезированных с радиоактивными изотопами, в сочетании с методом радиохроматографии, флюорометрии и другими позволило исследовать сложнейшие биохимические процессы, протекающие в растениях и насекомых под влиянием токсикантов.

На примере фосфорноорганических соединений типа тиофоса и метафоса была установлена скорость гидролиза этих инсектицидов в зависимости от фазы развития растения и его физиологического состояния, а также условий внешней среды. Определена длительность сохранения остаточных количеств токсикантов на сельскохозяйственной продукции в зависимости от сроков химических обработок. С помощью радиоактивных изотопов выявлены особенности метаболитических процессов, устойчивых и неустойчивых к гербицидам растений.

Внесение в пестициды изотопов с коротким периодом полураспада в индикаторных количествах дает возможность производить оценку качества обработки семенного материала и посевов сельскохозяйственных растений пестицидами при использовании различных систем опрыскивателей, а также определять оптимальные нормы их расхода.

Путем воздействия ионизирующих излучений на энтомопатогенные микроорганизмы (Beauveria bassiana Unill.) доказана возможность повысить их вирулентность в целях улучшения микробиологического метода борьбы с вредными насекомыми. Стерилизующее и летальное действие ионизирующих излучений может быть использовано как активное средство борьбы с вредными насекомыми. Применением гамма радиации установлены стерилизующие дозы для ряда насекомых-вредителей запасов (Calandra granaria L., Acanthoscelides obtectus Say, Pectinophora malvella Hb., Leptinotarsa decemlineata Say, Chlorides obsoleta F.), а также посевов.

Полученные данные исследований будут положены в основу разработки и усовершенствования методов борьбы с вредными насекомыми в условиях сельскохозяйственного производства.

UTILIZACIÓN DE LOS RADIOISÓTOPOS Y DE LAS RADIACIONES EN LA LUCHA CONTRA LOS INSECTOS NOCIVOS PARA LAS PLANTAS Y LOS ANIMALES. El problema de la lucha contra los insectos dañinos es de gran importancia para las economías nacionales. Los últimos descubrimientos de la ciencia pueden contribuir considerablemente a resolverlo. A fin de emplear de una manera más racional y eficaz los métodos de lucha contra las plagas de insectos, se ha recurrido a los radioisótopos y a las radiaciones.

Los radioisótopos y las radiaciones son auxiliares muy útiles para mejorar los métodos de lucha contra los insectos dañinos. Su empleo requiere un estudio detallado de toda una serie de cuestiones de bioecología, toxicología, etc. En bioecología, la marcación de los insectos es una técnica muy prometedora. El empleo de los radioisótopos a fin de marcar a los insectos dañinos para los cultivos de cereales (Eurygaster integriceps
de los radioisótopos a fin de marcar a los insectos dañinos para los cultivos de cereales (Eurygaster integriceps
Put, Hadena sordida Skh), y a sus parásitos (Meniscus agnatus Crow, Pseudogonia cinerascens Rond) ha permitido calcular la extensión de las migraciones, delimitar los reservorios, evaluar la importancia de las poblaciones y estudiar el modo de alimentación de los parásitos de los insectos nocivos.

Con este método se ha podido establecer la dinámica de la dispersión del escarabajo de la patata (Leptinotarsa decemlineata Say) y obtener los datos indispensables para aplicar medidas para su erradicación.

Se han podido también investigar los complicadísimos problemas del parasitismo y la predación (estudio de las cadenas alimentarias de la alimentación intermedia y de la quimiotaxia). Gracias a la automarcación de los roedores mediante cebos radiactivos se puede determinar la importancia de las zonas donde éstos se alimentan.

La utilización de los pesticidas marcados, en combinación con la radiocromatografía, la fluorometría y otras técnicas, ha permitido investigar los procesos bioquímicos más complejos que tienen lugar en las plantas y en los insectos bajo la acción de agentes tóxicos.

Se ha calculado la velocidad de hidrólisis para los compuestos fosforoorgánicos del tipo Tiofos y Metafos en función de la fase de crecimiento de las plantas, de su estado fisiológico y de las condiciones del medio ambiente. Se ha determinado el tiempo que los residuos de agentes tóxicos permanecen en los productos agrícolas en función de la época en que se procedió al tratamiento químico. Con ayuda de los radioisótopos se han investigado las características de los procesos metabólicos de las plantas resistentes y no resistentes a los herbicidas.

La marcación de los pesticidas con isótopos de período corto ha permitido estudiar cualitativamente el tratamiento de las semillas y de las plantas utilizando diferentes sistemas de aplicación de pesticidas pulverizados y establecer normas óptimas para su empleo.

Mediante la acción de las radiaciones ionizantes sobre los microorganismos entomopatogénicos (Beauveria bassiana Unill) se ha demostrado la posibilidad de aumentar su virulencia y, por tanto, mejorar los métodos microbiológicos de lucha contra las plagas de insectos. La acción esterilizadora y letal de las radiaciones ionizantes puede ser muy eficaz en la lucha contra las plagas de insectos. Utilizando rayos gamma se han establecido las dosis esterilizadoras para toda una serie de insectos nocivos para los productos agrícolas almacenados

(Calandra granaria L., Acanthoscelides Obtectus Say, Pectinophora Malvella Hb., Leptinotarsa decemlineota Say, Chloridea obsoleta F.) y los cultivos.

Los datos obtenidos con esas investigaciones servirán de base para elaborar y perfeccionar los métodos de lucha contra los insectos dañinos para la agricultura.

В Советском Союзе проводится интенсивная исследовательская работа по использованию радиоактивных изотопов и излучений в целях разработки теоретических и практических задач борьбы с вредными насекомыми. В этих исследованиях значительное место отводится изучению вопросов токсикологии, биоэкологии вредных насекомых и их паразитов, а также исследованию процессов, связанных с рационализацией методов борьбы.

Применение радиоактивных изотопов позволило по новому подойти к решению поставленных задач и выявить данные, которые не могли быть получены обычными методами исследования.

Наряду с этим приводятся также исследования по применению мощных источников ионизирующих излучений для целей активной борьбы с вредными насекомыми.

Правильная организация мероприятий по борьбе с вредителями возможна лишь на основе детального изучения особенностей их биологии (миграций, ареалов распространения, мест резерваций и ряд других вопросов).

Для изучения указанных вопросов успешно используется метод маркировки насекомых радиоактивными изотопами, который позволяет вести наблюдения над большим числом объектов. Это дает возможность получать более достоверные результаты, используя метод математического анализа.

Метод радиомаркировки нашел применение в паразитологии человека [1 - 6] и животных. В этом плане изучение характера и предельной дальности перемещений насекомых имеет большое значение для понимания их роли как переносчиков возбудителей инфекционных заболеваний человека и животных.

Одним из наиболее опасных вредителей сельскохозяйственных животных являются вольфартовые мухи (Wohlfahrtia magnifica Schin.), личинки которых паразитируют в ранах домашних животных. Зона распространения этих мух - южные районы Советского Союза.

Миазы, вызываемые личинками, приносят большой вред овцеводству. Эффективная борьба с вольфатиозом скота без знания биоэкологии мух весьма затруднительна. В связи с этим для изучения некоторых биологических особенностей биологии этих мух был применен метод радиомаркировки. Маркировка мух производилась путем скармливания им сахарного сиропа, содержащего радиоактивный изотоп фосфора с концентрацией 1,86 микрокюри в 1 мл. Маркированные мухи в количестве нескольких тысяч экземпляров были выпущены в местах выпаса овец. В дальнейшем производился отлов мух ловушками с мясными приманками, затравленными 0,02% раствором хлорофоса, с последующим анализом отловленных мух радиометрической аппаратурой. Ловушки расставлялись в зоне выпаса скота на расстоянии от 150 м до 10 км от места выпуска мух. Наибольшее количество мух было отловлено у водопоя, который находился на рас-

стоянии 2 км от места выпуска. Это позволило сделать вывод, что общий водопой является местом резервации мух и перелета их с одних отар на другие. Лабораторный и полевой опыты также показали, что у маркированных мух наблюдается транслярвальный переход радиоактивного фосфора от самок к личинкам. Последнее позволяет установить размеры распространения инвазии от отдельных особей [7]. Метод радиомаркировки может быть также применен при изучении миграций мелких объектов: тлей (Aphididae), трипсов (Thysanoptera), клещей (Acarina), блох (Aphaniptera) и др. В этом случае, помимо радиометрического анализа, может быть использован метод радиоавтографии. Радиоавтограммы насекомых, полученные при длительном (24 - 72 часа) экспонировании, дают отчетливую картину наличия радиоактивной метки в них. Указанный метод радиоавтографии был применен в исследованиях миграции блох (Xenopsilla heopis и Ceratophyllus fosciatus), паразитирующих на грызунах и являющихся переносчиками ряда опасных заболеваний.

Для осуществления маркировки блох производилась маркировка радиоактивным изотопом мышей, на которых эти блохи паразитируют. При маркировки мышам путем инъекции вводился препарат радиоактивного фосфора из расчета 4 микрокюри на 1 г веса животных. После выпуска маркированных мышей, в места их обитания на различных расстояниях от места выпуска производился массовый отлов мышей. После обработки мышей и находящихся на них насекомых, летальной дозой наркоза, собранные насекомые проверялись радиоавтографическим методом. Проведенные опыты позволили установить размеры зоны распространения блох от мышей, инъекцированных радиоактивным фосфором [8].

В исследованиях по защите растений, маркировка насекомых радиоактивными изотопами, была применена при изучении миграций опасного вредителя зерновых - клопа черепашки (Eurigaster integriceps put.), зерновой совки (Hadena sordide pxh), а также паразитов последней (pseudogonia cinerascens и Meniscus agnatus grov) [9 - 11]. Маркировка этих насекомых производилась различными специально разработанными методами: в личиночной стадии насекомые метились путем кормления их на пище, содержащей радиоактивные изотопы. Взрослые насекомые с твердым хитиновым покровом, метились путем погружения их в радиоактивные растворы.

Для маркировки бабочек и паразитических мух, во избежании возможных нарушений их покровов, применялся метод самомаркировки, заключающийся в использовании положительного эффекта фото- и хемо таксисов. Насекомые, привлеченные светом или запахом к приманкам, содержащим радиоактивные вещества, соприкасаясь или питаясь ими, становились маркированными.

Изучение особенностей биологии вредителей картофеля - колорадского жука (Leptinotarsa decemlimata Say) методом маркировки позволило изучить миграции перезимовавших жуков.

Выяснено, что после зимовки жуки, только что вышедшие из почвы, способны наряду с передвижением по земле совершать сравнительно большие перелеты, дальность которых достигает 500 и бо-

лее метров в сутки. Наибольшую активность жуки проявляют в период спаривания, пролетая за сутки более 1 км. В этот период происходит максимальное расширение площадей заражения. Во время яйцекладки подвижность жуков резко снижается, и маркированные жуки перемещаются 10 - 40 м в сутки.

С помощью маркировки радиоактивными изотопами была проверена существующая методика обнаружения очагов заражения и плотности их заселения жуками. В результате проведенных контрольных обследований было установлено, что даже при трехкратном обследовании, проводимых одно за другим посредством ручных сборов, обычно обнаруживается не более 80% жуков, находящихся на данном поле. Это позволило обосновать, в известной мере, причины частичного обнаружения очагов колорадского жука и объяснить причины образования многолетних очагов.

Применение радиоактивных изотопов, являющихся источником жесткого гамма-излучения (Co^{60}, Fe^{59}), дает возможность проводить наблюдения также и за миграциями скрытноживущих насекомых, обнаруживая с помощью радиометрических приборов под слоем коры короедов различных видов, а в почве - проволочников и др. [12]. Маркировка радиоактивными изотопами растений в период их цветения позволила решить вопрос о преимущественном и избирательном питании вредных насекомых и ряда их паразитов различными видами растений [13]. Кроме того, применение радиоактивных изотопов позволяет определять пищевые циклы, устанавливать хищничество и паразитизм (т.е. выбор хищниками и паразитами жертвы и хозяина).

Наряду с исследованиями в области прикладной энтомологии представляет практический интерес применение метода самомаркировки теплокровных биоценоза. Опыты проводились по оценке приманочного метода борьбы против грызунов, а также влияния этого метода на полезную фауну (промысловых птиц и зверей).

Метод исследования заключался в следующем. В местах предполагаемого обитания вредных грызунов разбрасывались отравленные приманки. В качестве приманок использовался овес с добавлением в него вторацетата бария и радиоактивного раствора $Na_2HP^{32}O_4$ из расчета 300 г приманки на 1 га.

Посещения грызунами мест обитания подтверждалось обнаружением экскрементов и трупов особей, питавшихся отравленными приманками, содержащими радиоактивный фосфор. Дозиметрия трупа являлась единственным средством установить, что животное погибло от отравленной приманки, так как обнаружить яд в трупном материале химическим путем не в полевых условиях было невозможно.

Проверка опасности отравленных приманок для промысловых птиц показала, что только незначительный процент отстреляных птиц содержал следы радиоактивности. Наряду с этим метод самомаркировки позволяет установить предпочитаемость грызунов в выборе типа кормовой основы приманки, в частности было установлено, что для суслика наилучшей приманкой является овес, а для водяной крысы - кукуруза.

Применение метода самомаркировки грызунов и промысловых животных радиоактивными изотопами дает возможность изучать слож-

ный комплекс вопросов теплокровных биоценоза и установления кормовых ареалов [14].

При проведении химической обработки сельскохозяйственных растений существенное значение имеет: а) определение качества обработки полей, б) установление оптимальных норм расхода пестицидов, в) определение сравнительных характеристик опрыскивателей различных систем.

Для решения этих вопросов был разработан новый метод, основанный на использовании радиоактивных изотопов.

О результатах применения метода более подробно было доложено в 1960 г. на симпозиуме в Бомбее, посвященным вопросам применения радиоактивных изотопов и радиации в энтомологии [15, 16]. В настоящее время этот метод используется в научно-исследовательской практике при проверке различных систем опрыскивателей при наземном и авиационном методах обработке посевов полевых культур, виноградников и садов.

Исследование сложнейших вопросов токсикологии насекомых и интоксикации растений (метаболических процессов, связанных с поступлением токсикантов в организмы, определение токсических характеристик пестицидов, длительности их сохранения, установление остаточных количеств токсических соединений в сельскохозяйственной продукции и многие другие) невозможно без привлечения общепризнанного в мировой практике метода радиоактивных индикаторов. Методом радиоактивных индикаторов получены данные о различной скорости проникновения пестицидов в организмы растений, вредных насекомых и грызунов; прослежена динамика распространения, пестицидов кишечного и контактного действия внутри организма насекомого, а также преимущественная локализация пестицидов в отдельных органах и тканях.

Исследования с помощью радиоактивных изотопов позволили выяснить ряд вопросов, связанных с практикой применения фосфорорганических соединений типа тиофоса и метафоса, а также изучить поведение этих химикатов в растениях, установить скорость гидролиза препаратов у различных растений в зависимости от условий их выращивания, а также определить остаточные количества токсикантов в сельскохозяйственной продукции. Методом радиохромотографии установлено, что тиофос и его метильный аналог обладают весьма слабым системным действием [9].

В практике применения инсектицидов в борьбе с вредными насекомыми недостаточно уделяется внимания влиянию пестицидов на растения при различных условиях их питания. Между тем, изменением условий питания, как подтверждают проведенные исследования, представляется возможным снизить степень токсического действия яда на защищаемое растение, особенно при неблагоприятных условиях произрастания. Применением метода радиоактиных индикаторов удалось установить, что хлорорганические препараты (ГХЦГ и ДДТ) оказывают существенное влияние на поступление фосфора в растение.

Так, например, при стимулирующих дозах хлорорганических препаратов процесс поступления P^{32} в растительные ткани активировался, и радиоактивный фосфор концентрировался в формирующихся органах в 1,5 - 2 раза интенсивнее, чем при поступлении его в необработанные токсикантом растения. Подобная активация поглощения радиоактивного фосфора является следствием усиления обмена веществ, вызываемого влиянием препарата. При воздействии же ингибирующих доз инсектицида наблюдалось нарушение нормального поступления фосфора P^{32} в растение, что явилось результатом угнетения токсикантом процессов жизнедеятельности.

Увеличение продуктивности отдельных органов и растения в целом было получено при совместном применении хлорорганических препаратов с фосфатами.

Совместное применение хлорорганических препаратов с фосфатами проверено в производственных условиях на различных культурах: пшенице, картофеле и других.

Таким образом, рациональное применение физиологически активных химических средств в сочетании с определенными удобрениями в практике защиты растений может обеспечить не только защиту культуры от вредных насекомых, но и дополнительно повысить урожай от 10 до 20 и более процентов [18].

В последнее время, как в СССР, так и в других странах большое внимание уделяется разработки методов лучевой дезинсекции продуктов запаса, а также лучевой стерилизации полевых вредителей в целях снижения численности их популяций.

Вопросу дезинсекции продуктов при их хранении посвящена довольно обширная литература. Рядом авторов [19 - 25] были проведены исследования по изучению действия ионизирующих излучений на насекомых вредителей запасов (Calandra granaria L., Calandra oryzae L., Acanthoscelides obtectus Say, Bruchus prisorum L).

Исследования летального действия ионизирующих излучений показали, что хотя гибель насекомых непосредственно под лучем наступает при 300 тыс. р, однако и значительно более низкие дозы (30 тыс. р) приводят к резкому сокращению продолжительности жизни насекомых.

При проектировании и осуществлении гамма-дезинсекторов большой производительности особое значение приобретает выбор оптимальной мощности источника облучения, соответствующей экономически наиболее целесообразному варианту. В связи с этим возникает необходимость в выборе метода облучения, обеспечивающего достаточную дезинсекцию при минимальных затратах на сооружение дезинсекторов и их эксплоатацию. Значительный выигрыш в этом отношении может быть получен за счет применения метода половой стерилизации насекомых. Дозы, необходимые для половой стерилизации насекомых, значительно ниже летальных.

Многочисленными исследованиями, проводимыми как в СССР, так и в других странах с различными вредителями запасов зерна, установлено, что оптимальными дозами для стерилизации насекомых вредителей являются дозы в 7 - 10 тыс. р. Эти данные явились исход-

ными при проектировании гамма-дезинсекторов. В настоящее время уже разработан ряд проектов гамма-дезинсекторов, часть которых осуществляется [26, 27].

В указанных дезинсекторах использованы различные приемы стерилизации. Одним из подобных решений является подача зерна к облучателю в контейнерах. В качестве источников гамма-излучения используются радиоактивные изотопы Co^{60} и Cs^{137}.

Наиболее рациональным является использование принципа облучения зерна при его движении непрерывным потоком мимо стержневого излучателя. Этот способ дезинсекции был положен в основу расчета гамма-дезинсектора зерна производительностью 30 т/час при суммарной активности источника излучения (Co^{60}) 60 кг эквивалент радия. На рис.1 показана схема подобной установки. Отличительной особенностью этого гамма-дезинсектора является применение водяной защиты и заглубление всего дезинсектора в грунт.

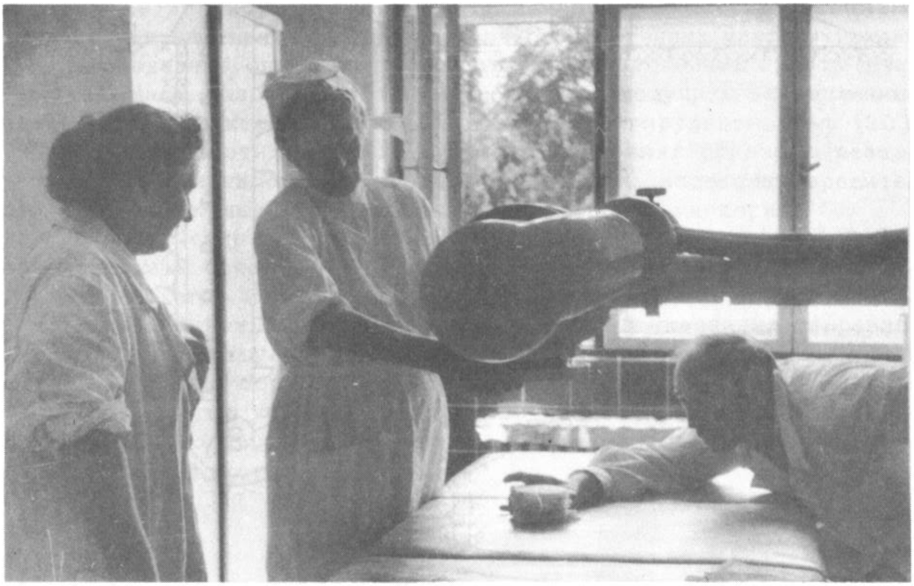

Рис.1

Подготовка к опыту по облучению куколок колорадского жука на рентгеновской установке, мощностью 200 - 650 р/мин.

В области борьбы с вредителями полевых культур в последние годы особый интерес энтомологов многих стран вызывает использование методов химической и лучевой стерилизации, как средства снижения численности естественной популяции.

Метод лучевой стерилизации позволяет значительно сократить или полностью исключить применение химических средств борьбы и, следовательно, устранить вредное действие на человека и сельско-

хозяйственных животных инсектицидов, остающихся в сельскохозяйственных продуктах после их обработки. Кроме того, этот метод является единственным, когда инстинктивное стремление насекомых к увеличению популяций направляется и используется человеком на самоуничтожение вредителя как вида.

В связи с этим в Советском Союзе проводятся поисковые исследования по оценке указанного метода на ряде объектов: хлопковой совке (Chloridae obsoleta F.), капустной мухе (Chortophila

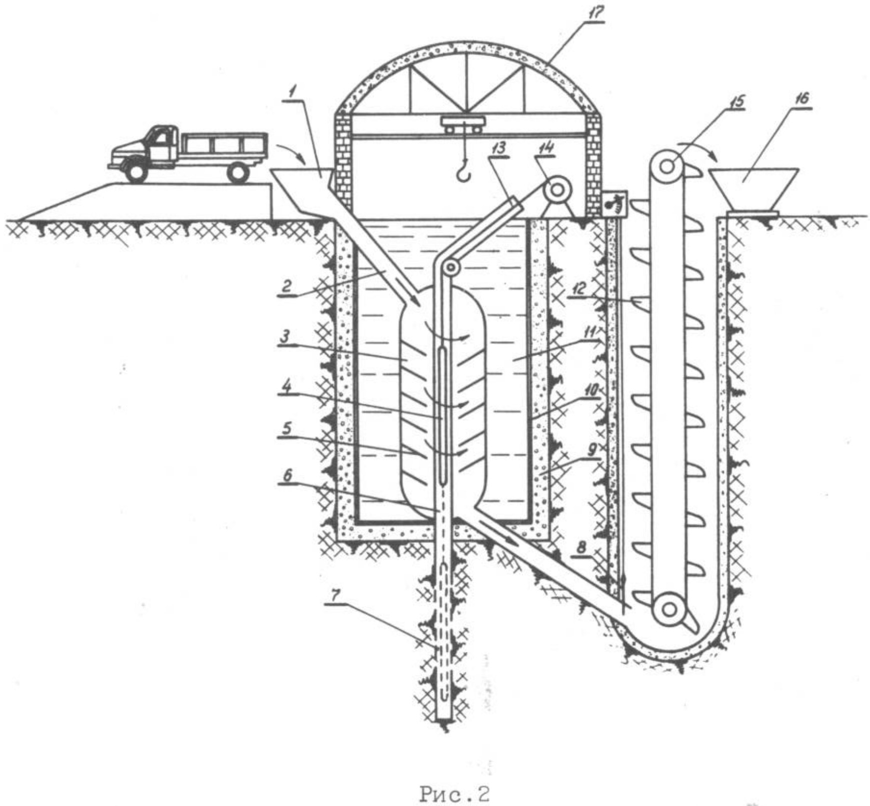

Рис.2

Принципиальная схема гаммадезинсектора зерна: 1 - приемный бункер; 2 - зернопровод; 3 - камера облучения; 4 - стержневой гамма-излучатель; 5 - спиралеобразные поверхности; 6 - металлическая труба; 7 - скважина; 8 - шибер; 9 - бетонированный колодец; 10 - слой гидроизоляции; 11 - вода; 12 - ковш транспортера; 13 - трос; 14 - лебедка; 15- разгрузочный транспортер; 16 - приемный бункер.

brassicae Bouche), колорадском жуке (Leptinotarsa decemlimata), мальвовой моли (Pectinaphora malvella Hb.).

Опыты по стерилизации этих насекомых показали, что стерилизующие дозы лежат в пределах 6 - 10 тыс. рентген [28, 29]. На рис.2 показана подготовка к облучению куколок колорадского жука на рентгеновском аппарате.

Проводятся исследования по изучению поведения стерилизованных особей в природе и определению их активности по сравнению с необлученными насекомыми, что необходимо для установления оптимальных соотношений между стерилизованными и нестерилизованными самцами в природе.

Особенное значение приобретает вопрос об установлении сравнительной эффективности метода при наличии у насекомых моно- или полигамии, а также при ограниченном числе генераций в году.Ионизирующие излучения являются также эффективным средством воздействия на микроорганизмы. Излучения могут быть успешно использованы в практике для борьбы с возбудителями заболеваний растений, а также для повышения вирулентности энтомопатогенных микроорганизмов.

Проведенные опыты по облучению энтомопатогенных грибов (Beaveria bassiana, Aspergillus flavus) позволили получить закрепленные изменения, характеризирующиеся повышенной вирулентностью [30].

Испытание этих штаммов в полевых условиях показало резкое увеличение смертности зараженных или вредных насекомых вредителей зерновых культур в местах их массового размножения.

В заключение следует отметить, что в моем кратком сообщении освещены лишь основные направления исследований в области использования изотопов и радиации в целях борьбы с вредными организмами.

О повышенном интересе к исследованиям в указанных направлениях свидетельствует организация 2-го симпозиума, посвященного острой проблеме борьбы с вредными насекомыми.

Содружество ученых многих стран является залогом успешного разрешения проблемы практического использования атомной энергии в сельском хозяйстве.

ЛИТЕРАТУРА

[1] ИЛЬИНСКАЯ Н.Б., ТРОШИН А.С. Маркировка мух и комаров при помощи радиоактивного фосфора. Зоол.журн., 33, 4 (1954).

[2] ШУРА БУРА Б.Л. Применение меченых атомов в энтомологии. Успехи современной биологии. XXXV.,1 (1957).

[3] ШУРА БУРА Б.Л. К вопросу изучения миграций комнатных мух при помощи радиоактивных изотопов. Чтения памяти Н.А. Холодковского. Изд. АН СССР (1952).

[4] ШУРА БУРА Б.Л. Опыт изучения миграций мух со свалки методом меченых атомов. Гигиена и санитария, № 9 (1955).

[5] ХУДАКОВ Г.Д. Сравнительная оценка трех методов мечения членистоногих 10 радиоактивными изотопами. Труды Центрального Научно-Исследовательского Дезинфекционного института, вып. 11 (1959).

[6] ХУДАКОВ Г.Д. К вопросу о применении радиоактивного фосфора при изучении миграций мух из жилых помещений. Труды Центрального Научно-Исследовательского Дезинфекционного института, вып.11 (1959).

[7] ТЕРНОВОЙ В.И. Изучение миграций вольфартовых мух методом радиомаркировки. Материалы симпозиума по применению биофизики в области защиты растений. Л., 1961.

[8] ШУРА БУРА Б.Л., ХАРЛАМОВ В.П. Применение радиоавтографии для выявления маркированных радиоактивными изотопами членистоногих. Материалы симпозиума по применению биофизики в области защиты растений. Л., 1961.

[9] АНДРЕЕВ С.В., МОЛЧАНОВА В.А., МАРТЕНС Б.К. Применение радиоактивных изотопов для маркировки насекомых. Защита растений от вредителей и болезней. № 2 (1960.

[10] АНДРЕЕВ С.В., МОЛЧАНОВА В.А., МАРТЕНС Б.К. Применение радиоактивных изотопов для маркировки бабочек зерновой совки. Зоол. журн. XL 1, 1 (1962).

[11] МОЛЧАНОВА В.А., МАРТЕНС Б.К., КАМЕНКОВА К.В. Радиомаркировка насекомых для изучения их миграций. Тезисы докладов на симпозиуме по применению биофизики в области защиты растений. Ленинград, 1961.

[12] ЧЕРЕПАНОВ А.И., ВОЛГИНА Н.Г. Определение перемещения проволочников в почве методом меченых атомов. Докл. АН СССР XCVIII, 2 (1954).

[13] КАМЕНКОВА К.В., МОЛЧАНОВА В.А. Установление фактов дополнительного питания насекомых с помощью радиоактивного фосфора. Материалы симпозиума по применению биофизики в области защиты растений. Л., 1961.

[14] ВЫКОВСКИЙ В.А. Применение радиоактивного фосфора для самомаркировки теплокровных биоценоза. Материалы симпозиума по применению биофизики в области защиты растений. Л., 1961.

[15] АНДРЕЕВ С., МАРТЕНС Б., МОЛЧАНОВА В. Новый метод определения качества обработки посевов ядохимикатами. Международный сельскохозяйственный журнал № 4 (1962).

[16] АНДРЕЕВ С.В., МАРТЕНС Б.К., МОЛЧАНОВА В.А. Применение радиоактивных изотопов при изучении вопросов защиты растений. Сборник "Radioisotopes and radiation in entomology" International Atomic Energy Agency, Vienna, 1962.

[17] АНДРЕЕВ С.В., ВОЕВОДИН А.В., МОЛЧАНОВА В.А., ХОТЯНОВИЧ А.В. Некоторые результаты применения радиоактивных изотопов в исследованиях вопросов защиты растений. Труды II Женевской конференции. Изд. АН СССР, 1959.

[18] БОГДАРИНА А.А., ЗАЗЕРИНА А.Н. О поступлении фосфора P^{32} и азота в растение при применении хлорорганических инсектицидов. Симпозиум по применению биофизики в области защиты растений. Л., 1961.

[19] ОЛЬХОВ О.А. Применение атомной энергии в сельском хозяйстве. Сельхозгиз, М., 1962.

[20] СУМАРУКОВ Г.В. Динамика радиационного поражения амбарных долгоносиков при разных условиях облучения. Биофизика № 2 (1957).
[21] ПЕРЕДЕЛЬСКИЙ А.А. Действие ионизирующих излучений на насекомых. Итоги науки, 1 радиобиология. Изд. АН СССР, 1957.
[22] ПЕРЕДЕЛЬСКИЙ А.А., РУМЯНЦЕВ П.Д., РОДИОНОВА Л.З., БИБЕРГАЛЬ А.В., ПЕРЦОВСКИЙ Е.С. Ионизирующие излучения как средство борьбы с насекомыми вредителями запасов зерна. Сборник трудов Всесоюзной Научно-Технической конференции по применению радиоактивных и стабильных изотопов и излучений в народном хозяйстве и науке. Изд. АН СССР, М., 1958.
[23] ПЕРЕДЕЛЬСКИЙ А.А., РОДИОНОВА Л.З., БИБЕРГАЛЬ А.В., РУМЯНЦЕВ П.Д., ПЕРЦОВСКИЙ Е.С. Разработка метода борьбы с насекомыми вредителями хранящегося зерна при помощи ионизирующих излучений. Биохимия зерна, вып. 35 (1958).
[24] РОДИОНОВА Л.З. Изменение активности питания жуков амбарного долгоносика, облученного Х-лучами. Сб. Биохимия зерна, вып. 35 (1958).
[25] АНДРЕЕВ С.В., МАРТЕНС Б.К., МОЛЧАНОВА В.А., СТЕПАНОВ А.С. Исследование влияния дозы облучения на смертность и половую стерилизацию амбарного долгоносика. Радиобиология, II (1962).
[26] РАДЗНЕВСКИЙ Г.Б. Гамма-облучение больших масс продуктов с применением движущихся препаратов. Биофизика 1, 5 (1956).
[27] БИБЕРГАЛЬ А.В., МАРГУЛИС У.Я., ПЕРЦОВСКИЙ Е.С. Использование мощных источников облучения для обеззараживания зерна. Атомная энергия II, 4 (1957).
[28] МАРТЕНС Б.К. Использование ионизирующих излучений в сельском хозяйстве. Л., 1960.
[29] АНДРЕЕВ С.В., САМОЙЛОВА З.И., МАРТЕНС Б.К., ИВАНСКИЙ Н.Л. Гамма-излучения и борьба с насекомыми. Защита растений от вредителей и болезней, №9 (1962).
[30] ЕВЛАХОВА А.А., МАРТЕНС Б.К. Использование ионизирующих излучений для повышения вирулентности энтомопатогенных грибов. Тезисы доклада на симпозиуме по применению биофизики в области защиты растений. Л., 1961.

DISCUSSION

K. K. NAIR (Chairman): In your paper you have referred to the grain irradiator recently fabricated in the Soviet Union. Could you tell me what exactly is the cost of operation of this unit? How much, that is, does it cost to irradiate one ton of grain at the proposed throughput of 30 t/h?

S. V. ANDREEV: The economic calculations have not yet been made, but it will be considerably cheaper if the irradiator is used in stationary conditions, close to a large silo where grain is kept for a long time. It is difficult to give the exact cost per ton. A number of plants are at present being set up in our country for the sterilization of grain and food products.

On the basis of the studies to be carried out once these plants are completed, an evaluation of their effectiveness and economic viability will be made. The plant I mentioned as one of the main ones should prove economically profitable.

M. S. QURAISHI: May I request you to throw some more light on the study of Eurygaster integriceps carried out in the Soviet Union?

S. V. ANDREEV: Study of the migration of Eurygaster integriceps has been carried out in the southern part of the Soviet Union. First larval migration, both vertical and horizontal, was studied under different climatic conditions. The larvae were reared on plants growing on soil sprayed with a solution of $Na_2H^{32}PO_4$. By labelling the larvae we were able to establish migration to field crops and wheat. We also found weather-dependent vertical migration; when conditions were favourable the larvae rose to the tops of plants, and when temperatures increased above normal they went into the ground and remained there until the next favourable period.

We also studied the migration of the adult insects. In summer they were collected in large numbers from grain by special mechanical collectors and then, by means of a special long stick with a hole in it, they were dipped in a tank containing a $Co^{60}Cl_2$ aqueous solution. After 1 min they were extracted and placed to dry in another tank filled with straw. This second tank was carried into the field and the insects were released within a given area.

Their radius of flight was then checked with field counters. It was found that in their final stages of development the insects collected in wooded areas in autumn for hibernation.

In spring, after over-wintering, they flew out of the forests, and during the early period, when their fat reserves were exhausted, were to be found at first close to the young wheat. During this period they stayed on the wheat for a few days and then when they had recovered their strength to some extent, began to carry out longer flights. We found that such flights may range over many tens of kilometres when there is no wind.

All their flight ranges were determined both in spring and autumn. We established the strength and direction of migratory flights at the various ecological stages and formulated techniques for control in these regions.

M. S. QURAISHI: I was unable to follow just when E. integriceps was labelled If I heard correctly they were labelled after hibernation and then released in the field.

If they were labelled at the time of migration from mountains to field, may I know the advantages of using a long-lived isotope like Co^{60}?

S. V. ANDREEV: The insects were labelled at the beginning of spring, on emerging from the forests. Afterwards, during the summer, we observed the generation which had been hibernating. Then the larvae appeared, non-labelled of course. After a certain period we obtained the adult insects from these and collected a new generation, which we again labelled in the spring or early summer. The reason we used Co^{60} was to be able to detect those hidden under leaves or topsoil in the forest, also to extend the observation period, in order to observe those emerging in the spring. The insects move about a good deal under the leaves, so that much of their radioactivity might have been lost if other techniques had been applied.

H. J. de FLUITER: In his introduction, the speaker mentioned Soviet investigations on the migration of Aphididae and Thysanoptera. We are very much interested in the investigations into the migration of Thysanoptera. Did these investigations deal with the migration of the adult thrips or with the migration of the larvae into the soil, where they often hibernate at various depths? How did you label the thrips and which species are involved?

S. V. ANDREEV: In addition to Eurygaster integriceps we have studied the migration of Hadena sordida and Pseudogonia. Our investigations have in fact been mainly concerned with soil pests. The soil pests are marked with metallic cobalt introduced into the anus. Vertical and horizontal migration is studied with a special counter and the vertical migration is studied according to absorption in the soil, so that the thickness of the layer under which the insect is to be found can be read. Horizontal migration is studied from the insect's range of movement over the surface of the soil, also by using a counter.

For the purpose of studying the migration of thrips on the cotton plant, they were labelled by being fed on P^{32} through the plant. Later, thrips collected from other plants in the neighbourhood of the experimental ones, and also from different plants from those used in the experiment, were transferred on to film on which they were exposed for 24 - 72 h. This made it possible to determine the extent of migration over 24 h, and also throughout their lifetime.

B. DARIS: On the subject of irradiation of grain in silos, I would like to ask how many curies does the Co^{60} source which you used give?

S. V. ANDREEV: In the main gamma-disinfestation source I have described, the entire estimate was based on an overall activity of 60 kg Ra equiv.

B. DARIS: How long does it take the grain to move from the entrance to the exit?

S. V. ANDREEV: At a throughput of 30 t/h the grain was no longer in the irradiation area than one minute.

R. KLJAJIĆ: You say you are studying questions of plant physiology as affected by the use of pesticides. This problem has been a frequent subject in modern phytopharmaceutical studies and modern scientific development has made possible the application of precise methods and criteria for the determination of plant behaviour in relation to pesticides. Classical methods, useful enough though they may be from the point of view of agricultural practice, do not always provide an objective and comprehensive picture of the most subtle phenomena in plants.

Research in our laboratory is aimed at applying more precise methods to the study of the effect of certain pesticides upon the living processes of treated plants.

In our paper: "Contribution to the study of P^{32} absorption in soybean and maize plants depending on application of some pesticides" *, by Kljajić R., Štrbac N., Šinžar B. and Ostojić N., certain data are given concerning soya beans and maize.

Soya bean varieties (Dickmans Grüngelbe, and Manchor Wisconsin) have been grown in sand culture in laboratory conditions (T = 20-22°C and

* Submitted to but not presented at the Symposium.

5000 lux). Treatment by pesticides, Kelthane W (conc. 0.0250 a.i.%;
5000 lux). Treatment by pesticides, Kelthane W (conc. 0.0250 a.i.%;
0.0500 a.i.%; 0.0650 a.i.%, Metasystox (0.0150 a.i.%; 0.0250 a.i.%; 0.0625 a.i.%) and Zineb WP (conc. 0.0975 a.i.%; 0.1300 a.i.%; 0.1625 a.i.%) has been carried out.
0.0625 a.i.%) and Zineb WP (conc. 0.0975 a.i.%; 0.1300 a.i.%; 0.1625 a.i.%) has been carried out.

Maize has been treated with Hg (MEMS 1.5% a.i.) and Hg (MEMS 1.5% a.i.) + Lindane (20% a.i.) pesticides.

All the results obtained for the absorption of P^{32} have been subjected to statistical analysis. They show that there are considerable differences in the effects of pesticides used upon the living processes of treated soya beans and maize. The degree of effect depends on the pesticide applied, its concentration, the kind or variety of plants investigated and the phases of growth and development.

If we could carry the experiments further by spectrophotometric, radiometric and other methods, including extraction of certain biological constitutents such as phospholipids, and by other chemical and biophysical studies I am sure a clearer picture of the effectiveness and quality of each individual pesticide would be obtained.

S.V. ANDREEV: Yes, I fully agree with you that at present specialists in pest control do not pay sufficient attention to the condition of the plant which is being protected. Many insecticides cause considerable physiological changes and damage to plant functions. In studying the influence of insecticides, therefore, it is necessary not only to study the effect on the insects but also on the plant itself, in order to be able to find the most appropriate methods and compounds.

H.J. BOROUGHS: Since it is known that injury to leaves may increase the absorption of viruses or nutrients, would you please explain how it is known that the increased absorption of phosphate in the presence of DDT is caused by interference with metabolism rather than just damage to the leaf substance?

S.V. ANDREEV: When chloro-organic insecticides are used against insect plant-pests in higher concentrations, the phosphorus uptake of the plant is damaged. With an increase in phosphate concentration, however, through the leaves, and not the roots, there is a sharp increase in phosphorus exchange, owing to the additional phosphorus. This considerably lowers the toxic effect of the chloro-organic insecticides which penetrate the plant and disrupt the course of its normal physiological processes. It is particularly important to apply these insecticides simultaneously under unfavourable conditions of plant growth. In our experiments the epidermis of the leaves was undamaged and the experiments were conducted only on normal plants.

E.E. TURTLE: I was interested in the reference to the use of a labelling technique to assess the amount of secondary damage to wild birds and mammals during the rodent control campaign. You used barium fluoracetate as your rodent control agent, but what did you use to label it?

S.V. ANDREEV: Yes, we used oats poisoned with barium fluoracetate and labelled with radioactive phosphorus. The presence of radioisotopes in the poisoned bait made it possible to determine accurately how much

poisoned bait was eaten by game animals and birds. If they died, it made it possible to determine the real causes, under field conditions, owing to the presence of radioactivity in the body.

We found what percentage of birds were taking the bait. By shooting them, we were able to establish that it was very small. We also found in certain cases that poisoned bait intended for rodents was in fact eaten by steppe antelopes, though the doses lethal for rodents were not lethal for antelope.

M. FRIED: I should like to ask three questions with regard to the grain disinfestation programme you mentioned.

Firstly, what is the efficiency of utilization of the radiation from the Co^{60} source and does all the grain receive the dose you referred to, i.e. 7000 r?

Secondly, can this method be applied to grain moving at 200 or more tons per hour, which is the present rate of movement in commercial plant operations?

Thirdly, have you any experience of using electronic accelerators to accomplish the same purpose?

S.V. ANDREEV: Yes, as I said, doses of 7 000 - 10 000 r ensure an overall activity of 60 kg Ra equiv.; to make sure of sterilizing all the insects we give 10 000 r. Uniformity of grain irradiation is obtained by stirring it up when it is moving forward, close to the rod source, by means of spiral planes inside the grain-pipe. The rod-type source ensures that the activity is used 100% because it is completely surrounded by moving grain. If we used a slot-type or a flat source, and the grain were to pass on one side of it only, we would have only 50% efficiency.

In reply to your second question, the gamma disinfestation plant is designed for a thoughput of 30 t/h. In general it operates on leguminous plants (peas, haricot beans, etc.) and is used to rid them of Acanthoscelides obtectus Say, Bruchus pisorum L. etc., though in principle the same kind of plant could be used to get rid of storage pests in grain. The principle remains the same. The capacity of the plant could be expanded by enlarging it, increasing the power source and lengthening the grain run.

As to the last question, electronic accelerators have not been used in our institute, though possibly they are being used in other institutes.

P. de PIETRI TONELLI: If I heard correctly, you said that an insecticide can influence the physiology of plants. Are you talking about phytotoxicity, cuticular phenomena, as you mentioned with regard to DDT and organo-phosphate absorption, or real internal physiological alterations? If so, may I have more details about this influence?

S.V. ANDREEV: In our work on the study of the effect of chloro-organic insecticides on physiological processes, we had in view mainly the effect produced by the penetration of toxic molecules on metabolism in plants, and in particular on phosphorus metabolism. We did not investigate the microscopic changes in the leaves, since the insecticide doses mentioned were within standard limits and a distinct change was observed at these amounts as well as inhibition of the uptake of phosphates by the plant from the soil.

C.H. SCHMIDT: You have told us that the possibility of using the sterile-male technique is being actively studied in your country. Have any

of these investigations reached the field stage and if so, with what insects?

S. V. ANDREEV: Research has begun on sterilization doses for such insect pests as Chloridea obsoleta F., Chortophila brassicae B., Leptinotarsa decemlineata and Pectinophora malvella Hb. We are also trying to determine the required ratio of sterilized individuals to the normal, as found in nature.

Research is also being carried out on polygamous and monogamous females and on the effectiveness of applying the method in these and other cases. In the forthcoming season it is intended to carry out a test of the sterilization technique and to use it against the Colorado potato beetle in its local habitat, i.e. in places where the potato fields are surrounded by woods or hilly country. Experiments will be conducted in isolated insectaries in field conditions in order to clarify the effect of various sterilization procedures on males and females. The fundamental difficulties of using this technique against agricultural pests include the difficulty of obtaining enough insects under artificial conditions and the limited number of generations obtainable from plant pests.