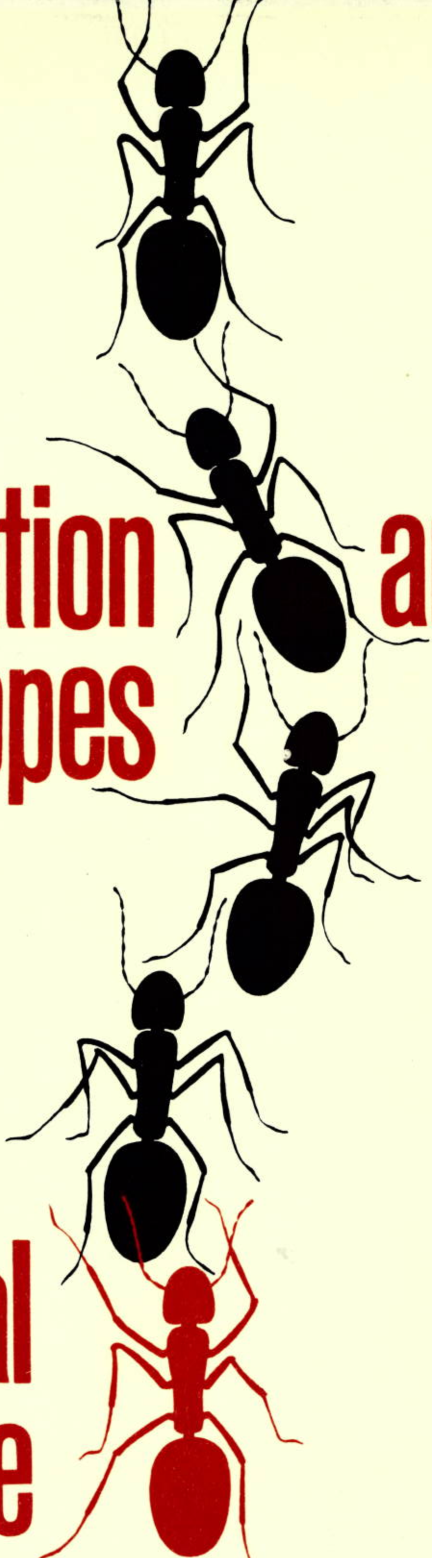
PROCEEDINGS OF A SYMPOSIUM,
ATHENS,
22-26 APRIL 1963
JOINTLY ORGANIZED
BY THE
IAEA AND FAO

Radiation and Radioisotopes Applied to Insects of Agricultural Importance



INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1963

RADIATION AND RADIOISOTOPES APPLIED TO INSECTS OF AGRICULTURAL IMPORTANCE

PROCEEDINGS SERIES

RADIATION AND RADIOISOTOPES APPLIED TO INSECTS OF AGRICULTURAL IMPORTANCE

PROCEEDINGS OF THE SYMPOSIUM ON THE
USE AND APPLICATION OF RADIOISOTOPES AND RADIATION
IN THE CONTROL OF PLANT AND ANIMAL INSECT PESTS
JOINTLY ORGANIZED BY THE
INTERNATIONAL ATOMIC ENERGY AGENCY
AND THE FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS
AND HELD IN ATHENS, 22 - 26 APRIL 1963

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1963

RADIATION AND RADIOISOTOPES APPLIED TO INSECTS
OF AGRICULTURAL IMPORTANCE, IAEA, VIENNA, 1963
STI/PUB/74
Printed by the IAEA in Austria
September 1963

FOREWORD

Since the pioneer work of the United States Department of Agriculture in the application of radiation and radioisotopes in the control of insect pests to cattle, many countries and organizations have pursued the advantages which might be gained in this field. Two years ago the IAEA organized the first international symposium in Bombay to study this problem, since when a considerable amount of basic research on the application of nuclear science in entomology and insect pest control has been undertaken. The potential gain of these studies, which would be in the form of an increased output of better food, is obvious to all Governments; hence the extensive international interest in the subject of this present Symposium, which was attended by 100 participants from 26 countries and 5 international organizations.

The proceedings consist of 37 papers presented by experts from 10 countries, together with a record of the discussions, and cover the use of radioisotopes in the study of the ecology of insects, such as their dispersal, migration and life-cycle. The application of radioisotopes to insecticides covers such subjects as labelling, application, uptake, translocation, metabolism, mode of action, and the determination of residues in plants and animals. The present position on the effects of radiation on insects is dealt with, including mutation, sterilization and the use of the sterile-male technique for the control and eradication of insect pests, and the need is emphasized for integration of chemical, biological, radiation and other methods of insect control.

The emphasis of this Symposium has been mainly on aspects of crop protection and it is hoped that the next symposium will also deal with aspects of livestock protection.

The sponsoring organizations wish to express their gratitude to the Government of Greece for its invitation to hold the Symposium in Athens, for its active assistance in the preparation and organization of the meeting, and for its generous hospitality to both participants and secretariat.

Appreciation is also due to Dr. E. Horber, of the Swiss Federal Experimental Station for Agriculture, Zürich-Oerlikon, who kindly assisted in the preparation of the Introduction to these proceedings and in the scientific editing of the discussion records.

EDITORIAL NOTE

The papers and discussions incorporated in the Proceedings published by the International Atomic Energy Agency are edited by the Agency's editorial staff to the extent considered necessary for the reader's assistance. The views expressed and the general style adopted remain, however, the responsibility of the named authors or participants.

For the sake of speed of publication the present Proceedings have been printed by composition typing and photo-offset lithography. Within the limitations imposed by this method, every effort has been made to maintain a high editorial standard; in particular, the units and symbols employed are to the fullest practicable extent those standardized or recommended by the competent international scientific bodies.

The affiliations of authors are those given at the time of nomination.

The use in these Proceedings of particular designations of countries or territories does not imply any judgment by the Agency as to the legal status of such countries or territories, of their authorities and institutions or of the delimitation of their boundaries.

The mention of specific companies or of their products or brand-names does not imply any endorsement or recommendation on the part of the International Atomic Energy Agency.

CONTENTS

INTRODUCTION

The contributions of the atomic sciences to entomological research are broad and continue to amaze and attract scientists interested in agricultural, silvicultural, industrial and medical entomology. Entomological research is an appropriate showcase to demonstrate the ever-widening range of the versatility of the atom's inherent possiblities.

A great deal of atomic energy research is dedicated to basic problems concerned not so much with immediate practical benefits, but with the development of techniques or materials which may be applied subsequently. Such exploratory studies have in many cases advanced to the stage of field tests. Break-throughs in one sector have to be considered against the increasing flow of background information in most other sectors which, through accretion, solidify the foundations for more striking progress in the future. This is the accepted procedure even for the more conventional and long-established branches of science.

From the papers presented and discussed at the Symposium at Athens it is evident, however, that the different fields of entomological research have shared unequally in the benefits of atomic energy.

Insect ecology depended on tracer application to study food exchange in social insects such as bees, wasps, ants and termites. Isotopes were also used to assess insect plant relationships quantitatively or to study the distribution of insecticides in a forest ecosystem. To trace the migration of aphids, grasshoppers, thrips, the Colorado potato beetle and soil insects, or the dispersal of the adult olive fly and the Mediterranean fruit fly, these insects were tagged with radioisotopes. The behaviour and biology of cotton insects, the Senn pest and malaria-carrying mosquitoes were observed with similar techniques. Yet in this field of application atomic energy research might profitably be intensified.

Isotopes have contributed to a high degree to the fast progress in insect physiology and insecticide research. The techniques of labelled insecticide and fungicide studies have been elaborated in order to study the contamination of insects by insecticide solutions or to calibrate dosage of minute amounts of labelled residual insecticides on test surfaces. Radioautography may be appreciated as a delicate tool of high resolution power to trace the radioisotopically tagged insecticides down to the cellular level if applied properly and evaluated conscientiously.

The increased emphasis given to insecticide and fungicide toxicology and residues is reflected in the widespread use of labelled compounds. They were used to follow the fate of insecticides and fungicides to determine the residues in plants and animals, to characterize surface deposits, areas of concentration and the diffusion and degradation of systemic insecticides in plants.

Insect metabolism, as well as insecticide toxicology, has felt the impact of isotope techniques, as shown by the key role played by radiotracers in studies on carbamate insecticides, other systemic and residual insecticides,

as well as on the metabolism and utilization of sterols in the house fly, tyrosine metabolism in the blowfly and tegumentary pigmentation in Orthoptera.

Irradiation of insects has received wide attention in all aspects, from the fundamentals of genetics, through the different approaches of exploratory development to technology and successful application to limited areas or to country-wide insect-pest eradication programmes. Radiation studies on specific effects have been carried out on male and female germ cells in Diptera, Hymenoptera, Coleoptera and Hemiptera in order to measure variations in response and sensitivity to radiation at different stages of oogenesis and spermatogenesis. The principles and applications of the irradiated-male technique were demonstrated on a wide variety of insects and other arthropods. Among the Diptera studied are a horn fly, the Mediterranean fruit fly, the melon fly, the Mexican fruit fly, the olive fly, the oriental fruit fly, several species of mosquito, the screw-worm and the stable fly. In Coleoptera, data are now available on the boll weevil, the Colorado potato beetle, the cowpea weevil, the grain-borer, the Mexican bean beetle, the rice weevil, the rust-red flour beetle, the tomato lady beetle and a white grub. Among the Lepidoptera were considered the European corn-borer, the gypsy moth, the pink bollworm and among other arthropods the Lone Star tick and a scorpion. Great efforts are directed towards the control of the olive fly. Mass-rearing techniques need to be elaborated before it will be possible to release sufficient numbers of irradiated males.

Insect pests of stored commodities are a very important problem, especially in sub-tropical and tropical countries; this was reflected in the number of contributions in that area of research. Putting into practice the irradiation of foodstuffs, e.g. the irradiation of grain to render it insect-free, depends not only on acquiring the necessary basic radiobiological knowledge and on advances in irradiation technology, but also on health and safety considerations.

The harrowing question of whether arthropods are able to build up resistance to radiation as they do to insecticides remains to be answered.

An attempt has been made by the sponsoring organizations to assemble representative experts at regular intervals to review progress in the field. The papers selected for the Athens Symposium varied greatly in scope, approach and level of contribution, but this too is representative of the stages reached in the various aspects of such research.

It has been our aim to reduce the time-lag between the meeting and the publishing of the Proceedings to a minimum. Only then can the Proceedings be a valid tool of communication enhancing or catalysing progress instead of being relegated to a cemetery for forgotten facts or obsolete ideas. The Symposium reported here should be regarded as a milestone marking a successful research period and staking out the directions in which more effort has to be concentrated before measurable progress can be expected. It can then be assumed that the Athens Symposium of 1963 is serving its purpose as fully as the previous one held in Bombay in 1960*.

* Proceedings published as Radioisotopes and Radiation in Entomology, IAEA, Vienna (1962) 307 pp.

I.

INSECT ECOLOGY: TRACER APPLICATIONS

QUELQUES EMPLOIS DES RADIOÉLÉMENTS ET DES RAYONNEMENTS EN ENTOMOLOGIE

G. COURTOIS
CENTRE D'ÉTUDES NUCLÉAIRES DE SACLAY,
ET
J. LECOMTE
INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE,
STATION DE RECHERCHES SUR L'ABEILLE ET LES INSECTES SOCIAUX,
BURES-SUR-YVETTE, FRANCE

Abstract — Résumé — Аннотация — Resumen

SOME USES OF RADIOISOTOPES AND RADIATIONS IN ENTOMOLOGY. The paper reviews the applications of radioisotopes in entomology that have been developed at our two centres during the last few years.

Early work (Au^{198}-labelling) related to the bee and more particularly to the radius of dispersion of worker bees from a colony. After investigations on the individual dose received in tagging of this kind, the radioresistance of the bee was determined, the lethal dose being estimated at about 90 kr. Au^{198} was also used to study exchange of food within a bee-hive. On the other hand, P^{32} was used for studies of exchange of food, in small hives, between individuals of different functions (males, workers and queens) or different colonies. Similar trophallaxic studies have recently been performed on wasps.

Au^{198} was likewise the basic radioisotope used in work on ant's nests. The most interesting finding from one of the early studies was that exchange of food takes place between nests more than 50 m apart and belonging to different species (Formica rufa and Formica polyctena). A later study, in which an ant run and not the nest itself was labelled, revealed a division of responsibility within the nest: the tagged ants were found invariably to explore the same run and to have little contact with other individuals of the same colony. In the same experiment abnormal radioactivity was noted in the ants before labelling, due in particular to $(Sr+Nb)^{95}$. This discovery would seem to point to accumulation of radioactive fall-out in ant's nests. At a period of low fall-out, natural radioactivity attributed to K^{40} was observed and was used for purposes of potassium determination in ants and bees.

An attempt was made to label acridians with Ir^{192} and the findings are described in the paper.

Lastly, an autoradiographic study has been made of the distribution of certain radioisotopes (P^{32} and S^{35}) in the body of the bee.

QUELQUES EMPLOIS DES RADIOÉLÉMENTS ET DES RAYONNEMENTS EN ENTOMOLOGIE. Le mémoire passe en revue les applications des radioéléments en entomologie qui ont été mises au point au CEN et à l'INRA durant ces dernières années.

Les premiers travaux concernèrent l'abeille, plus particulièrement la dispersion des butineuses en provenance d'une colonie; l'étude a été réalisée par des marquages à ^{198}Au. Suite à ces considérations sur la dose reçue par l'individu dans de tels marquages, la radiorésistance de l'abeille a été déterminée et la dose létale estimée à 90 kr environ. ^{198}Au a également servi à étudier les échanges de nourriture à l'intérieur d'une ruche. Par contre, c'est ^{32}P qui fut utilisé pour des études d'échanges de nourriture à l'intérieur de ruchettes entre individus de fonctions (mâles, ouvrières, reines) ou de colonies différentes. Des études analogues de trophallaxis ont récemment été faites sur des guêpes.

^{198}Au a été également le radioélément de base de travaux sur les fourmilières. Le résultat le plus intéressant d'une première étude a été la découverte d'échanges de nourriture entre fourmilières distantes de plus de 50 m et d'espèces différentes (Formica rufa et Formica polyctena). Dans une deuxième étude, par marquage d'un chemin de fourmis et non de la fourmilière elle-même, on a mis en évidence une division des responsabilités à l'intérieur de la fourmilière, les fourmis marquées prospectant toujours le même chemin et n'ayant que peu d'échanges avec les autres individus de la même colonie. Dans cette même expérience, on a constaté avant tout marquage une radioactivité anormale des fourmis, due notamment à $^{95}(Sr + Nb)$. Cette découverte aurait tendance à montrer un amassage des retombées radioactives dans les fourmilières. En période

de faibles retombées, une radioactivité naturelle attribuée au ^{40}K avait été constatée et avait servi à faire un dosage du potassium dans les fourmis et dans les abeilles.

Un essai de marquage d'acridiens à ^{192}Ir a été fait et les résultats obtenus sont décrits dans le mémoire.

Enfin, une étude de la répartition de certains radioisotopes (^{32}P, ^{35}S) dans le corps de l'abeille au moyen de la méthode autoradiographique a été effectuée.

НЕКОТОРЫЕ ВИДЫ ПРИМЕНЕНИЯ РАДИОЭЛЕМЕНТОВ И ОБЛУЧЕНИЯ В ЭНТОМОЛОГИИ. Статья содержит обзор методов применения радиоэлементов в энтомологии, разработанных в наших двух центрах в течение последних лет.

Первые работы относились к пчелам и в особенности к дальности полетов пчел-сборщиц из пчелосемьи. Исследование проводилось с помощью мечения изотопом Au^{198}. Продолжением работ по определению дозы, полученной особью в процессе мечения, явилось определение радиорезистентности пчелы и летальной дозы, лежащей в пределах 90 000 р. Au^{198} был также использован для исследования обмена пищей внутри улья. Вместе с тем, для изучения обмена пищей внутри ульев между рабочими особями (мужскими особями, рабочими пчелами, матками) или между различными роями применялся P^{32}. Недавно аналогичные исследования трофилаксии были проведены на осах.

Au^{198} был также основным радиоэлементом в работе с муравейниками. Наиболее интересным результатом первого исследования явилось открытие обмена пищей между муравейниками, отстоящими друг от друга на расстоянии свыше 50 м, и разными видами муравьев (Formica rufa и Formica polyctena). В процессе второго исследования, с помощью мечения пути движения муравьев, а не самого муравейника, было выявлено распределение обязанностей внутри муравейника. Меченые муравьи следовали по одному и тому же маршруту и редко обменивались с другими особями той же колонии. В процессе этого опыта уже до мечения была установлена аномальная радиоактивность муравьев, обусловленная прежде всего наличием $(Sr + Nb)^{95}$. Это открытие говорит о тенденции к накоплению в муравейниках радиоактивных вещества из осадков. В период слабых осадков определяли естественную радиоактивность, связанную с наличием K^{40}, и ее использовали для определения содержания калия в организме муравьев и пчел.

Была предпринята попытка мечения саранчовых насекомых. Приводятся полученные результаты.

Наконец, было проведено исследование распределения некоторых радиоизотопов (P^{32} и S^{35}) в организме пчел с помощью метода авторадиографии.

ALGUNAS APLICACIONES DE LOS RADIOELEMENTOS Y DE LAS RADIACIONES EN ENTOMOLOGÍA. La memoria reseña las aplicaciones de los radioelementos en entomología, ensayadas en los últimos años en los dos centros a que pertenecen los autores.

Los primeros trabajos se dedicaron a la abeja, estudiándose particularmente con marcaciones de ^{198}Au la dispersión de las colectoras provenientes de un enjambre. Tras considerar la dosis recibida por cada individuo como resultado de las marcaciones, se determinó la radiorresistencia de la abeja y se calculó que la dosis letal ascendía a unos 90 kr. También se utilizó el ^{198}Au para estudiar el intercambio de alimentos en el interior de una colmena. Ahora bien, para estudiar el intercambio de alimentos dentro de pequeñas colmenas entre individuos de función (machos, obreras, reinas) o enjambres diferentes se utilizó el ^{32}P. Recientemente se han hecho estudios análogos de trofalaxia sobre las avispas.

El ^{198}Au se ha empleado también como radioelemento básico para el estudio de hormigueros. El resultado más interesante del primer estudio efectuado fue el descubrimiento de un intercambio de alimentos entre hormigueros distantes más de 50 m y correspondientes a especies diferentes (Formica rufa y Formica polyctena). En otro estudio, se marcó un camino de hormigas en lugar del hormiguero y se pudo comprobar que en el interior de éste existe una división de funciones, pues las hormigas marcadas exploraban siempre el mismo camino y tenían muy escasos contactos con los demás individuos de la colonia. En este mismo experimento, antes de proceder a la marcación se observó en las hormigas una radiactividad anormal, debida en particular al $^{95}(Sr + Nb)$. Esto parece demostrar que las precipitaciones radiactivas se acumulan en los hormigueros. En período de precipitaciones escasas se había podido observar una radiactividad natural imputable al ^{40}K, que sirvió para dosificar el potasio en las hormigas y en las abejas.

Los autores han ensayado la marcación de acrídidos con ^{192}Ir y describen en la memoria los resultados obtenidos.

Se ha hecho también un estudio de la distribución de ciertos radioisótopos (^{32}P y ^{35}S) en el cuerpo de la abeja por medio del método autorradiográfico.

Le présent article passe en revue les applications des radioéléments dans l'écologie et la biologie des insectes, développées dans nos deux centres ou avec leur collaboration, durant ces dernières années.

I. ÉTUDE SUR LES ABEILLES

1. Etude du secteur de butinage [1, 2, 3]

La première étude réalisée a été celle du rayon d'action et de la dispersion des abeilles butineuses en provenance d'une ruche. Cette étude, commencée dès 1958, a été effectuée grâce à un marquage total de l'ensemble de la population de la colonie, soit environ 40 000 individus. Différentes considérations de caractéristiques radioactives, de nocivité et de facilité d'assimilation nous ont fait choisir comme traceur, incorporé à la nourriture, le ^{198}Au (période: 2,7 j), émetteur entre autre d'un gamma de 0,411 MeV.

L'activité d'environ 1 μc par individu a été choisie en fonction de la période biologique de ^{198}Au (2 à 3 j) et des hétérogénéités de marquage d'une abeille à l'autre. Ainsi une quarantaine de millicuries de ^{198}Au, permet-il de faire le marquage d'une colonie entière dont chaque individu peut être repéré pendant 4 à 5 j après début du marquage. Les échanges de nourriture entre insectes aidant, au bout d'un temps variable, mais toujours inférieur à 48 h, la quasi totalité des abeilles possède une activité suffisante pour permettre la détection dans la nature. Dans le secteur de butinage, la détection s'effectue à l'aide d'un détecteur portatif à scintillation (cristal NaI(Tl) 1"1/2 × 1"), chaque insecte étant préalablement attrapé à l'aide d'un filet à papillon à mailles fines. Ce procédé permet l'examen d'insectes posés dans des endroits inaccessibles à la sonde détectrice, branches hautes des arbres par exemple, et donne loisir d'examiner attentivement les cas douteux.

Le terrain expérimental toujours le même comportait un rucher de trente colonies parmi lesquelles était choisie celle marquée, huit autres ruches se trouvant dans un rayon d'environ 1 km.

En 1958, 17 butineuses marquées furent retrouvées sur 1027 examinées; en 1960, le pourcentage fut de 81 butineuses marquées sur 2442 ouvrières attrapées. On constate que dans les conditions de l'expérience, aucune butineuse ne dépasse sensiblement 1 km de distance, le gros de la troupe se trouvant à l'intérieur d'un cercle de 600 m de diamètre (figure 1 et tableau I). D'autre part, l'hétérogénéité de la distribution dans l'espace des butineuses marquées est très frappante. Ainsi, nous avons pu trouver un champ de crépis où la proportion d'abeilles marquées était de 33%. Un certain nombre de facteurs conditionnant le choix des secteurs de butinage a pu ainsi être mis en évidence tel que l'effet répulsif du relief et celui des grandes étendues stériles.

En 1959, un marquage similaire fut tenté, mais avec des conditions météorologiques mauvaises, abondantes chutes de pluie et température assez basse. Malgré une forte miellée de robiniers et bien que le sirop de sucre ait été absorbé plus rapidement que dans les autres cas, les pourcentages de butineuses marquées furent loin d'être aussi importants et ne

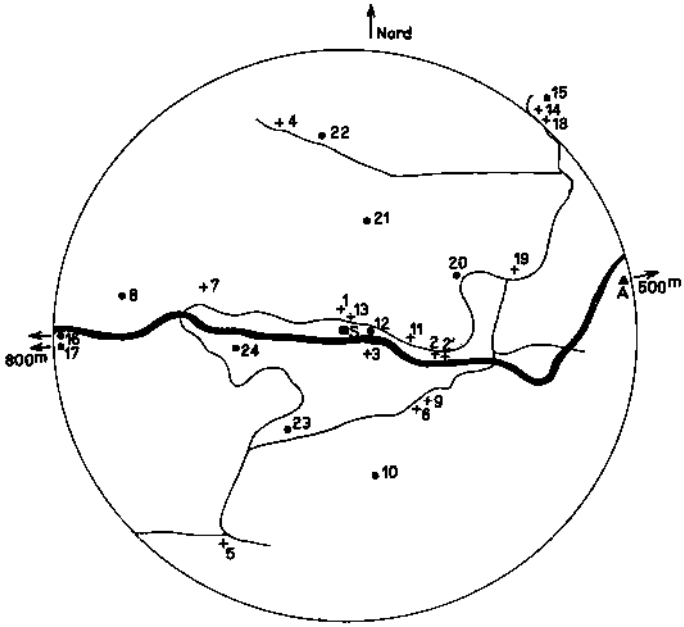


Figure 1

Etude du secteur de butinage. *

- Rivière
- Route
- \+ Abeilles marquées
- ● Pas d'abeilles marquées
- ▲ Fleurs sans abeilles
- ■ Emplacement de la ruche
- S Source
- ϕ Diamètre 1000 m

dépassèrent pas 70%, l'activité individuelle étant d'ailleurs plus faible. Une seule abeille active put être retrouvée sur le secteur de butinage.

2. Résistance au rayonnement gamma de l'abeille ouvrière [4]

Lors de tels marquages, nous nous sommes inquiétés de connaître les doses reçues par abeille et l'influence que pourraient avoir de telles doses sur le comportement des individus. Un calcul approximatif, considérant que l'émission β de 1 MeV de ^{198}Au est dissipé dans un individu, montre que la dose maximum reçue par une abeille marquée par 1 μc de ^{198}Au est de 600 r.

Nous avons donc soumis des ouvrières à l' action croissante du rayonnement du ^{60}Co(450 c de ^{60}Co fournissant une dose d'environ 100 000r/h sur un volume de 500 cm^{3}).

Les abeilles ainsi irradiées furent comparées à deux cagettes de témoins. Aucun effet décelable n'apparaît en-dessous de 18 000 r, au moins

* Les chiffres renvoient au tableau I.

TABLEAU I *

ÉTUDE DU SECTEUR DE BUTINAGE

Prélève-ments	Plantes visitées	Abeilles capturées	Abeilles marquées
1	Ciste (Cistus albidus)	125	5
2	Crepis sp.	100	33
2'	Mélilot (Melilotus officinalis)	100	8
3	Sainfoin (Onobrychis sativa)	212	3
	Ciste - Thyn (Thymus sp.)		
4	Moutarde (Synapis arvensis)	101	1
5	Renoncule (Ranunculus sp.)	121	1
6	Acacia	150	1
7	Ciste	108	7
8	Renoncule, Vesce, Moutarde	3	0
9	Ciste	100	10
10	Cistes (C. albidus et Monspelliencis)	100	0
11	Acacia	103	6
12	Acacia	75	0
13	Thym	56	2
14	Thym	57	1
15	Vesce (Vicia sativa)	122	0
16	Sainfoin	105	0
17	Thym	105	0
18	Ciste	125	2
19	Ciste	49	3
20	Ciste	40	0
21	Ciste	101	0
22	Crepis, Mélilot, Ciste	100	0
23	Thym	29	0
24	Sainfoin	111	0

* Voir figure 1.

au cours des huit premiers jours, soit à des doses bien supérieures à celles reçues lors d'expériences par traceurs radioactifs. Entre 18 000 et 70 000 r, les dommages subis semblent dépendre des conditions physiologiques des individus, les abeilles les plus âgées semblant plus sensibles au rayonnement.

La dose létale (mort de la moitié des individus) semble être atteinte vers 90 000 r, 200 000 r étant la première dose qui entraîne la mort immédiate de tous les insectes.

3. Echange de nourriture à l'intérieur d'une ruche [5, 6]

A l'intérieur d'une ruche expérimentale d'observation formée d'un cadre unique et ayant des parois transparentes, une certaine quantité de ^{198}Au a été fournie à cinq à huit butineuses. La paroi de cette ruche étant munie d'un quadrillage de repère, une prospection a été continuellement faite après le nourrissement. L'examen a été fait à l'aide d'un détecteur à scintillation, sans collimateur, l'expérience ayant montré que la mesure était peu affectée par le rayonnement des zones voisines.

Contrairement à ce qu'on aurait pu attendre, les échanges de nourriture ne se font pas à partir du nourrisseur vers le centre de la grappe d'abeilles, mais il apparaît de manière certaine que les ouvrières, après avoir rempli leur jabot de miel radioactif, se dirigent rapidement vers le centre de la grappe, point privilégié à partir duquel s'effectue par la suite la presque totalité des échanges et la diffusion de la nourriture à travers l'ensemble de la population. Ce point correspond au centre thermique de la ruche où pendant la période d'hivernage se tient généralement la reine. Il fut également constaté que, contrairement à ce qu'on obtenait en période active en été, le nombre d'individus ayant ingurgité de la nourriture active était faible, soit 15 à 20% des individus. Il est vrai que la radioactivité avait été initialement fournie à un nombre très restreint d'insectes.

Sur ces bases, Mlle SALLERON [6] a étudié les échanges de nourriture entre individus de castes (reines, butineuses, mâles, etc.) ou de colonies différentes. Le travail a été effectué en ruchettes expérimentales, adossées l'une à l'autre et munies de trous permettant l'échange de nourriture sans autoriser le passage d'un insecte d'une ruchette à l'autre. Dans ce cas, les abeilles n'ayant pas accès à l'extérieur, le traceur employé fut le ^{32}P, émetteur β pur, sous forme de phosphate monosodique. Il fut incorporé à une nourriture solide à base de miel et de sucre préférable pour l'entretien des abeilles élevées en cagettes. Les comptages furent effectués soit sur des insectes morts soit sur des insectes vivants à l'aide d'un compteur G-M cloche de 1,5 mg/cm² de paroi. Trois questions furent étudiées à l'aide de cette technique:

1° Echange entre ouvrières

De par les études faites, il n'est pas apparu évident, contrairement à l'opinion déjà émise, que des ouvrières préfèrent échanger de la nourriture avec des ouvrières de la même colonie plutôt qu'avec des étrangères en provenance de ruche différente. Si cette tendance existe, elle est faible. Il semble que les échanges s'effectuent plus volontiers avec les abeilles

sœurs pendant les deux premiers jours, pour s'inverser ensuite et se faire plutôt avec les étrangères. Peut-être les nourrices moins généreuses avec elles au début les ont-elles obligées à demander davantage?

2° Echange entre mâles et ouvrières

Ce travail a montré sans ambiguïté que les mâles sont capables dans une certaine mesure de se nourrir seuls mais qu'en présence d'ouvrières, ils préfèrent se faire nourrir par ces dernières. D'autre part, il y a transfert de radioactivité des mâles vers les ouvrières, ce transfert pouvant être dû dans certains cas à l'absorption par les ouvrières des régurgitations des mâles déposés sur le substrat, dans d'autres cas à un échange direct de nourriture, échange qui a d'ailleurs pu être directement observé.

3° Echange entre reines et ouvrières

L'existence d'un transfert de radioactivité des reines aux ouvrières paraît maintenant une chose certaine. D'autre part, ces échanges ne sont le fait que d'un très petit nombre d'abeilles spécialisées même au sein de colonies expérimentales restreintes composées d'une vingtaine d'ouvrières.

4. Répartitions de certains radioéléments dans le corps de l'abeille.

Par la méthode autoradiographique, on s'est proposé de localiser les radioéléments dans le corps de l'abeille. Un certain temps après ingurgitation du traceur, les abeilles sont placées à −35° et incluses dans une solution de gelose. L'insecte est ensuite coupé en totalité au moyen d'une scie circulaire très fine; sur la coupe ainsi obtenue on place le film dont l'exposition a lieu à −35°.

Cette technique a été utilisée pour le ^{32}P sous forme de phosphate monosodique et pour le ^{35}S sous forme de $^{35}SO_4\ H_2$, introduits tous deux dans de la nourriture solide.

Le rayonnement trop énergique du ^{32}P ne permit pas l'obtention de bons clichés comme par exemple celui de la figure 2 qui montre la localisation du ^{35}S.

II. ÉTUDE SUR LES FOURMIS

Les résultats obtenus à l'aide des radioéléments dans l'étude des abeilles nous ont incités à l'utiliser pour d'autres insectes sociaux et notamment pour les foumis.

1. Transmission d'isotopes radioactifs entre deux fourmilières d'espèces différentes [7] .

Chauvin étudiant depuis six ans de nombreuses fourmilières d'un certain bois, le terrain nous apparu propice à l'étude de la transmission de nourriture entre fourmilière à l'aide de traceurs radioactifs.

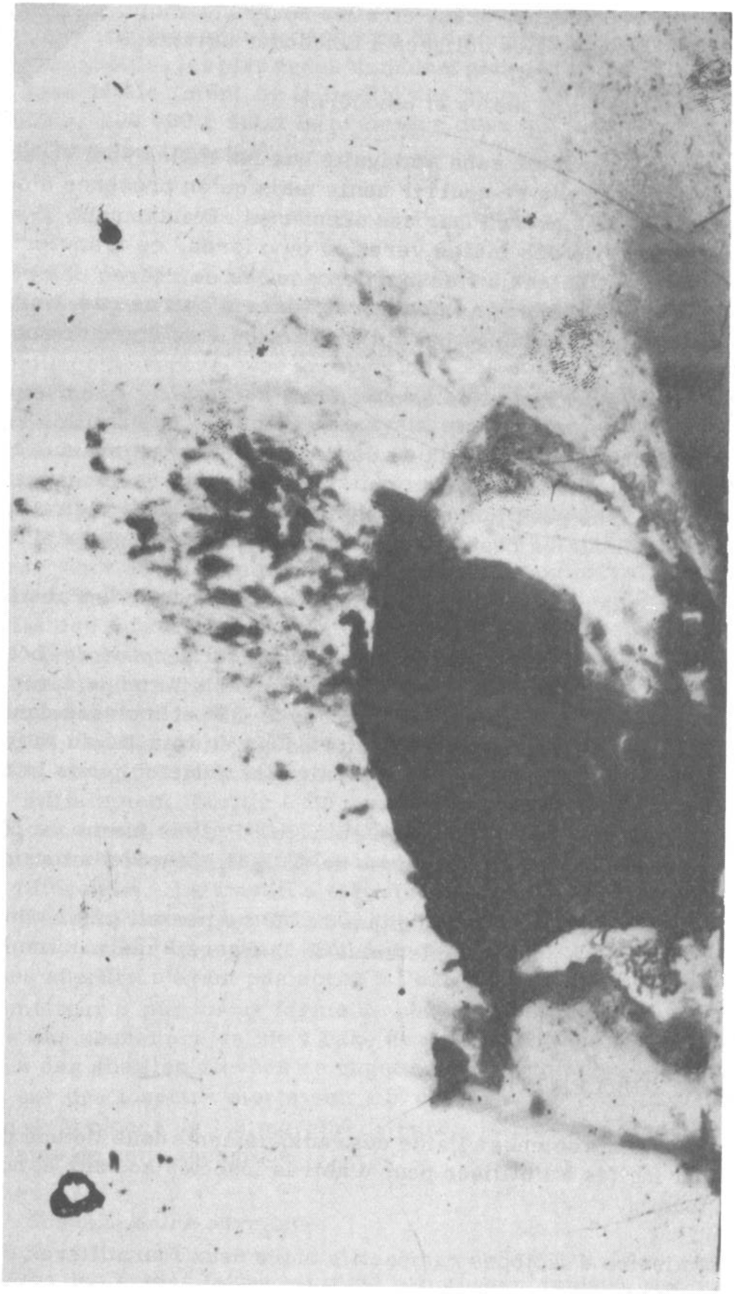


Figure 2

Autoradiographie d'une abeille après absorption de ^{35}S.

Une grosse fourmilière de Formica polyctena fut marquée, un soir, en versant sur elle 50 mc de ^{198}Au mélangées à 50 cm^3 d'une solution sucrée épaisse. Si l'on considère cette colonie comme peuplée d'un million d'individus et si l'activité initiale se répartit entre toutes les fourmis, nous arrivons à une activité de 0,05 μc par insecte très largement décelable même par le moins sensible des détecteurs dont nous disposions: un détecteur portatif à scintillation et un ensemble de comptage à scintillation à poste fixe, muni d'un cristal creux 1"3/4 × 2".

Le lendemain matin, le comptage de prélèvements de fourmis sur les différentes pistes partant de la fourmilière marquée donnent des résultats variables, l'activité des individus du milieu des pistes étant toujours plus grande que celle des extrémités. Ces inégalités s'atténuent d'ailleurs avec le temps mais restent cependant notables. On constate sur une fourmi particulièrement radioactive que l'activité est interne, l'abdomen portant 99,9% de l'activité totale.

Le résultat le plus intéressant obtenu lors de ces essais a été la découverte d'une certaine activité dans trois fourmilières d'une espèce voisine, Formica rufa, situées à une ciquantaine de mètres du nid des polyctena. Nous avons pu exclure l'hypothèse que les rufa se contaminaient en traversant les pistes de polyctena car ces pistes elles-mêmes sont inactives, ensuite la population de polyctena qui s'y déplace est si dense qu'on ne voit pas la possibilité pour une fourmi d'une autre espèce de la traverser sans se faire houspiller d'importance. Reste alors des attaques possibles des polyctena par les rufa qui pourraient les dévorer et emporteraient ainsi dans leurs colonies des matières radioactives. Mais nous n'avons pu constater ces attaques dans la nature et d'autre part, les auteurs généralement s'accordent à admettre que c'est polyctena, l'espèce la plus agressive, qui attaquerait rufa. Peut-être aussi les rufa pourraient-elles emporter des matériaux de construction souillés des excréments radioactifs de polyctena; nous sommes dans l'incapacité d'infirmer cette dernière hypothèse que nous considérons pourtant comme assez improbable. On pourrait penser aussi que les rufa fréquenteraient les mêmes pins que polyctena et lècheraient les mêmes pucerons pour en obtenir du miellat, mais nous n'avons pu, jusqu'ici, mettre en évidence des pistes de rufa se dirigeant vers les pins de polyctena et nous n'avons jamais vu de rufa sur le tronc de ces pins; nous pensons donc qu'il faut rejeter l'hypothèse d'une régurgitation de matières radioactives auprès des pucerons qui seraient ensuite absorbés par les ouvrières de rufa. Il semble donc assez probable qu'il y ait échange de nourriture entre des fourmis d'espèce différente appartenant à des fourmilières voisines.

2. Echanges de nourritures entre individus de la même fourmilière [8]

Durant l'été dernier, un nouveau marquage a été réalisé en plaçant la nourriture non plus sur la foumilière, mais au milieu d'une piste de butinage à environ 50 m du nid.

Les comptages effectués sur des prélèvements de 50 fourmis ont toujours montré une très nette supériorité du taux de comptage pour les prélèvements effectués sur la piste initialement marquée (voir fig. 3) et les

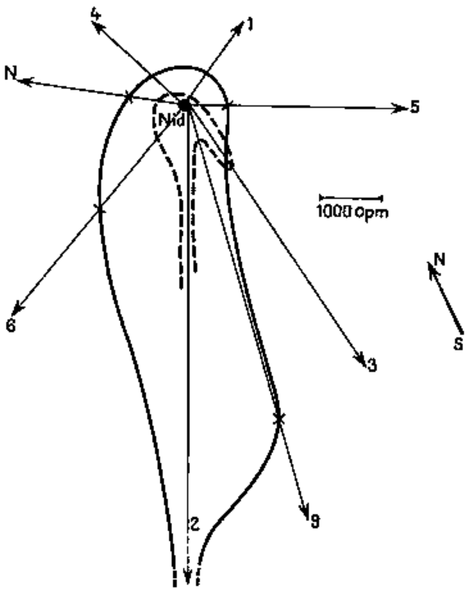


Figure 3

Représentation schématique des pistes rayonnant autour du nid.
Sur chaque piste, on trace un vecteur proportionnel à la moyenne des cpm pour 50 fourmis. Les deux tracés obtenus en réunissant l'extrémité distale de ces vecteurs correspondent à la distribution quantitative de la radioactivité pour deux journées de mesure. Le marquage a été effectué sur la piste 2. Le vecteur de la piste 2 devrait, pour la journée du 25, se prolonger de 92,5 et, pour la journée du 26, de 130 fois la valeur du ségment symbolisant 1000 cpm

- - - Journée du 25
—— Journée du 26

échanges de nourriture restent très faibles entre ouvrières travaillant sur des pistes différentes. On observe cependant une augmentation générale des taux de comptage d'un jour à l'autre et la transmission de nourriture est lente, mais certaine, et intéresse l'ensemble de la population de la fourmilière.

Il semble donc possible de supposer l'existence à l'intérieur de la fourmilière de groupes de travail ayant relativement peu de contact pendant les périodes de butinages. Le fait que cette coupure soit moins forte entre les chemins voisins peut tenir au fait que les groupes de travail butinant dans des secteurs voisins occupent dans la fourmilière un emplacement généralement voisin, soit être due à ce qu'un certain nombre de butineuses peuvent changer de lieu de travail en le faisant toutefois dans des limites relativement étroites.

3. Teneur en potassium des fourmis et des abeilles

Lors des expériences précédemment décrites, nous avions constaté que le bruit de fond relevé au scintillomètre portatif sur les fourmilières était légèrement supérieur de l'ordre de 1 choc/s au bruit de fond ambiant.

Des prélèvements effectués ont été analysés et comptés au détecteur à bas bruit de fond (ensemble Philips à couronne d'anticoïncidence, détecteur G-M à circulation de bruit de fond 0,8 cpm environ).

La radioactivité trouvée a été attribué au ^{40}K. Elle correspondrait à une teneur d'environ 1% en poids de potassium dans la fourmi.

Une étude analogue chez l'abeille conduit à une teneur de 1,5% en poids dans l'abeille préalablement desséchée.

4. Les fourmis indicateurs possibles de retombées radioactives [9].

Cependant lors d'une dernière expérience, fin juillet 1962, nous constatons avant tout marquage une différence de bruit de fond entre fourmilière et ambiance de 25 chocs/s très visible avec le détecteur portatif et ceci sur toutes les fourmilières sans exception. La radioactivité se trouve dans les fourmis et non dans les matériaux constitutifs de la colonie, car une fourmilière désertée depuis un mois est la seule à ne pas présenter ce phénomène.

Un prélèvement de 880 g de fourmis est suivi d'un examen à l'aide d'un spectromètre gamma 60 canaux. Le spectre obtenu (fig. 4) présente trois pics:

- un pic à 780 keV ± 20 keV attribuable à la filiation $^{95}(Zr + Nb)$ (période: 65 j);
- un pic aux alentours de 140 keV attribuable au ^{141}Ce (période: 33 j);
- un léger pic vers 500 keV attribuable au ^{103}Ru (période: 40 j).

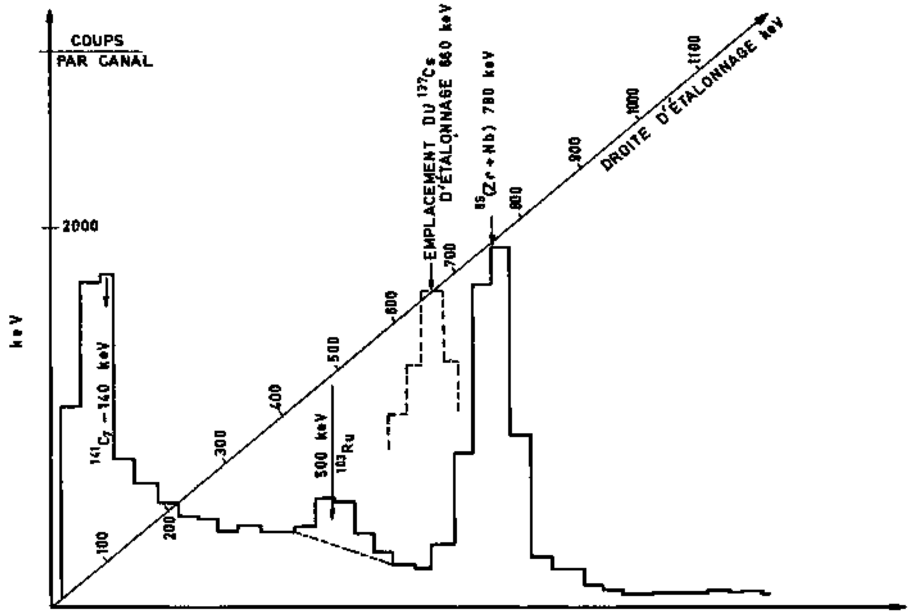


Figure 4

Spectre d'un prélèvement de fourmis en juillet 1962.
880 g de fourmis. Stockage 15 min. Bruit de fond déduit.

Une évaluation de l'activité due au $^{95}(Zr + Nb)$ donnait $3,5 \cdot 10^{-9}$ c/kg de fourmis.

Le mécanisme par lequel les fourmis fixent les retombées paraît en liaison directe avec leur habitude de prélever les excréments sucrés des pucerons des arbres, ou miellat. Les multiples gouttelettes gluantes ainsi exposées à l'air doivent capter une très grande quantité de poussières atmosphériques et, par conséquent, celle des retombées. D'ailleurs, les gouttelettes de miellat qui tombent sur les feuilles sont récoltées aussi par les abeilles, et l'on sait depuis longtemps que le miellat d'abeilles est bien plus riche en poussières que les miels floraux (où les nectaires sont bien abrités en général, ou même au fond de cavités étroites). Il est possible aussi et même probable que la résine des conifères fixe aussi ces poussières, et que les fourmis en entraînent quelque peu au cours de leurs innombrables marches et contremarches, sur les troncs. D'autre part, le fait que les fourmilières abandonnées ne montrent pas d'activité supérieure au bruit de fond, même quand l'abandon est très récent (c'était le cas dans notre premier prélèvement) prouve que les fourmis ne défèquent pas à l'intérieur de la fourmilière: on le pensait depuis longtemps, mais nous croyons que, pour la première fois, la démonstration en est apportée dans la nature.

Quant à l'importance des fourmis comme détecteurs des retombées, elle reste à évaluer, par rapport aux méthodes traditionnelles de comptage du bruit de fond sur échantillons de végétaux prélevés au hasard. Nous pensons qu'il serait nécessaire pour évaluer la sensibilité de la méthode par les fourmis, de suivre comparativement l'histoire d'une retombée dans la fourmilière, par comparaison avec les méthodes classiques.

III. ÉTUDES SUR LES ACRIDIENS MIGRATEURS*

A la suite d'un problème posé par M. Descamps du Centre de défense des cultures, nous avons regardé les possibilités de marquage radioactif d'un grand nombre d'acridiens (jusqu'à 500 000) pendant une période suffisamment longue de deux à trois mois pour faire une étude d'un secteur de grégarisation au Niger.

Le problème posé conduisait à prendre un radioélément de période radioactive et biologique suffisante. Les essais en laboratoire ont été effectués avec de ^{192}Ir sous forme d'une solution de chloroiridate d'ammonium dispersée sur de jeunes feuilles de blé servant de nourriture aux lots d'insectes étudiés. Chaque lot était constitué de 100 insectes d'âge différent d'un lot à l'autre (fig. 5).

Les résultats obtenus peuvent être divisés en éléments favorables et défavorables à un marquage de grande envergure.

1. Eléments favorables

Période apparente satisfaisante: La période apparente est de l'ordre de six jours indépendamment de l'âge des insectes; cependant la dispersion

* Etudes effectuées en collaboration avec M. Descamps, Centre de défense de cultures.

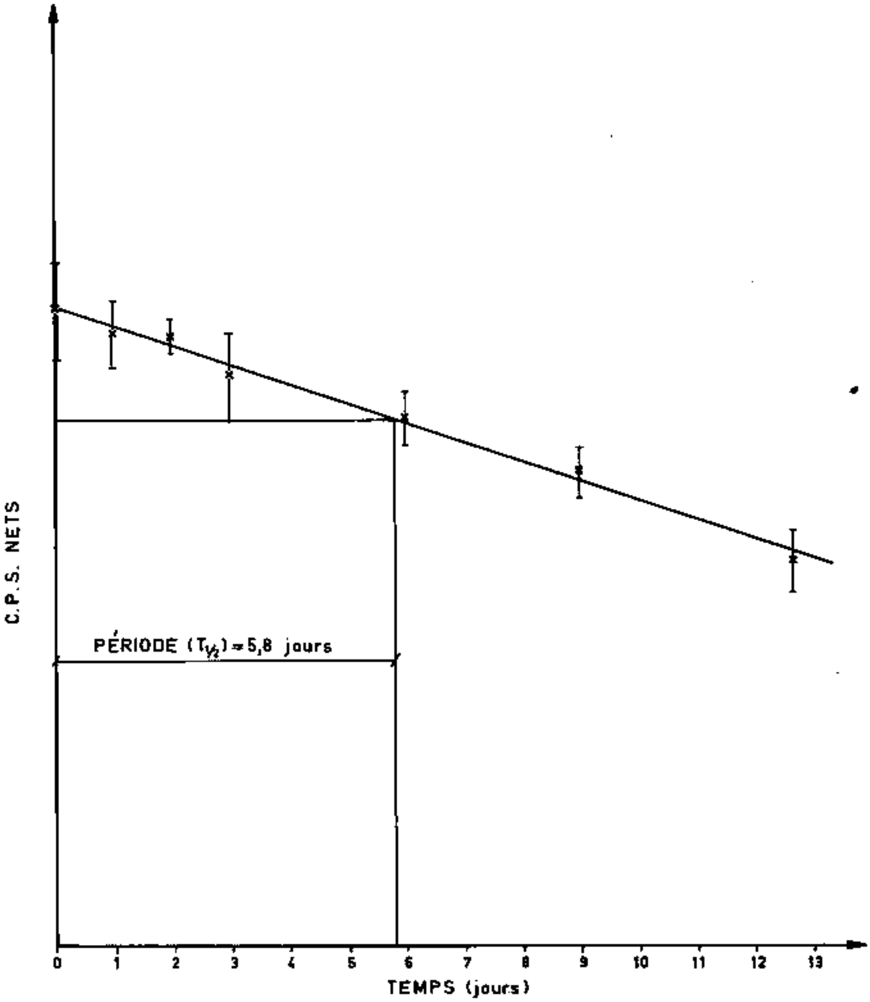


Figure 5

Période biologique de ^{192}Ir chez la sauterelle.

des résultats semble plus élevée pour les insectes les plus jeunes. D'autre part, suivant les experts, le métabolisme étant moins important dans la nature, il se pourrait que dans un marquage dans la nature, la période apparente soit encore plus importante (fig. 6).

Comportement satisfaisant des acridiens marqués: Il n'y eut pendant la période étudiée, ni mortalité ni changement de comportement notable.

Dispersion raisonnable de l'activité absorbée: Il y eut peu de sauterelles non marquées et aucune ayant absorbé 10 fois plus que la moyenne.

Individus non contaminants en dehors des excréments: Plus de la moitié de l'activité reste localisée dans la tête.

Pas de diminution de l'activité au passage à l'état adulte.

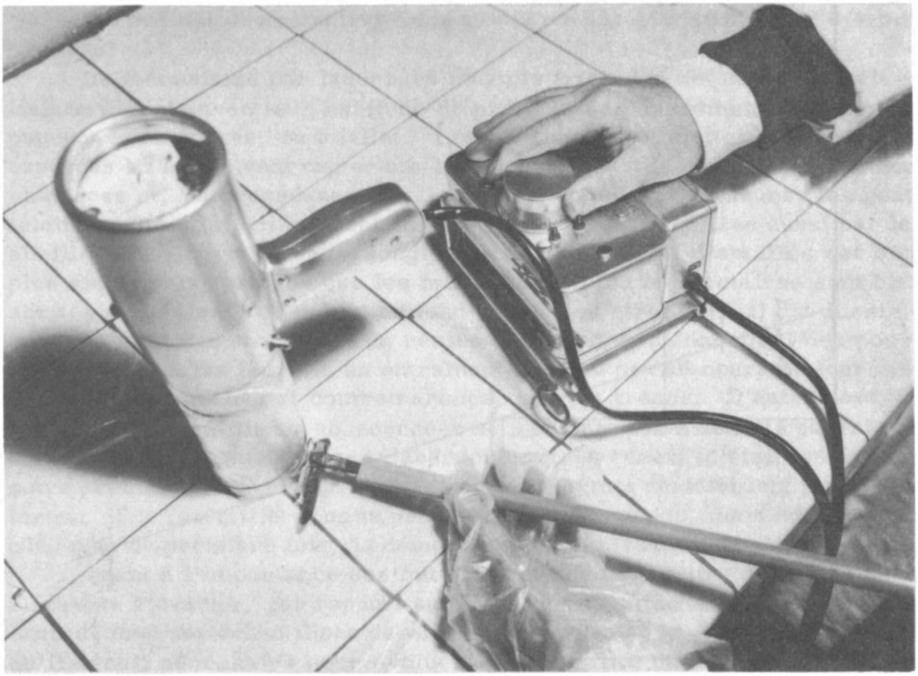


Figure 6

Marquage expérimental de sauterelles.

2. Eléments défavorables

Rendement du marquage faible: A peine 10% du traceur mis en œuvre semble avoir été absorbé par les insectes. Ceci est dû en partie à la mauvaise utilisation du traceur sur le blé. Ceci peut être, pense-t-on, fortement pallié par l'emploi de son, qui absorbe mieux le liquide traceur, comme nourriture marquée.

L'activité totale utilisée est élevée: Avec les détecteurs actuellement à notre disposition et le rendement de marquage précédent, il faudrait utiliser une trentaine de curies de ^{192}Ir pour le marquage de 500 000 individus, ce qui semble excessif. Encore faut-il noter qu'avec une telle activité, au bout de deux mois un insecte ne serait décelable qu'au contact même de l'appareil (au lieu de 50 cm réclamés par le demandeur).

Pour ces raisons entre autres, le marquage au Niger n'a pas eu lieu.

IV. ÉTUDES SUR LES GUÊPES*

Une étude assez semblable à celle entreprise avec les abeilles et concernant les échanges trophallactiques a été effectuée sur les guêpes Para-

* Etudes effectuées en collaboration avec M. Montagner, Faculté des sciences de Nancy.

vespula vulgaris, germanica et Dolichovespula media. Une dizaine d'individus ayant été nourris avec de l'or radioactif ont été re-introduits dans le guépier et nous ont servi à étudier la vitesse de transmission de la nourriture marquée dans le nid. Ces vitesses sont en général très grandes.

Ces études ont permis de dégager les principaux aspects des relations interindividuelles au cours des échanges alimentaires et d'éclaircir les rapports entre couvain et adultes.

1. Echanges entre ouvrières

Cette étude a été effectuée dans des cagettes à 3 compartiments, dont celui du milieu contenait seul la nourriture marquée; il était séparé des deux autres par une cloison perforée en plexiglass. Nous avons essentiellement montré que les ouvrières du compartiment central approvisionnent beaucoup mieux leurs sœurs que les étrangères. Ainsi, nous avons établi, pour deux séries d'expériences, que les pourcentages d'activité des ouvrières sans nourriture, rapportée à celle des nourrices du compartiment central, étaient, après 24 heures, de 45,1 et 43% pour les sœurs et de 19,4 et 22% pour les étrangères. D'autres expériences effectuées dans le même sens ont toujours confirmé ces résultats. Mais cette discrimination nutritionnelle disparaît avec le temps et n'est plus nettement décelable après 2 jours de vie commune.

2. Alimentation des mâles – leurs rapports avec les autres membres de la société

Les capacités de «self-alimentation» des mâles dépendent non seulement de leur âge, mais aussi du degré d'évolution du nid. Ainsi, les jeunes sont parfaitement capables de bien s'alimenter tout seuls, mais les adultes semblent refuser la prise de nourriture à une époque qui coïncide avec l'apparition des fondatrices filles ou la décroissance de l'activité sociale. Il semble donc qu'il se produise un arrêt dans l'alimentation des mâles, au moment des fécondations.

En outre, ils sont peu alimentés par les ouvrières. Nous avons montré que, dans le nid, ils se nourrissent essentiellement en provoquant les régurgitations des larves. Il semble donc que dans le nid les individus mâles vivent en parasites sur ces régurgitations larvaires, qu'ils savent obtenir facilement.

3. Les rélations alimentaires entre les ouvrières et le couvain

Les ouvrières nourrissent les larves en fonction de leur taille et de l'intégrité de leurs cellules. Lorsque des larves d'ouvrières et de fondatrices sont élevées en compétition, ces dernières sont toujours approvisionnées de façon plus abondante. Ajoutée à d'autres résultats cette constatation nous incline de plus en plus à penser que la quantité de nourriture donnée aux larves doit jouer un rôle essentiel dans leur différenciation en deux castes femelles.

V. ÉTUDE DE RÉPARTITION D'UN FONGICIDE*

Bien que ce travail sorte du cadre imposé par le titre de cet article, nous en dirons quelques mots car il s'inscrit dans le programme de ce colloque.

Dans les plantations de bananiers, des huiles minérales sont utilisées en tant que fongicide sous forme de diffusion lente de quelques centimètres cubes en un point déterminé de la plante. L'emploi d'une huile fongicide marquée au tritium a permis d'en étudier la distribution à l'intérieur du bananier, la vitesse d'écoulement dans différents tissus, et de comparer l'efficacité de différents modes d'injection.

La simple dissolution d'un élément marqué dans une huile n'a pas été utilisée car le métabolisme de l'élément dissous peut être très différent de celui de l'huile elle-même. La solution adoptée a donc été la fixation de tritium sur les doubles liaisons du squalène pour obtenir une huile comparable aux huiles minérales.

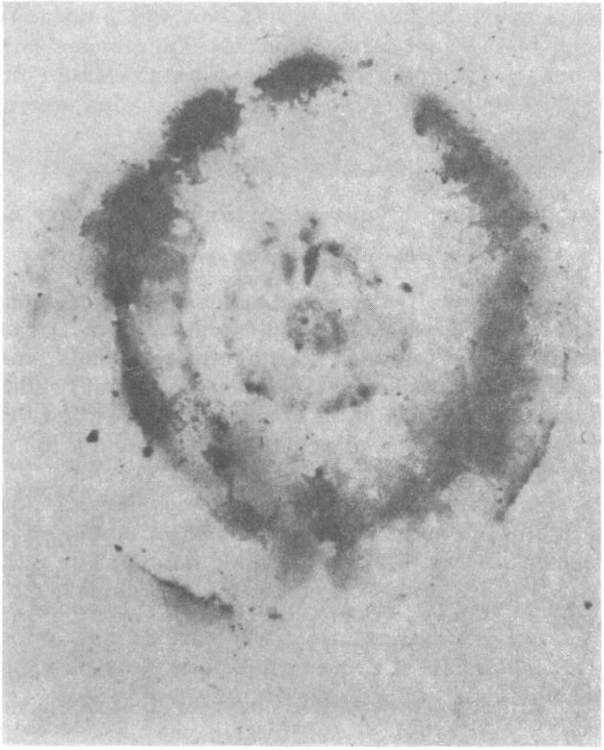


Figure 7

Autoradiographie d'une hampe de bananier marqué au squalène tritié.

---

* Etudes effectuées en collaboration avec MM. Cuille et Laville, Institut français de recherches fruitières d'Outre-Mer [10].

Après une étude en laboratoire démontrant la validité de la méthode proposée, une plantation de 30 bananiers, située à la Guadeloupe, a été traitée à raison de 50 mc de squalène tritié par arbre. On a fait varier les conditions d'injection (activité spécifique de l'huile, emplacement, durée et date de l'injection) et le traitement des arbres.

Périodiquement, des échantillons ont été prélevés sur les arbres dans différentes régions (limbes et nervures des feuilles, pseudotronc, bulbe et racine). Ces échantillons ont été brûlés, l'eau de combustion récupérée et comptée par scintillation liquide. L'activité de l'eau de la terre avoisinante a également été mesurée.

Simultanément, en laboratoire, de jeunes pousses ont été marquées à forte activité spécifique (250 mc/cm^3) et les diffusions étudiées par autoradiographie après congélation des échantillons dans l'azote liquide (fig. 7).

Les résultats obtenus montrent notamment que l'huile séjourne assez longtemps à l'endroit d'injection, puis se déplace préférentiellement vers les feuilles et les fruits, et qu'une faible fraction traverse le bulbe avant d'être éliminée par les racines.

RÉFÉRENCES

[1] COURTOIS, G. et LECOMTE, J., C. R. Acad. Sci. 247 (1958) 147-149.
[2] COURTOIS, G. et LECOMTE, J., Int. J. appl. Rad. and Isotopes, 5 (1959) 265-268.
[3] LECOMTE, J., Ann. de l'abeille 4 (1960) 317-327.
[4] COURTOIS, G. et LECOMTE, J., Rapport CEA n° 1377.
[5] COURTOIS, G., LECOMTE, J. et SALLERON, F., C. R. Acad. Sci. 252 (1961) 1057-1059.
[6] SALLERON, F. (à paraître dans les Annales de l'abeille).
[7] CHAUVIN, R., COURTOIS, G. et LECOMTE, J., Insectes sociaux VIII, 2 (1961) 99-107.
[8] COURTOIS, G. et LECOMTE, J., Insectes sociaux IX, 4 (1962).
[9] CHAUVIN, R., COURTOIS, G. et ANGUENOT, F., C. R. Acad. Sci. 256 (1963) 508-511.
[10] COURTOIS, G., CUILLE, J. et al., Fruits 17,7 (1962).

DISCUSSION

W. KLOFT: Your paper deals with work similar to what we have been doing ourselves since 1956, and it is very interesting to hear that you have also found that honey-bee drones are able to regurgitate crop food to workers. We first published our findings on this subject in 1958, but since they were not generally accepted, we repeated the study last year, using tracers and film analysis, and we found quite definitely that the drones are able to regurgitate. One great source of error lies in the distribution of radioactive drone excrement in the honey-bee community: as you know, drones can excrete their food two hours after intake and their radioactive excrement is taken up by the workers and spread through the colony very quickly. By using a special experimental set-up, however, we were able to exclude this source of error.

At the suggestion of Mr. Lecomte, the results of the experiments we carried out last year will be submitted to the journal Annales de l'abeille with a view to publication.

J. LECOMTE: I was most interested to learn that you had continued your work on this particular problem. The fact that similar phenomena have been observed in a different laboratory confirms our findings.

M. FERON: I was very interested in what the authors of this paper had to say on food exchange in social insects, and I think the same methods could also be applied to non-social insects. In the case of Diptera, the process of food exchange by frequent regurgitations is well known and the part it plays in the spread of pathogenic germs may be of importance both to man and animals and to the insects themselves. Perhaps this labelling method could provide useful information regarding such phenomena.

J. LECOMTE: That is true. However, the tracer study should then be coupled with other observations such as film analysis, designed to verify the absorption of regurgitated material and to eliminate the possibility of contamination from other sources.

R. CAVALLORO: In investigating the mechanism by which ants fix radioactive fall-out, did you measure the radioactive spectrum of the honeydew of aphids?

J. LECOMTE: No, but this has been done in Freiburg in Germany, using the honeydew taken up by bees from aphids.

R. CAVALLORO: What species of Acrididae did you use in your experiments?

J. LECOMTE: The Locusta migratoria.

W. KLOFT: We too have done field experiments on food distribution in ant colonies, especially in Formica polyctena and related species, and we obtained similar results with a spread of activity up to 200 m. However, in our field experiments on natural colonies of Formica polyctena with interspersed nests of Formica rufa, the Formica rufa did not take part in the exchange. I know this does not conflict with your own results, because we have done a lot of laboratory experiments which prove that there is good exchange between different species of the genus Formica. The explanation is to be found in the complex conditions existing in the field: relations between nests are influenced by the saturation of single nests, isolation, the distances involved, etc.

J. LECOMTE: We were indeed surprised at the exchange of food between different colonies of ants, but the determination of species was carried out in your own laboratories and I do not think we can question the results.

W. KLOFT: I did the sample analysis myself and the results are undoubtedly correct. Regarding your interpretation of the way in which ants have concentrated radioactive fall-out, I should like to say that I have done a lot of work on trophobiosis and have demonstrated that ants usually take food directly from the anus of aphids. Only in rare cases do they take honeydew from leaves, whereas the honey-bees have no direct relations with the aphids and always take the honeydew from leaves. I think, therefore, that in the case of predacious ant species the concentration of this fall-out may be due to the ingestion of leaf-eating insects.

J. LECOMTE: To reach some definite conclusions on this point, one would have to study in the same area and at the same time not only the radioactivity of the ants and bees but also that of the aphids, caterpillars, leaves,

etc. Our supposition is perhaps not correct, but we felt that honeydew taken from leaves probably plays an important role. The radioactivity spectrum of the honeydew collected by the honey-bee is very similar to what we observed in ants.

G.T. SCARASCIA: Have you any experimental evidence regarding the possibility that the resin of conifers may fix radioactive fall-out? Work carried out in Italy by the Agricultural Chemistry Station (Ministry of Agriculture) and by the National Commission for Nuclear Energy has shown no difference between the fall-out radioactivity of pines and that of other plants.

J. LECOMTE: We have no experimental evidence on the role of the resins. Our observations were limited to honeydew only, in view of the fact that resin is not collected by ants or bees.

J. HALBERSTADT: I noted in the paper that there is a difference in uptake of Au^{198} and Ir^{192} by bees, ants and grasshoppers. I also understand that there is a big difference in the location of these isotopes in the different insects after uptake. Could you give an explanation of these differences? Perhaps it is because gold is used in the form of a colloidal solution while iridium is used as a suspension and is therefore taken up poorly.

J. LECOMTE: The form in which the radioisotope is administered will certainly have some effect on the fixation rate and the biological half-life. We did not try to find out where the gold and the iridium are localized, but it appears that gold is mainly found in the walls of the digestive tract. We were interested in gold because of its ease of use, and we obtained good results with the colloidal form without appreciable difficulty. It was not necessary for us to try to improve the rate of fixation. As regards iridium, we have so far done only preliminary experiments. We have not gone very far in this field yet, and possibly by varying the method of administration we could improve the rate of fixation and the biological half-life, but this has not been studied as yet.

TRACER EXPERIMENTS ON FOOD EXCHANGE IN ANTS AND TERMITES

K. GÖSSWALD AND W. KLOFT
INSTITUTE OF APPLIED ZOOLOGY, UNIVERSITY OF WÜRZBURG,
FEDERAL REPUBLIC OF GERMANY

Abstract — Résumé — Аннотация — Resumen

TRACER EXPERIMENTS ON FOOD EXCHANGE IN ANTS AND TERMITES. One of the most important aspects of social insects, such as ants, honey-bees and termites, is their tendency to trophallactic food exchanges, which can be studied well by the use of labelled food. In comparative studies we found the most rapid rate of exchange in honey-bees, but extreme differences were observed among the different subfamilies of ants, which must be taken in consideration in case of tracer field-experiments. The greatest tendency towards trophallactic exchanges was found in the subfamily Camponotinae. Ants of the genus Formica, especially Formica polyctena Först. and related polygenous and polycalous species known to be important factors in the preventive biological control of forest insect pests, were intensively analysed. It was found that the rate of food exchange within one nest is conditioned by temperature, time, number of individuals and saturation. Another question is whether food exchanges also take place between the different nests of ant-colonies of the same species. By labelling single nests with radioactive food we were able to find intensive food exchanges with different nests in the colony, up to distances of 200 m. We obtained similar results in three different colonies and in different years. The existence of such long-range food exchanges is very important for the estimation of the regulating effect on insect pests of useful Formica species, since these exchanges prevent the quick saturation of any nest through local mass-infestation of insects in their predating area. It was shown by the tracer experiments that the collected food flows in most of the surrounding nests of the ant-colony: thus colonies of those useful wood-ants act as a complex system with high ecological effectiveness.

Using labelled food, we studied in termites (Kalotermes flavicollis Fabr.) which stages and castes are capable of direct feeding or are receptors of stomodeally or proctodeally given trophallactic food. Pseudoworkers are most effective. We also attempted to use tracer methods to explain the greater longevity and greater aggressiveness of termites when in groups than as single individuals. Pseudoworkers of Kalotermes flavicollis were labelled with I^{131}. After feeding, some of these labelled termites were caged individually, while other groups were formed which also included unlabelled individuals. By measuring the effective half-life and calculating the biological half-life, which primarily depends on the rate of excretion, we found that the tracer remained longer in groups - when the group is viewed as one unit — than in isolated individuals. The result can be explained by the measured trophallactic exchanges of food and repeated circulation among individual members comprising a group. Similar results were obtained with two ant-species from different subfamilies. The greater economy in the use of food and other substances may be a contributory factor to the "group-effect" in social insects.

Special problems of comparable measuring techniques for living single individuals and insect groups are dealt with.

EMPLOI DES RADIOINDICATEURS DANS L'ÉTUDE DE LA TROPHALLAXIS CHEZ LES FOURMIS ET LES TERMITES. Un aspect particulièrement important de la vie des insectes sociaux comme les fourmis, les abeilles et les termites est la tendance à la trophallaxis, qu'il est facile d'étudier au moyen de nourriture marquée. Des études comparatives ont montré que la trophallaxis est le plus rapide chez les abeilles, mais qu'elle varie considérablement d'une sous-famille de fourmis à l'autre, facteur qui doit être pris en considération dans les expériences faites sur le terrain à l'aide de radioindicateurs. La plus forte tendance à la trophallaxis a été relevée chez les individus de la sous-famille Camponotinae. Les auteurs ont étudié tout particulièrement les fourmis du genus Formica, en particulier la Formica polyctena Först (fourmi rousse) et les espèces polygènes et pluridomes apparentées, qui représentent un facteur biologique important dans la lutte contre les insectes nuisibles aux forêts. Les auteurs ont observé que la trophallaxis à l'intérieur d'une fourmilière est conditionnée par la température, l'époque, le nombre d'individus et la saturation. Un autre problème est de savoir s'il y a également trophallaxis entre différentes fourmilières de colonies appartenant à la même

espèce. En introduisant de la nourriture marquée dans diverses fourmilières, les auteurs ont pu détecter des échanges intensifs entre des fourmilières séparées par des distances allant jusqu'à 200 m. Ils ont obtenu des résultats semblables pour trois colonies différentes, au cours de plusieurs années. Cette trophallaxis à grande distance a beaucoup d'importance pour l'évaluation du rôle des espèces Formica dans la lutte contre les insectes nuisibles; elle empêche toute fourmilière d'être rapidement saturée par l'infestation massive d'insectes dans la zone d'activité des fourmis: les expériences faites à l'aide de radioindicateurs montrent que la nourriture accumulée est échangée entre la plupart des fourmilières voisines. Ainsi, les colonies de fourmis rousses utiles constituent un complexe qui peut jouer un rôle important sur le plan écologique.

A l'aide de nourriture marquée, les auteurs ont étudié quelles formes et castes de termites (Kalotermes flavicollis Fabr.) interviennent par voie buccale ou anale dans la trophallaxis. Les résultats les plus intéressants ont été enregistrés chez les pseudo-ouvriers. Les auteurs se sont aussi efforcés d'étudier, à l'aide de radioindicateurs, les causes pour lesquelles la longévité est plus élevée et l'agressivité plus forte chez les termites en groupes que chez les termites isolés. Ils ont marqué des pseudo-ouvriers de Kalotermes flavicollis à l'iode-131. Après les avoir nourris, ils ont placé dans des cages individuelles un certain nombre des individus marqués et ils ont formé en même temps d'autres groupes qui comprenaient aussi des individus non marqués. En mesurant la période effective et en calculant la période biologique, qui dépend essentiellement du taux d'excrétion, ils ont constaté que le radioindicateur persistait plus longtemps chez les individus en groupes — le groupe étant considéré comme l'unité — que chez les individus isolés. Ce résultat peut être attribué à la trophallaxis et à la circulation répétée entre les individus du groupe. Les auteurs ont obtenu des résultats semblables pour deux espèces de fourmis appartenant à des sous-familles différentes. Il se peut qu'une plus grande économie dans l'utilisation de la nourriture et d'autres substances contribue à «l'effet de groupe» chez les insectes sociaux.

Les auteurs étudient les problèmes particuliers que pose la mise au point de méthodes de mesure comparables pour les insectes isolés et les groupes d'insectes.

ИЗУЧЕНИЕ С ПОМОЩЬЮ ИНДИКАТОРОВ ОБМЕНА ПИЩЕЙ У МУРАВЬЕВ И ТЕРМИТОВ. Самым важным для таких общественных насекомых, как муравьи, медоносные пчелы и термиты является их стремление к трофоллактическому обмену пищей, который может быть хорошо изучен с помощью меченной радиоактивными веществами пищи. При сравнительном изучении самая высокая скорость обмена была обнаружена у медоносных пчел, в то время как у различных подсемейств муравьев она была чрезвычайно разнообразной, что должно быть учтено при проведении полевых экспериментов с применением индикаторов. Самое сильное стремление к трофоллактическому обмену мы обнаружили у подсемейства Camponotinae. Усиленно изучались муравьи Genus Formica, особенно Formica polyctena (Först.), и родственные полигенные и polycalous виды, которые играют важную роль в предупредительной биологической борьбе с насекомыми-вредителями леса. Установлено, что скорость обмена пищей в одном муравейнике обусловлена температурой, временем, количеством особей и насыщением. Другой проблемой является вопрос о том, существует ли обмен пищей между различными муравейниками колонии муравьев одного вида. Пометив отдельные муравейники с помощью внесения радиоактивной пищи, мы смогли обнаружить интенсивный обмен пищей между различными муравейниками колонии, отстоящими друг от друга на расстояние до 200 м. Аналогичные результаты мы получили для трех различных колоний в различные годы. Существование такого дальнего обмена пищей является очень важным для оценки регулирующего действия полезных видов муравьев в отношении насекомых-вредителей. Дело в том, что обмен препятствует быстрому насыщению популяции любого муравейника вследствие местной массовой инвазии насекомых в их предыдущем гнезде. Как показали эксперименты с применением индикаторов, собранная пища попадает в большинство окружающих муравейников колонии муравьев. Поэтому колонии этих полезных лесных муравьев действуют как сложная система с высокой экологической эффективностью.

С помощью меченной радиоактивными веществами пищи мы изучили, какие категории и касты термитов (Kalotermes flavicollis Fabr.) способны к прямому питанию и какие питаются трофоллактической пищей, отрыгиваемой кормильцами изо рта в рот или получаемой из прямой кишки последних. Наиболее эффективными являются псевдорабочие. Мы также пытались объяснить с помощью индикаторных методов причины большей продолжительности жизни и большей агрессивности групп термитов по сравнению с отдельными изолированными особями. Псевдорабочие Kalotermes flavicollis метились J^{131}. После кормления некоторые из меченых термитов были изолированы поодиночке, а из других термитов образованы группы, включающие также немеченых особей. Измерив эффективный период полураспада и рассчитав биологический полупериод, который прежде всего зависит от скорости выделения, мы обнаружили, что индикатор дольше остается в группе (когда группа рассматривается как единое це-

лое), чем у изолированных особей. Это можно объяснить измеренным трофаллактическим обменом пищей и повторной циркуляцией среди отдельных особей, составляющих группу. Аналогичные результаты мы получили с двумя группами муравьев, относящимися к различным подсемействам. Большая экономия в потреблении пищи и других веществ может быть фактором, способствующим "групповым действиям" общественных насекомых.

Рассматриваются специальные проблемы методов сравнительных измерений живущих отдельно особей и групп насекомых.

EMPLEO DE INDICADORES RADIACTIVOS PARA ESTUDIAR LA TROFALAXIA EN LAS HORMIGAS Y LOS TERMITES. En los insectos sociales – hormigas, abejas y termites – es de suma importancia la tendencia a la trofalaxia, que puede estudiarse bien mediante alimentos marcados. Los autores de la memoria han podido comprobar en estudios comparativos que la trofalaxia es más rápida en las abejas, pero han observado diferencias considerables entre las diversas subfamilias de hormigas, lo que debe tenerse en cuenta cuando se efectúan experimentos con indicadores. La subfamilia Camponotinae es la que tiene una tendencia más marcada a la trofalaxia. Los autores estudiaron hormigas del Genus Formica, en particular la Formica polyctena Först, y otras especies poligénicas afines que desempeñan un importante papel en la lucha biológica preventiva contra las plagas de insectos forestales. La trofalaxia dentro de un nido está condicionada por la temperatura, el tiempo, el número de individuos y la saturación. Otro problema es el de saber si existe también trofalaxia entre diferente nidos de colonias de hormigas de la misma especie. Introduciendo en algunos nidos alimentos marcados, los autores observaron un considerable intercambio de alimentos entre los nidos de la colonia hasta 200 metros de distancia. Lograron resultados análogos en tres colonias diferentes y en años distintos. La trofalaxia a grandes distancias es un factor muy importante para evaluar la acción destructiva que ejercen las especies útiles de Formica sobre los insectos nocivos, ya que impide la rápida saturación de los nidos por una infestación local masiva de insectos en la zona de merodeo. Los experimentos con indicadores muestran que los alimentos acumulados pasan a la mayoría de los nidos vecinos de las colonias de hormigas. Por tanto, las colonias de la Formica rufa actúan como un sistema complejo de gran eficacia ecológica.

Con ayuda de alimentos marcados, los autores estudiaron en los termites (Kalotermes flavicollis Fabr.) qué fases y castas intervienen en la trofalaxia bucal o anal. Los seudoobreros son los más eficaces. Utilizando indicadores radiactivos, los autores trataron de descubrir por qué razón los termites en grupo son más agresivos y tienen mayor longevidad que los termites solitarios. Los seudoobreros fueron marcados con ^{131}I. Después de alimentarlos, algunos de estos termites marcados fueron encerrados en cajas por separado, y al mismo tiempo se formaron otros grupos que comprendían termites no marcados. Midiendo el período eficaz y calculando el período biológico, que depende ante todo del índice de excreción, los autores observaron que el indicador permanecía más tiempo en los individuos en grupo – considerando el grupo como una unidad – que en los individuos aislados. Este resultado puede explicarse por la trofalaxia y una circulación frecuente entre los individuos del grupo. Se obtuvieron resultados análogos con dos especies de hormigas de dos subfamilias distintas. La mayor economía en el empleo de alimentos y otras sustancias puede contribuir al «efecto de grupo» en los insectos sociales.

En la memoria se examinan algunos problemas especiales de las técnicas de medición comparables para grupos de insectos e insectos aislados.

INTRODUCTION

The exchange of food and other substances between individuals of the colony is one of the most important integrative mechanisms among social insects. Especially among the highly organized social insects forming insect-states, the labile equilibrium between one or a few reproductives and a very large number of non-reproductives possessing a more or less latent sexual potentiality (worker ants and honey-bees) or potentiality for development into reproductives (termite larvae) is maintained primarily through such mutual exchanges. Social food exchanges are among the preconditions of division of labour, of brood-rearing in the optimal ecological conditions of the nest,

and of any other development into higher forms of social life among insects. They may be also a means of communication between individuals. The study of these exchanges is not only of great scientific interest but also of practical interest, since many of the social insects have great economic importance in agriculture and forestry. The use of radioactive isotopes as tracers offers excellent possibilities for the basic study of these processes. In 1952, NIXON and RIBBANDS [1] introduced the tracer method into the study of food transmission within the honey-bee community; we followed in our laboratory with a series of papers from 1956 on concerning ants [2-15, 37-38], termites [4, 9, 16, 17, 18] and honey-bees [4, 9, 19]. The present paper outlines the results of our tracer studies with special reference to unpublished experiments on ants and termites.

A. FOOD EXCHANGE IN ANTS

1. Methods

In the experiments, P^{32} and I^{131} were used as tracers. They were administered to the ants in honey-water, saccharose solutions or pure water [20] at specific activities of 1 μc/ml up to 1 mc/ml, depending on the specific experimental purposes. The liquid food was offered on small glass dishes or the ants were individually supplied through glass pipettes, and the greatest possible care was taken to prevent external contamination. If necessary we decontaminated the radioactively fed ants that were to act as food donors by a special method, bathing them first in watery solutions of the inactive compound (sodium phosphate or sodium iodide) and then in pure water. They were dried quickly by letting them run about on layers of soft absorbent cellulose, which were changed several times. Measurements were carried out with G-M end-window counting tubes (window thickness 1.2-1-4 mg/cm^2) or with a scintillation counter, both in conjunction with electronic scalers and (if necessary) with automatic sample-changers.* Live ants were enclosed for counting in small measuring cages consisting of a ring of glass covered by a thin membrane. The diameter of these rings was adapted to the size of the ant-species and ensured the same geometrical arrangement in comparative studies.

2. Food exchange among worker ants

The most intensive studies were carried out with ants of the genus Formica, especially the Formica rufa group (F. rufa L., F. polyctena Foerst., F. pratensis Retz. (syn. F. nigricans Em.), F. cordieri Bondr.). If one freshly fed worker is placed as donor within a group of hungry individuals, the donor supports through direct feeding (regurgitation of crop-content) the major part of the food ingested up to 6-8 ☿☿ [2, 3, 7]. Between 7° and 35°C the percentage of transmitted crop activity is the same, but the time required depends on the temperature [12]. The primary recipients of food act as

* Made by Frieseke & Hoepfner, Erlangen

secondary donors and so on, and the food exchanges continue to produce a multilateral food chain. In this way up to 80 ☿☿ can take part of the content of one crop. With honey-bee workers we found such distributions from one crop-content only up to 50 individuals, since the individual food consumption
crop-content only up to 50 individuals, since the individual food consumption
(crop → midgut → resorption) is higher than in ants. The distribution rate is influenced by time, temperature and the number of individuals within the group. Under conditions of constant temperature, the temporal course of food distribution follows an exponential function, so that after some time all individuals, up to a maximum of about 80, have been reached. Food exchange starts at 5-7°C, reaches its highest frequency between 25 and 30°C and then falls until the limit of 45-50°C is attained [12]. This optimal temperature is identical with the temperature in the centre of the nest-hill. The greater the group the more individuals can take part in the distribution of radioactivity. By measuring not only the number of radioactive ants but also the activity rate of each individual, we find that at first the distribution is very heterogeneous: some individuals have very high activities while others show extremely low activities. Through often repeated secondary regurgitations, the distribution reaches after some time a statistical equalization, following a Gauss "normal distribution" (Fig. 1) [4, 6, 7]. Over a longer period

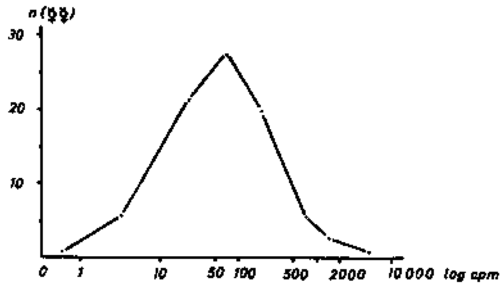


Fig. 1

Distribution of radioactive food within worker-groups of Formica polyctena Foerst.
The graph summarizes two experiments with groups of 50 workers (☿☿) each to which was given a single radioactive worker as food-donor for 20 h at 25°C. Counts per minute, showing the amount of ingested food, are plotted against number of individuals receiving a certain amount of this food.

of time, it is found that the "normal distribution" does not persist but gives way to a single-peak distribution with preference for progressively smaller exchange units. In this way each individual of the group or colony is assured of an adequate food supply. An increase in the number of donors results in a corresponding increase in the speed of distribution. We have not yet done such detailed experiments in honey-bee groups, but we can state that the individual food exchanges through regurgitation from proboscis to proboscis take place more quickly among bees than among ants.

In comparative tracer studies with more than 20 species of ant we found extreme differences between the four subfamilies available in our region and also between biological different species within the same subfamily. The

highest tendency to trophallactic exchanges was found in the subfamily Camponotinae (genera Camponotus, Formica, Lasius), although there is one species of this subfamily that has been shown in our laboratory to act only as receptor of labelled food [7, 13]: this is Polyergus rufescens Latr., the Amazon ant, which keeps in its nest workers of other species (G. Serviformica) as slaves responsible for food supply and distribution. While the workers of the Amazon ant are still able to ingest some food directly, other social-parasitic species have lost the capacity to do this, as has been proved by tracer techniques [7]. In tracer experiments with colonies of Epimyrma gösswaldi Men. living on a social-parasitic basis with colonies of the genus Leptothorax, it was possible to demonstrate a significant preference on the part of the parasitic ants for food supply through donors of the host ant (L. unifasciatus in our case). This result points the way towards one possible explanation for the process of selective evolution from normal independent ant species through social parasites of varying degrees up to the end-type, the workerless ant. Workers of the subfamily Monerinae have only very weak food exchanges among themselves; the individuals seem to be self-supporting. One species of Dolichoderimae that was studied showed relatively good food exchanges. Great differences were found among Myrmicinae; this agrees with the results of WILSON & EISHER [21], who carried out tracer studies with five American species. A high trophallactic tendency among workers seems, according to our experiments, to be correlated with trophobiotic habits. The demonstrated differences must be taken into consideration in field tracer experiments with ants. In the light of our experience we recommend that some distribution experiments should first be carried out among worker groups before starting with species not yet studied.

3. *Food distribution among different castes and stages*

Old queens, young queens before and after mating and males of F. polyctena feed directly on radioactive honey-water in amounts similar to those for workers. We also proved the capacity of sexuals to distribute food by regurgitation in the following experimental combinations: ♀♀$^{+}$; ♀♀, ♀♀$^{+}$; ♂♂, ♀♀$^{+}$; ☿☿, ♂♂$^{+}$; ♂♂, ♂♂$^{+}$; ♀♀ and ♂♂$^{+}$; ☿☿. In all cases the experiments were positive, the rate of distribution of ♀♀ being similar to that of workers, while that of males was smaller. The most notable observation was the capacity of Formica males for food distribution. We obtained similar results with Lasius and especially with Camponotus ligniperda ♂♂ [40]. These males remain for about nine months in the imaginal stage in their mother's nest and not only distribute food to other ♂♂, ♀♀ and ☿☿ but even transfer radioactivity to larvae. This trophallactic tendency is less marked when the overwintering and the subsequent mating phases are reached. Using tracer methods, we obtained similar results with the drones of honey-bees which are undoubtedly also able to regurgitate and distribute crop food [4, 9, 19]. A source of error are radioactive excrements which are fed and distributed through workers. Mated queens of Apis mellifica distribute radioactive crop substances among workers. In further experiments that we carried our with newly founded colonies of Lasius niger L., the queen showed a

decreasing tendency to supply larvae with radioactive food through herself after the first imaginal workers (pygmies) had hatched: The pygmean workers and the queen acted mutually as both donors and receptors of food. The larvae of the species Camponotus ligniperda L. and C. herculeanus L. have during the overwintering phase a growth diapause which under laboratory conditions is maintained even at relatively high temperatures. They do not normally receive food but at higher temperatures they were supplied with small amounts of labelled food [14].

In agreement with WILSON & EISNER [21], we often found that queens in relatively large groups or colonies showed no preference for the supply of radioactive crop food introduced through foraging ants. By the application of P^{32}, it was found that the reason was transfer of glandular substances. These substances cannot be labelled until the ingested radiophosphorus is assimilated throughout the body. This process begins 1-2 h after feeding and takes 24-48 h, depending on the temperature [4, 7].

In special experiments we were able to distinguish between primary crop food and transfer of glandular secretions [15]. Radioactively fed workers were isolated for 48 hr, after which they were allowed to empty their crop through repeated regurgitations. Then they were fed several times with inactive food solution which they were permitted to regurgitate to feed further hungry workers. By measuring these receptors we were able to determine the point at which the crop was completely cleared of the primary crop activity. After some time, especially in Spring, the crops of such workers acquired secondary radioactivity due to secretion of pharyngeal glands. Since these glands open into the pharynx and have no reservoirs of their own, their secretions are swallowed and may be stored in the crop, which thus acts as a kind of reservoir for the pharyngeal glands. This secondary crop content is regurgitated, especially during the early springtime, more or less pure as a special food for queens and for rearing the sexual brood. It seems to have caste-determining or differentiating effects on the female larvae [38]. After external foraging begins, the external food is mixed with gland secretions through repeated regurgitations and thus becomes more or less ameliorated before reaching brood or queens. Thus we have an explanation for the often very weak transfer of radioactivity to queen ants in colonies.

4. *Field experiments on food exchange among different nests of Formica colonies*

As shown in the previous sections we have intensively analysed, using tracer methods, the trophallactic exchanges of ants of the genus Formica, especially Formica polyctena Foerst. and related species. These ants, especially polygynous and polycalous species, are known to be important factors in the biological prevention and control of forest insect pests [22]. An important question is whether food exchanges also take place between the different nests of ant colonies of the same species. It is known that natural colonies of the polygynous F. polyctena develop by forming daughter nests. It is apparent from the occasional exchanges of brood, workers and queens that close relations at first continue to exist between mother-nests and daughter-nests. Later the nests seem to be more or less independent except

for periodical migrations between nests which are at present under investigation. Our special problem was to find out whether exchanges of food take place under normal conditions, i.e. in the absence of visible migrations and exchanges of population. The tracer method was first used for problems of this kind by KANNOWSKI [23] who employed labelled food to determine whether each of the numerous nest-mounds of the subterranean ant Lasius minutus Emery in a swamp was an independent unit or not. He found that usually four or five neighbouring mounds seemed to form a colony with close feeding relations. Similar studies were carried out by MORTREUIL and BRADER [24] in African pineapple plantations in order to elucidate the dimensions of ant nests, their underground relations and their associations with harmful coccids. As was pointed out during the discussion of Mortreuil and Brader's paper at the Bombay Symposium (1960) [25], we have carried out this type of ecological tracer study for several years with interesting results. Beginning in 1959 and continuing through 1961/62 we labelled single nests of F. polyctena in one natural and two artificially founded colonies [22].

The tracer (P^{32} and/or I^{131}) was given in honey-water solutions. To prevent external contamination we offered the food in small special glass or plastic vessels which were introduced into the nest-mounds; this prevented the contamination of other insects. During the first experiments we gave only small activities, e.g., in a natural colony, 1 mc I^{131} dissolved in 10 ml honey-water. The activity was introduced in a single food-vessel into the south-west sector of nest-mound A (31.5.1959, 11 a.m.). After 46 h we took several samples of 20 ♀♀ each out of all 5 nests, which were counted in the laboratory with a scintillation counter. Secondary radioactivity was found in ants from nests C and D (Fig. 2); though weak, it was significantly different from the background activity and from the activity of ants from untreated nests in the same area. Surprisingly, we detected no transfer of radioactive food into nest B and the very near newly founded daughter-colony a. We suggest that a single food vessel was insufficient for labelling the whole population of the nest. Meanwhile, CHAUVIN, COURTOIS and LECOMTE, who independently from us did similar work with Formica [26, 27],

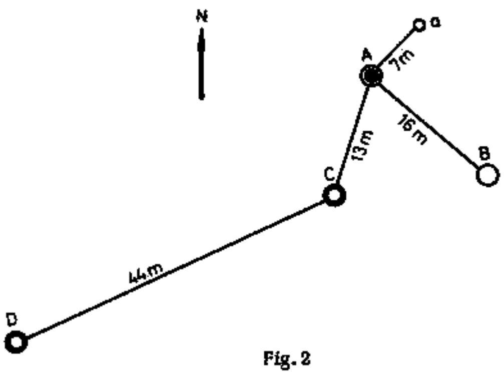


Fig. 2

Distribution of radioactive food among nests of a natural colony of Formica polyctena Foerst.

◉ primary labelled nest, labelled with 1 mc I^{131}

O nest showing secondary radioactivity after 46 h

O nest inactive after 46 h

showed in their second paper similar sectorial preferences of food transmission within ant-nests. In all later experiments we divided the tracer into 6-10 vessels which were well distributed over the primary labelled nest-mound.

Further experiments were done in artificially founded colonies [22]. One artificially founded nest (No. 59/1) showed two months after foundation migrations of changing intensity over a distance of 75 m to a secondary daughter-nest (No. 59/3a) which had been established through the artifically founded nest No. 59/3. Nest 59/1 was labelled with 0.12 mc P^{32} + 0.2 mc I^{131} in 4 ml honey-water (5. 6. 1959). After 96 h samples of 25 ☿☿ each were taken from 59/1, 59/3a and two other old-established nests (No. 104, No. 5) 70 m away from the primary-labelled 59/1. They were counted with G-M tube and scintillation counter in the laboratory. The results can be seen in Table I, which gives the counts per minute, including statistical error, obtained with two different counting methods. The transmission of radioactivity was positive only to nest 59/3a, in which the level of radioactivity was about two thirds of the counts in 59/1. The transmission may have been conditioned in this case by mixing of the nest population as well as by food exchange.

TABLE I

TRANSMISSION OF RADIOACTIVITY
AMONG DIFFERENT NESTS OF F. POLYCTENA
Nest No. 59/1 primary-labelled with P^{32}+ I^{131},
transfer positive only to nest 59/3a.

Sample	G-M tube (counts/min)	Scintillation counter (counts/min)
Background chamber	19.2 ± 1.8 18.9 ± 1.9	133.7 ± 3.6 138.0 ± 8
25 ☿☿ from nest 59/1	154 ± 8 169 ± 7	210 ± 8 195 ± 6.9
25 ☿☿ from nest 59/39	100 ± 7 89 ± 6.2	163 ± 8 174 ± 8.2
25 ☿☿ from nest 104	19.8 ± 2 18.6 ± 1.8	143 ± 6 141 ± 6
25 ☿☿ from nest 5	18.9 ± 1.7 19.7 ± 2	139 ± 7 138 ± 8

The next experiment, carried out with an artificially enlarged colony of F. polyctena, showed long-range transmissions of radioactivity over distances up to 200 m. The primary-labelled nest No. 7 (Fig. 3) received 10 mc I^{131} in 30 ml honey-water (12 June 1959). Countings of samples consisting of 100 ♀♀ each and taken 72 h after primary labelling were carried out with the scintillation counter in the laboratory. Seven of 13 nests carried significant secondary labelling. Visible connection, through migration of

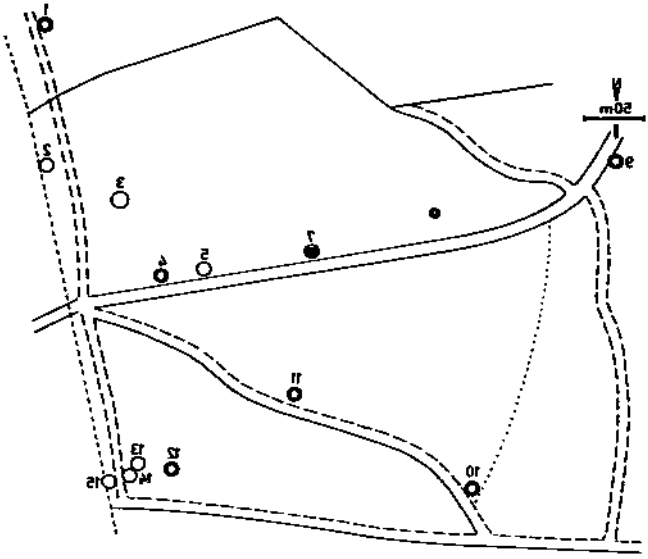


Fig. 3

Transmission of radioactive food from a single primary labelled nest to others
- primary labelled nest
- nest with secondary radioactivity after 72 h
- nest inactive after 72 h
- small unnumbered daughter nest, radioactive after 72 h

workers in both directions, existed only between No. 7 and No. 9, including the small unnumbered daughter nest.

During the same summer season we started a twice-repeated experiment in an artificially founded colony of F. polyctena in an area containing single nests of a natural population of F. rufa. Radioactivity was transmitted during June into most surrounding nests up to a distance of 70 m in a direct line, except for one nest of F. rufa which was at the shortest distance (30 m) from the primary-labelled nest of F. polyctena (the tracer dosage was the same as in the experiment described earlier). In the surroundings of the primary-labelled nest were three newly founded (two months old) artificial daughter nests. These did not partake of the radioactive food. But when we repeated the experiment under the same conditions three months later (4 September 1959) we found radioactivity in the daughter nests too. Especially this last result is very important since it shows that new daughter colonies, founded with ants of the same species but strange origin, will be integrated in the colony-system in the course of a few months. The experiment also confirms an observation published in 1940 [39] that ants of artificially founded daughter nests established in the mother nest's area migrate back, since they have too close social relations. Daughter nests of the same species but strange origin need some months to develop such relations and become well-established in the meantime.

The results of these field tracer experiments are of great importance in evaluating the regulating effect of useful Formica species on insect

pests [28], since these exchanges prevent the quick saturation of any nest by local mass infestation by insects in its predating area. The food collected flows, as shown by the tracer experiments, into most of the surrounding nests of the ant colony. Thus colonies of these useful wood-ants are acting as a complex predating system with high ecological effectiveness. In this connection it should be mentioned that we also have tested the distribution of radioactive caterpillars (activated by feeding on radioactive leaves), which were taken as living prey. Though the specific activity of the caterpillars was not very high, we observed good transmission within small colonies of F. polyctena under laboratory conditions.

We do not know exactly how the exchanges of radioactivity among different nests operate. Mixing of populations through migrations in both directions may play a part, as well as food transmission through regurgitation between workers of different nests meeting outside the nests. Both events can be observed, but may be different from case to case under the complex natural conditions. They should be analysed by labelling the ants with a combination of externally visible colours and radioactive food.

In some further experiments we tried to determine whether the food transmission operates better among artificially founded nests of the same origin than among these of different origin. Since these experiments were carried out at the beginning of the overwintering phase we found only weak transmissions of radioactivity between nests (1-15 October 1962). However these experiments yielded another very interesting result: during the overwintering phase the population stores food reserves in the hypertrophied fat-bodies of a part of the population [12, 20], especially of young workers, as has been suggested [20].

These "fat" individuals with swollen gasters are in the depth of the nests. During overwintering their behaviour pattern changes: their metabolism is reduced and they do not accept radioactive food [12]. Though they are already in the deep parts of the nests, they show a significant preference for supply with radioactive food offered at the nest surface. The storing individuals seem to be filled up as well as possible during this period of preparation for the winter.

For the last two years we have been using a portable counter independent of current mains. The apparatus was developed in conjunction with an engineering bureau* for our special purposes. The apparatus is fully transistorized and equipped with Ni-Fe batteries and a 220-V AC generator. It supplies the G-M tube with direct current up to 1800 V and the counter (which works electronically in two decades and mechanically in three) with 220-V alternating current (see Fig. 4). A start-stop device with time-intervals of 1 to 10 min is also fitted.

B. FOOD EXCHANGE IN TERMITES

Since termites are hemimetabolic insects, all stages - apart from the egg - are mobile and have well-developed mouth-parts, though specialized in the case of soldiers. For that reason it is more difficult to obtain an

* Radiomed (Firma Erich Jaeger), Würzburg

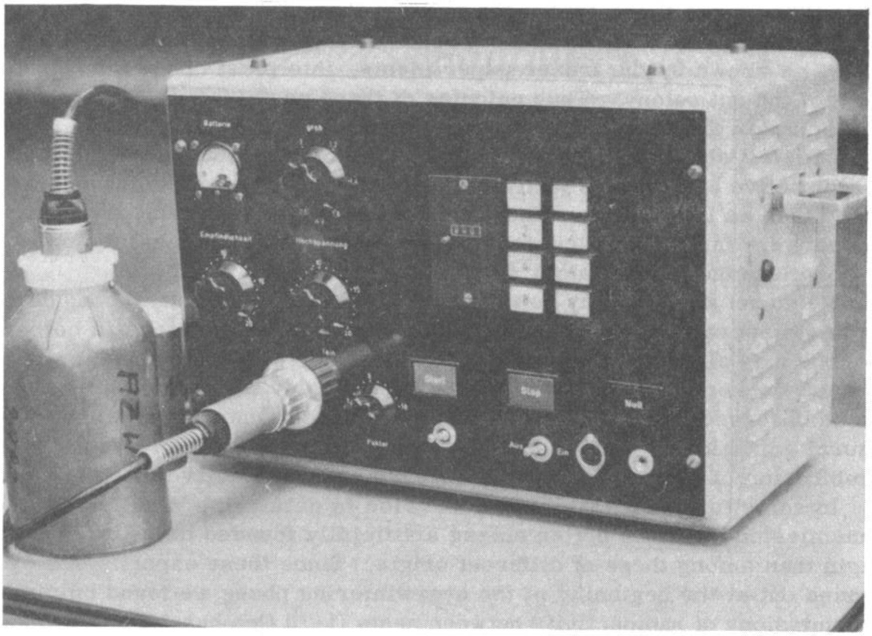


Fig. 4

Portable counter for field measurements

insight into the food relations among the members of a termite-colony than with social Hymenoptera. However, the tracer method offers excellent possibilities of studying these problems in termites. We began work of this type in 1958 in our laboratory with the drywood termite Kalotermes flavicollis Fabr. [4, 16, 9, 17, 18]. This species is very suitable as a laboratory termite for testing the termite-resistance of materials [29, 30, 17, 18].

It first had to be determined which stages were capable of direct uptake of foodstuffs. The termites were carefully separated into the different stages. We offered them as food filter paper soaked with P^{32} solution (specific activity 1 mc/ml). To avoid contamination, the termites were forced to eat through a small hole (diameter corresponding to the head width of the state studied) in a plastic foil stretched over the radioactive filter-paper. The experiments showed that larvae of the first stage, soldiers and preimagines (short interstage between second nymph and adult), and also alate termites never feed directly. Larvae of the second stage take up only very small amounts. The most active feeders are larvae of the third, fourth and fifth stages and pseudergates. In relation to their size, the two last-mentioned stages eat the largest amount of radioactive paper, thus demonstrating their suitability for test purposes [17]. Nymphs of both stages are also very active feeders, but they need a pre-adaption time of two days. In a similar way imaginal sexuals, more than three years old, regained the capacity to feed directly after an isolation time of 65-75 h.

Food exchange among individuals of K. flavicollis takes place relatively slowly. Putting radiolabelled termites at 27°C into normal colonies (ratio

labelled: unlabelled = 1 : 10), we found that after 12 h 40%, after 20 h 70%, and after 35 h 100% of the individuals were radioactive. The older larvae and young nymphs, especially the pseudergates, play the main part as donors, while young larvae, older nymphs, sexuals and soldiers are receptors of food. Moulting individuals do not feed two days before and two days after this event [18]. The exchange of food and other substances between termites takes place both stomodeally (i.e. by mouth-to-mouth feeding of regurgitated crop content or glandular secretions) or proctodeally (by feeding of excreted rectal content with a more or less fluid consistency)*. Such protodeal exchanges are known also among ants of the subfamily Dolichoderinae [31] and tracer methods enabled them to be demonstrated also in larvae of Myrmicinae [7].

C. STUDIES ON THE EXCRETION OF I^{131} IN ANTS AND TERMITES: COMPARISON OF SINGLE ISOLATED INDIVIDUALS WITH INDIVIDUALS IN A GROUP

As shown in the previous sections social insects have a high tendency to trophallactic changes of food and other substances, e.g. cuticularly excreted P^{32} [11, 32]. We supposed that, as a consequence of such repeated transmissions among individuals, the utilization of substances in groups might be more economic than in single individuals. A comparison of measurements of the biological half-life of a tracer in single isolated individuals and in individuals within a group seemed to be a convenient method for this problem. We studied the biological half-life of a γ-emitter, I^{131}, because as a result of changing body distribution and body absorption of β-particles precise determinations of the real effective half-life through repeated measurements of living insects are nearly impossible with β-detecting counters [32], unless the insects are very small and have thin cuticles, e.g. aphids [33]. However, all these complications are greatly reduced if γ-sources are used as tracers and the measurements are made with scintillation counters.

Pseudo-workers of the termite K. flavicollis and workers of the ant Formica pratensis Retz. (syn. Formica nigricans Em.) (Camponotinae) and Myrmica scabrinodis Nyl. (Myrmicinae) were fed with I^{131} by the methods described above (termites with labelled filter paper, ants with labelled honey-water). After the insects had eaten, the labelled ones were separated and kept in containers which were changed daily to avoid contamination through excreted radioactivity, either singly or in groups with unlabelled individuals of their nest (K. flavicollis: 1 labelled + 4 unlabelled; ants: 1 labelled + 5-15 unlabelled) under constant conditions of temperature (27°C) and air humidity (98% r.h.). During the experimental time fresh inactive food was always available.

Both the initial and the subsequent measurements of the radioactivity of the insects were taken with the help of a scintillation counter under the same geometric and other physical conditions. Before each measurement the background activity was first measured and subsequently subtracted from the measurements of the radioactivity of the insects. The insects were kept

---

* Shortly before the opening of the Symposium, a paper by McMAHAN was received, dealing with similar tracer studies of termite feeding relationships carried out with the drywood termite Cryptotermes brevis Walker [41].

under the scintillation counter enclosed in a small glass ring. Its diameter was different depending on whether single insects or a group of insects as a single unit were to be measured. It is interesting to note that the sum of the measurements of the individual insects composing a group was found statistically to be the same as the measurement of the radioactivity of the group when viewed as a unit.

The biological half-life (T_{biol}), which primarily depends on rate of excretion, was calculated from measured effective half-life (T_{eff}) and the known physical half-life (T_{phys}) by means of the following relationship:

$$\frac{1}{T_{eff}} = \frac{1}{T_{phys}} + \frac{1}{T_{biol}}.$$

The results are given in Table II. More details will follow in special papers [10, 34].

TABLE II

BIOLOGICAL HALF-LIFE (T_{biol}) OF I^{131} IN ISOLATED INDIVIDUALS AND IN GROUPS OF TERMITE PSEUDOWORKERS AND WORKER ANTS

	T_{biol} (h) Isolated single individuals	Groups	No. of experiments	Statistical constant (t)*	Probability
K. flavicollis	354.3	580.9	21	2.84	0.02
F. pratensis (syn. F. nigricans)	19.3	43.9	24	2.15	0.05
M. scabrinodis	83.0	191.2	14	2.21	0.05

* Student's t-test (1928)

The summarized results show that the biological half-life is shorter in isolated individuals than in groups. Since the measurements of the single individuals clearly showed the repeated interindividual exchanges of radioactivity among individuals, it became apparent that the prolonged T_{biol} was a consequence of this circulation. The greater economy in the use of food and other substances may be a contributory factor to the "group-effect" by which GRASSE and his group [35, 36] tried to explain the higher longevity and greater vigour of social insects in groups than as single individuals. Nevertheless these results cannot be generalized, since sometimes through storage in certain individuals (the above-mentioned fat ants during overwintering phase or "honey-pot" ants), the situation may be quite reversed. Tracer experiments carried out with replete workers of Proformica nasuta Nyl. in our laboratory [37] seen to indicate this.

ACKNOWLEDGEMENTS

These studies were supported by grants from the Bundesministerium für Atomkernenergie und Wasserwirtschaft and the Bundesministerium für Ernährung, Landwirtschaft und Forsten of the Federal Republic of Germany, and from the Bayerisches Staatsministerium für Unterricht and Kultus. To these Ministries we wish to express our best thanks. Our gratitude is also due to our collaborators and guests, especially to Mme. J. Alibert of Paris, Dr. R. Stumper of Luxembourg and Mr. P.K. Sem-Sarma of Dehra Dun, India.

REFERENCES

[1] NIXON, H.L. and RIBBANDS, C.R., Proc. Roy. Soc., Ser.B, 140 (1952) 43.
[2] GÖSSWALD, K. and KLOFT, W., Waldhygiene 1 (1956) 200.
[3] GÖSSWALD, K. and KLOFT, W., Proc. X. Int. Congr. Entomol., 1956, 2 (1958) 543.
[4] GÖSSWALD, K. and KLOFT, W., Umschau 58 (1958) 743.
[5] GÖSSWALD, K., Natur und Volk 89 (1959) 209
[6] GÖSSWALD, K. and KLOFT, W., Entomophaga 5 (1960) 33.
[7] GÖSSWALD, K. and KLOFT, W., Zool. Beiträge, N.F. 5 (1960) 519.
[8] GÖSSWALD, K., Math. Naturwiss. Unterricht 12 (1960) 208.
[9] GÖSSWALD, K. and KLOFT, W., Imkerfreund 16 (1961) 7.
[10] GÖSSWALD, K. and KLOFT, W., Atti IV Congresso U.I.E.S. Pavia 1961, Symposia genetica et biologica italica X (1962) (in press).
[11] BERWIG, W., Naturwiss. 46 (1959) 610.
[12] KNEITZ, G., Atti IV Congresso U.I.E.S. Pavia 1961, Symposia genetica et biologica italica X (1962) (in press).
[13] BECK, H., Insectes sociaux 8 (1961) 1.
[14] HÖLLDOBLER, B., Insectes sociaux 8 (1961) 13.
[15] NAARMANN, H., Experientia, Basle (1963) (in press).
[16] ALIBERT, J., C.R.Acad. Sci. (Paris) 248 (1959) 1040.
[17] GÖSSWALD, K. and KLOFT, W., Entomol. exp. et appl. 2 (1959) 268.
[18] GÖSSWALD, K., Proc. UNESCO Int. Symp. on Termites in the Humid Tropics, New Delhi 1960 (1962) 169.
[19] GÖSSWALD, K., KLOFT, W. and KÖHLER, F., in preparation (1963).
[20] KIRCHNER, W., Diss. Würzburg (1963).
[21] WILSON, E.O. and EISNER, T., Insectes sociaux 4 (1957) 157.
[22] GÖSSWALD, K., Die rote Waldameise im Dienste der Waldhygiene, Metta Kinau Verlag, Lüneburg (1951).
[23] KANNOWSKI, P.B., Ecology 40 (1959) 162.
[24] MORTREUIL, M. and BRADER, L.M., Radioisotopes and Radiation in Entomology, IAEA, Vienna (1962) 39.
[25] KLOFT, W., Discussion comment to [24], Radioisotopes and Radiation in Entomology, IAEA, Vienna (1962) 44.
[26] CHAUVIN, R., COURTOIS, G. and LECOMTE, J., Insectes sociaux 8 (1961) 99.
[27] COURTOIS, G. and LECOMTE, J., Insectes sociaux 9 (1962) No.4
[28] GÖSSWALD, K. and KLOFT, W., Waldhygiene 5 (1963) (in preparation).
[29] GÖSSWALD, K., Mitt. Biol. Reichsanst. 65 (1941) 34.
[30] GÖSSWALD, K., Composite Wood 3 (1956) 65.
[31] TOROSSIAN, C., Insectes sociaux 6 (1959) 369; 7 (1960) 171.
[32] KLOFT, W., Radioisotopes and Radiation in Entomology, IAEA, Vienna (1962) 163.
[33] KLOFT, W. and EHRHARDT, P., Radioisotopes and Radiation in Entomology, IAEA, Vienna (1962) 181.
[34] SEN-SARMA, P.K. and KLOFT, W., Entomol. exp. et appl. 6 (1963) (in press).
[35] GRASSE, P.P. and CHAUVIN, R., Rev. scient. 82 (1944) 461.
[36] GRASSE, P.P., Experientia 2 (1946) 77.
[37] STUMPER, R., Naturwiss. 48 (1961) 735.

[38] GÖSSWALD, K., Atti IV Congresso U.I.E.S. Pavia 1961, Symposia genetica et biologica italica X (1962) 106.
[39] GÖSSWALD, K., Mitt. Forstwirtsch. u. Forstwiss. (1940) 97 and 291.
[40] HÖLLDOBLER, B., unpublished
[41] McMAHAN, E.A., Ann. Entomol. Soc. Amer. 56 (1963) 74.

DISCUSSION

J. LECOMTE: Although your studies on the important question of transmission of food in the form of labelled caterpillars have been confined to the laboratory, do you consider that such exchanges can be as intense as the exchange of sugared foods?

W. KLOFT: Your question is an important one, and we did take this problem into consideration. As I stated, the laboratory experiments with labelled caterpillars were positive throughout, and ought to be repeated under outdoor conditions. These caterpillars were given in the form of living prey. The specific activities of the caterpillars were not very high, but we known from our experiments that the genus Formica prefers haemolymph when feeding on insects, and takes up the cell complexes suspended therein particularly from the fat-body, and of course other tissues also. This more or less liquid precipitate can then be directly distributed in the same way by regurgitation.

D.A. CROSSLEY: I would like to ask Dr. Kloft what percentage of the P^{32} in the body would be excreted through the salivary gland and consequent passage through the gut.

W. KLOFT: We carefully measured these percentages. I do not know the exact figures but they will be published by our colleague, H. Naarmann. The total amount excreted in this way in the course of one day is about 1-5% of the total body activity remaining after there is no more primary activity in the crop. It is important to bear in mind that this only occurs with individuals during a certain phase of life, and especially with over-wintered young workers from the previous year. Workers which are born this year can do this next year, in April to May. During that time the young workers which have over-wintered feed the young queen-larvae with glandular secretions and in this way substances coming from the fat-body are used for rearing young sexual forms, especially young queens.

D.A. CROSSLEY: May I ask a further question? If you now consider the concentration of radioisotopes in various steps in the food chain, would it not be possible to measure the flow of energy through an ant nest, that is the amount of transfer of stored energy from one phase to another? You should be able, should you not, through this type of distribution, to calculate how much trophallaxis occurs, in theory at least?

W. KLOFT: We have not yet made this calculation, though it should be possible, using your special techniques for summation of the ingested activity of one queen larva, for example.

Mme. S. FUZEAU-BRAESCH: May I ask Dr. Kloft something about his experiment on measuring activity in isolated animals and grouped animals? He finds that the biological half-life of isolated animals is less than that of animals in groups, and mentions a group-effect phenomenon in that con-

nection. I would like to ask him whether he thinks that it is a real group-effect — that is, an effect which goes through sensorial and nervous stimuli and acts on the whole physiology of the animal — or simply a result of the grouping of several individuals leading to recontamination, trophallactic or otherwise.

W. KLOFT: Naturally I am aware that the group-effect must to a large extent be conditioned by sensorial and nervous stimuli. We regard it as a complex phenomenon in which the mutual exchange of food and other substances plays a contributory part, as one single factor. Through the continuous exchange of crop-content with or without glandular secretions by licking and so on, essential substances as well as pure calorie providers, (i.e. pure energy foods) are distributed evenly throughout the group; not every individual has these or is always in a physiological condition to produce them. Radioactive phosphorous was excreted through the cuticula of queens and the excreted P^{32} was licked up by the workers and distributed among each other. We think this food transfer between individuals within a group is a main cause of their longer expectation of life.

As you know, if we isolate one single individual, a termite, an ant or a honey-bee worker, and give her all the energy substances she needs, she cannot live as long as the same individuals with the same food supply in a group. These things are well described by Grassé and his group in Paris. Through repeated exchanges of such food substances and also glandular excretions, all individuals have about the same equilibrium in foodstuffs and other substances. I know they need the stimuli, including all the olfactory and other nervous stimuli, but I think the longer biological half-life is undoubtedly related to the so-called group-effect.

G. COURTOIS: I should like to add that by using Ir^{192} we have also found this increase in the biological half-life in grasshoppers, depending on whether they are kept isolated or in groups.

D.A. CROSSLEY: Dr. Frank Gaully reports a similar increase for millipedes. He can produce the effect with an individual animal by preventing it from eating its own faeces. The particular millipede with which he is working has a strong tendency to eat a good number of its own droppings. If the animal is prevented from doing this, its measured biological half-life becomes much shorter.

Mme. S. FUZEAU-BRAESCH: Could part of the difference in the biological half-life be due to immediate recontamination between individuals, that is to a phenomenon similar to the one mentioned in the millipede?

W. KLOFT: Undoubtedly, we also have external contamination because individuals lick each other externally. We cannot prevent that. I take the biological half-life as a total effect for the whole group. These external excreted substances are always taken up from one individual to the other, and this type of external contamination is undoubtedly included, but using gamma-emitting tracers and measuring with scintillation counters, we had no problems as regards absorption effects. In other cases, as I reported at the Bombay Symposium*, we get higher rates of radioactivity, after excretions, on the cuticula. At the beginning of the experiment, using gamma-emitters, we

* Radioisotopes and Radiation in Entomology, IAEA, Vienna (1962), 163.

had no such problems, but I agree with you that we do have some external contamination. The substance, however, is not lost to the community. Individuals take it from one another.

Mme. S. FUZEAU-BRAESCH: I believe I could suggest a useful experiment. This prolonged sensorial effect, which is a real group-effect, concerns a whole group of insects.

To eliminate contamination I suggest that isolated animals should be measured. Some of them could be isolated from a long time, and the others kept in groups before the experiment. Since the group-effect is an effect which is prolonged, any difference in the half-life occurring will be due solely to a "group-effect".

W. KLOFT: Mutual contamination with the radioactive group is of course unavoidable. In fact, the technique is based directly on it. The animals lick each other constantly and — as we have been able to show by the tracer method — they distribute, by regurgitation-feeding, substances excreted by the cuticula. Thus in actual fact a substance already excreted returns again to the intestinal tract of other individuals or, by means of regurgitation, even enters into the metabolism of the original producer.

Nevertheless I agree with you that further differentiation should be made in the experiments, in order to solve these complex problems.

J. HALBERSTADT: In connection with biological half-life problems, and in order to get more exact data, would it not be helpful to run parallel experiments using two isotopes of the same element, P^{32} and P^{33}, for instance, or I^{131} and I^{132}? Would that not give you much more exact information?

W. KLOFT: We have not yet done the experiment with two isotopes of the same element, but our field experiments on ants were done partly with two different tracers, P^{32} and I^{131}, and we had different half-lives for these two, because P^{32} is very well assimilated in the body while I^{131} is less well assimilated. However, I will bear your suggestion in mind.

USE OF RADIOACTIVE TRACERS IN THE STUDY OF INSECT-PLANT RELATIONSHIPS

D. A. CROSSLEY, JR.
RADIATION ECOLOGY SECTION, HEALTH PHYSICS DIVISION,
OAK RIDGE NATIONAL LABORATORY, OAK RIDGE, TENNESSEE

Abstract — Résumé — Аннотация — Resumen

USE OF RADIOACTIVE TRACERS IN THE STUDY OF INSECT-PLANT RELATIONSHIPS. In early uses of radioactive tracers in ecological investigations of pests insect, dispersion and migration phenomena were studied with radioisotopes as markers for individual insects. A more recent development is the use of radioisotopes to evaluate insect-plant and predator-prey associations through estimates of food utilization. Biological elimination of radioisotopes, a nuisance in dispersion studies, is being utilized as a measure of feeding rates. In both field and laboratory experimentation, insects are allowed to reach steady-state concentrations of radioisotopes through feeding. Then the rate of intake is equal to the rate of elimination of the radioisotope: (rate of ingestion) = (steady-state amount) × (fractional rate of loss). Measurements of elimination rates (biological half-lives) permit the steady-state concentrations to be translated into intake rate functions.

Food consumption studies have been performed for single-insect-species populations and for multiple-species populations. In a single-species investigation, radiocaesium in a tagged field site was used to estimate the consumption of willow leaves by populations of the beetle Chrysomela knabi. Direct measurements of food consumption made in the laboratory showed good agreement with field estimates of feeding rates based on the radioisotope techniques. Biological half-lives differed for the larval stages and these had to be considered separately. Radioisotopes provided a means of separating overwintering adults from newly emerged ones, through the more rapid elimination of caesium from overwintering adults.

In multiple-species work, the relationship between size of insect and elimination rate was used to derive an average biological half-life for radiocaesium elimination from herbivorous insects in a field site tagged with caesium-137. This average rate, used in conjunction with data on plant and insect biomasses and concentrations of radiocaesium, permitted an evaluation of plant consumption by an entire insect community. Similarly, the utilization of insects as food by predaceous arthropods was estimated from steady-state concentrations of radiocaesium in predators and prey, biomasses, and an average elimination rate.

EMPLOI DES RADIOINDICATEURS DANS L'ÉTUDE DES RELATIONS INSECTE-PLANTE. Dans les premiers travaux écologiques sur les insectes nuisibles effectués à l'aide de radioindicateurs, les chercheurs ont étudié les phénomènes de dispersion et de migration en utilisant les radioisotopes pour le marquage d'individus. Plus récemment, ils s'en sont servis pour étudier les relations insecte-plante et prédateur-proie, en procédant à des évaluations de l'utilisation des aliments. L'élimination biologique des radioisotopes, qui constitue un inconvénient dans les études sur la dispersion, est utilisée dans ce cas comme mesure des taux d'alimentation. Dans les expériences en plein champ ou en laboratoire, on attend que la concentration des radioisotopes ingérés par les insectes parvienne à l'équilibre. Le taux d'absorption des radiosotopes est alors égal à leur taux d'élimination: (taux d'ingestion) =(quantité à l'équilibre)×(taux de pertes fractionnaire). Les mesures des taux d'élimination (période biologique) permettent de traduire les concentrations à l'équilibre en fonctions exprimant les taux d'ingestion.

Les auteurs ont procédé à des études sur la consommation d'aliments dans des populations composées d'une seule espèce d'insectes et dans des populations composées de plusieurs espèces. Dans une étude en plein champ sur une seule espèce, ils ont procédé au marquage par le radiocésium afin d'évaluer la consommation de feuilles de saule par des populations de chrysomèles (Chrysomela knabi). Les mesures directes de la consommation d'aliments faites en laboratoire ont bien concordé avec les évaluations des taux d'alimentation faites en plein champ au moyen des méthodes radioisotopiques. Les périodes biologiques ont été différentes pour les stades larvaires, qu'il a fallu étudier séparément. Les radioisotopes ont permis de distinguer les adultes ayant survécu à l'hiver de ceux qui venaient d'apparaître; en effet, le césium est éliminé plus rapidement par les premiers.

Dans les études portant sur plusieurs espèces, on s'est servi du rapport entre la taille de l'insecte et le taux d'élimination pour établir une période biologique moyenne d'élimination du radiocésium chez les insectes herbivores dans un champ marqué au césium-137. En rapprochant cette moyenne des données relatives aux ensembles biologiques de végétaux et d'insectes et aux concentrations du radiocésium, il a été possible d'évaluer la consommation de végétaux pour toute une population d'insectes. De même, à partir des concentrations à l'équilibre du radiocésium dans les prédateurs et leurs proies, ainsi que dans les ensembles biologiques, et du taux moyen d'élimination, on a évalué l'importance des insectes dans la nourriture des arthropodes prédateurs

ИСПОЛЬЗОВАНИЕ РАДИОАКТИВНЫХ ИНДИКАТОРОВ ДЛЯ ИЗУЧЕНИЯ ВЗАИМНОЙ СВЯЗИ МЕЖДУ НАСЕКОМЫМИ И РАСТЕНИЯМИ. При более раннем использовании радиоактивных индикаторов в экологических исследованиях насекомых-вредителей их рассеяния и миграции изучались при помощи радиоизотопов, которыми маркировались отдельные насекомые. В последнее время получило распространение использование радиоизотопов для изучения биологической ассоциации насекомых и растений, а также хищных насекомых и их добычи, исходя из определения потребления ими пищи. Биологическое выделение радиоизотопов, которое мешает изучению рассеяния насекомых, используется в настоящее время для измерения количества потребления пищи. Как в полевых исследованиях, так и в лабораторных экспериментах насекомым дается возможность достичь в процессе питания состояния устойчивой концентрации радиоизотопов. В этих условиях темп поглощения данного радиоизотопа равен темпу его выделения: (темп поглощения) = (величине устойчивого состояния) × (скорость потери). Измерения темпов выделения (биологического полураспада) позволяют переводить состояния устойчивой концентрации в функции темпов поглощения.

Изучение потребления пищи было проведено как для популяций насекомых одного только вида, так и для популяций смешанного типа. При изучении насекомых одного вида на меченом поле использовался радиоактивный цезий для определения потребления ивовых листьев популяциями жука Chrysomela knabi. Проведенные в лаборатории непосредственные измерения потребления пищи показали хорошее совпадение с результатами изучения темпов питания, полученными на опытном поле на основании радиоизотопного метода. Однако для личинок биологический период полураспада оказался иным, так что эти результаты должны рассматриваться раздельно. С помощью радиоизотопов удалось разделить перезимовавших взрослых особей от вновь появившихся на свет, благодаря более быстрому выделению цезия перезимовавшими взрослыми особями. При работе со смешанными популяциями было использовано соотношение между размером насекомого и темпами выделения радиоизотопов для определения среднего биологического периода полураспада при выделении радиоактивного цезия травоядными насекомыми, меченными цезием-137. Эти средние темпы совместно с данными о биомассах растений и насекомых, а также о концентрациях радиоактивного цезия позволили определить размеры потребления растений всей популяцией насекомых. Аналогичным образом, количество насекомых, съедаемых хищными членистоногими, было определено на основании состояния устойчивой концентрации радиоактивного цезия у хищников и у их добычи, их биомасс и средних темпов выделения.

EMPLEO DE MARCADORES RADIACTIVOS PARA ESTUDIAR LAS RELACIONES INSECTO-PLANTA. Las primeras aplicaciones de los indicadores radiactivos en los estudios ecológicos sobre las plagas de insectos y sobre los fenómenos de dispersión y migración insectiles consistieron en marcar con radioisótopos a algunos insectos. Más recientemente se han llegado a utilizar los radioisótopos para estudiar las relaciones insecto-planta y depredador-víctima a través del consumo de sustancias nutritivas. La eliminación biológica de los radioisótopos, elemento perturbador en los estudios sobre la dispersión, sirve para medir los índices de alimentación. Tanto en las campañas experimentales como en los estudios de laboratorio se logra una concentración estacionaria de radioisótopos en los insectos por ingestión. El índice de ingestión de radioisótopos es entonces igual al de eliminación; la fórmula aplicada es la siguiente (índice de ingestión) = (cantidad estacionaria) × (índice fraccional de pérdida). La medida del índice de eliminación (período biológico) permite determinar el índice de ingestión a partir de los datos relativos a la concentración estacionaria.

Se han estudiado el consumo de alimentos en poblaciones insectiles formadas por una sola especie y formadas por varias especies. En el estudio de una de las primeras, se utilizó el radiocesio aplicándolo en una zona determinada para calcular el consumo de hojas de sauce por poblaciones del escarabajo Chrysomela knabi. Las medidas directas del consumo de alimentos, realizadas en laboratorio, dieron resultados que coincidían con los obtenidos en las campañas experimentales emprendidas para calcular los índices de alimentación mediante el uso de radioisótopos. En la fase larval, el período biológico de los insectos es diferente, por lo que hubo de ser objeto de un estudio aparte. Los radioisótopos permitieron distinguir a los adultos que habían pasado la hibernación de los adultos nuevos, porque la eliminación del cesio en los primeros era más rápida.

En los estudios sobre poblaciones formadas por varias especies, la relación entre el tamaño del insecto y el índice de eliminación sirvió para calcular el período medio biológico para la eliminación del radiocesio por insectos harbívoros en una zona tratada con ^{137}Ce. Este período medio y los datos sobre las masas biológicas vegetales e insectiles y sobre las concentraciones de radiocesio han permitido calcular el consumo de vegetales de una población entera de insectos. Del mismo modo, se ha podido calcular el consumo de insectos de los artrópodos depredadores basándose en las concentraciones estacionarias de radiocesio en los depredadores y en las víctimas, las masas biológicas y el índice medio de eliminación.

INTRODUCTION

In modern insect-control practices, an integration of chemical, biological, cultural and other manipulative procedures is increasingly visualized as the ideal means for minimizing the depredations of pest insects and mites. From the purely biological aspects, autecological work on pest insects has always been considered meritorious and ecological phenomena were prominent in the attempts at "biological control" during the past few decades. Recent concepts, however, transcend the older ideas of biological control in that pest insects are regarded as members of more extensive ecological systems. For example, SMITH [1] has listed three principles for an integrated control concept which may be restated briefly as follows: (1) Crops, pest insects, and other biota and environment should be treated as a functional unit — the ecosystem; (2) Control measures should aim to keep pests below economic levels rather than attempt complete eradication; and (3) Disruption of other parts of the ecosystem must be considered in the evaluation of control procedures.

Implicit in such concepts is a consideration of insect-crop relationships as part of a unit system and the evaluation of control measures as a shifting of balances within the system. Such an integrated control procedure is not always necessary and may even be undesirable in some instances. The evaluation of even a very effective chemical control procedure must be made on more than destruction of pest insects, however, and such evaluation falls within the realm of ecology.

Radioisotope techniques provide an effective means of studying insect-plant relationships within an ecosystem context, and the full value of these techniques evidently is yet to be realized. Early uses of radioisotopes in economic entomology involved studies of dispersal and migration phenomena [2]. The unique value of radioactive materials as markers for individual insects has become a universally appreciated technique [3]. In mass tagging experiments, the loss of ingested radioisotope due to biological elimination was noted and deemed to be of nuisance value only. DAVIS and FOSTER [4] seem to be the first ecologists to suggest that biological elimination of radioisotopes be used to measure feeding rates, although this concept is inherent in numerous physiological studies [5]. The new doctrine of health physics also has contributed functional models and terminology. Essentially these methods use elimination rates to estimate accumulation rates, so that the concentration of a radioisotope in an insect (for example) can be used to infer rates of plant consumption or prey consumption by predaceous insects. The elimination rate technique offers a widely applicable and uncomplicated means for trophic analysis.

ELIMINATION RATES AND BIOACCUMULATION

The amount of a radioisotope which is retained in an insect following ingestion of a single dose decreases exponentially, because of biological elimination. Fig. 1 shows the retention of caesium-137 by a female grasshopper (Melanoplus differentialis); the exponential decrease produces a straight line in a semilogarithmic graph. Expressions of such rates of elimination are usually made in terms of the biological half-life. This is defined as the time required for the amount of a radioactive element in the body to decrease to one-half its initial value as the result of biological processes. The data in Fig. 1 suggest a biological half-life of approximately 2.5 d. Since these data were not corrected for radioactive decay, the result is technically an effective half-life; however, the physical half-life of Cs^{137} is so long in comparison with the observed half-life (25 yr as against 2.5 d) that radioactive decay can be ignored. In the general case these three rates are related as follows:

$$T_{eff} = \frac{T_b \, T_r}{T_b + T_r} \tag{1}$$

where T_{eff}, T_b, and T_r are the effective, biological, and radioactive half-lives respectively.

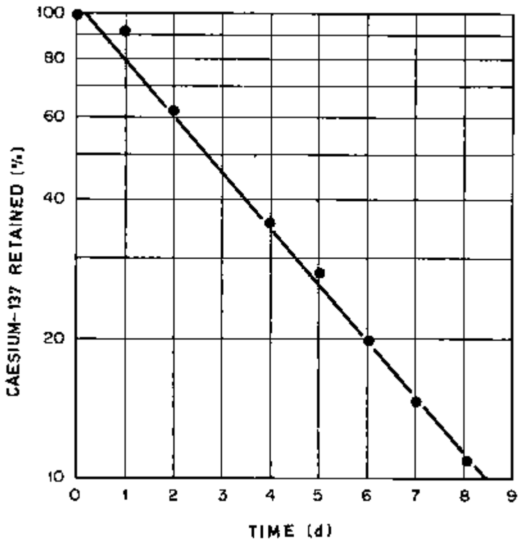


Fig. 1

Retention of Cs^{137} by a single grasshopper, Melanoplus differentialis.

By analogy with radioactive decay, the loss of radioactivity from the insect's body can be described from the relationship:

$$A_t = A_0 \, e^{-kt} \tag{2}$$

where A_t is the radioactivity at any time t, A_0 is the initial radioactivity of the insect at time t = 0, and k is an elimination constant equal to $0.693/T_b$ (or $0.693/T_{eff}$ in the general case). For the case illustrated in Fig. 1, k is 0.693/2.5 d, or 0.277 d^{-1}.

The amount of radioisotope eliminated during any given day, in this example, is equal to the radioisotope content of the insect times the elimination constant k. Thus when an insect feeds continually on radioactive plants the daily rate of elimination will increase until daily intake is balanced by daily elimination. At that time the concentration of radioisotope in the insect may be said to have reached a steady state. The relation between intake and elimination in a steady state is:

$$r = kA_q \tag{3}$$

where r is the rate of feeding (μc/d), k is the elimination constant (d^{-1}), and A_q is the whole-body radioactivity (μc) in steady-state equilibrium. Thus, if the steady-state radioactivity of the insects is measured and the biological half-life for the particular radioisotope is known, the rate of feeding r can be calculated directly. If the concentration of radioisotope in the food of the insects is known, then r can be expressed as the amount of food consumed per day. If only a fraction of the radioisotope ingested is assimilated, a correction must be applied. The right-hand side of Eq. (3) may be divided by the fraction assimilated, or the elimination of unassimilated material may be treated as an additional exponential factor. No such correction is necessary for the results discussed in this report since they are based on radiocaesium, which is almost completely assimilated [6].

The approach to the steady-state equilibrium concentration is asymptotic; the time required to reach 50% of the equilibrium value is equal to one biological half-life. Practically, equilibrium is considered to be attained after a time equivalent to five biological half-lives. This would be 97% (31/32) of the final value.

APPLICATION TO FIELD POPULATIONS

Two types of field application of these radioactive tracer methods will be discussed, the single-species population and the multiple-species population, since each presents its own set of requirements.

Single-species population

Feeding rates were estimated for the third-instar larval stage of Chrysomela knabi Brown, feeding upon willow leaves. This beetle species overwinters in the adult stage. Eggs are laid on young willow leaves in early May, in the East Tennessee area. A single generation of beetles develops each year. All three larval stages as well as adults feed exclusively on willow leaves. Development is completed usually by mid-June. The life history of this beetle species has been described by BROWN [7].

Laboratory studies showed that biological half-lives for radiocaesium in this insect increased with age and size during development. Elimination

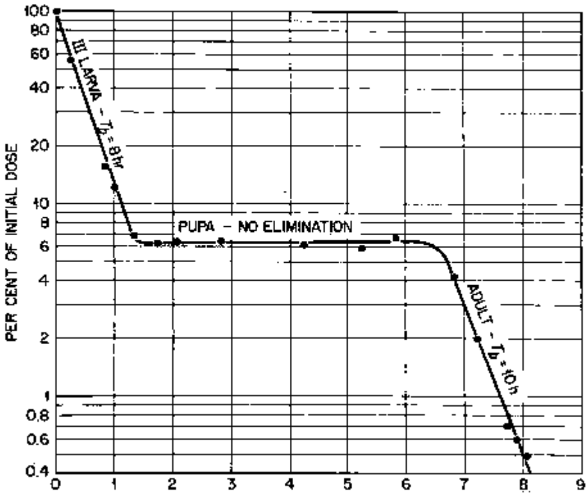


Fig. 2

Retention of Cs^{134} by an individual beetle (Chrysomela knabi)
Third-instar larva, pupa, and adult stages are shown.

of Cs^{134} occurred at rates corresponding to a 7-h biological half-life for first-instar larvae (mean of 4 individuals), an 8-h T_b for second-instar larvae (10 individuals), an 8-h T_b for third-instar larvae (14 individuals), and a 10-h T_b for newly-emerged adults. Fig. 2 illustrates Cs^{134} retention by one individual. Loss during the third instar, no elimination during the pupal stage, and further loss by the newly emerged adult are shown. These short biological half-lives suggest that a steady-state concentration would be reached in less than two days of feeding. However, the average duration of the larval stages also is short, and only during the third instar (5-d average duration) and the adult stage might a steady-state equilibration be expected.

Beetles containing Cs^{137} were obtained from willows growing on the White Oak Lake bed. White Oak Lake was formerly a part of the Oak Ridge National Laboratory's low-level waste disposal system. The lake was drained in 1955, and the plants and animals which invaded the lake basin accumulated various radioisotopes from the sediments [8]. Cs^{137} accumulated by willows (Salix nigra) growing in these sediments is transferred to insects feeding upon these plants. The consumption of leaves was calculated for third-instar larvae, which were more readily collected than were adults. The Cs^{137} content of a sample of 122 third-instar Chrysomela knabi larvae was 72.9 ± 14.6 pc/g dry wt. This value represents the steady-state equilibrium concentration. The elimination constant k, based on a biological half-life of 8 h (= 0.333 d), is estimated as $0.693/0.333 = 2.08\ d^{-1}$. By substitution in Eq. (3), $r = (2.08)(72.9) = 151 \pm 30.4$ pc/g beetle per day. The average Cs^{137} concentration in willow leaves was 37.8 ± 2.38 pc/g dry wt., on the basis of leaves from which the beetle larvae were collected. Dividing 151 pc consumed per day by 37.8 pc/g gives 4.01 ± 0.84 g plant consumed/g beetle per day. The average weight of third-instar larvae in the sample was 4 mg;

TABLE I

AVERAGE WEIGHTS OF FOOD CONSUMED BY THIRD-INSTAR LARVAE OF CHRYSOMELA KNABI

Values are means for 12 measurements.

Age of larvae (days in third instar)	Mean weight of beetles (mg dry wt.)	Mean weight of food eaten (mg dry wt.)
1	1.9	6.3
2	2.4	10.7
3	4.6	15.0
4	7.4	15.4
5	9.0	5.3

the consumption of willow leaves thus would be estimated at 16.0 ± 3.4 mg dry wt. per beetle larva per day.

Table I shows the results of direct laboratory measurements of food consumption (willow leaves) by third-instar larvae. This instar is a period of rapid growth; weights of larvae increased from a mean of 1.9 mg to 9.0 mg in the 5-d period. Food consumption showed an increase from 6.3 to 15.4 mg/d, followed by a sharp drop to 5.3 mg/d on the day preceding pupation. The average weight of the third-instar larvae collected from willows on White Oak Lake bed was 4 mg; in Table I this corresponds to a daily food consumption of about 15 mg, as compared with the field estimate of 16 mg inferred by the radiocaesium technique.

These results show the accuracy which can be obtained for field data by this radioisotope technique. Biological half-lives are probably slightly different for each day of prepupation in the third larval instar, as well as between instars, but additional refinements in estimates of elimination constants would appear unnecessary. The results may be exceptionally good in this example, since the insects sampled could be related to a particular leaf on which they were feeding and thus sampling error was reduced for estimates of leaf concentrations of Cs^{137}. Had the sampling been based on adults, the motility of that stage might have reduced the accuracy in field estimates. An anomaly in the biological half-lives of Cs^{137} in adult beetles was also noted: the average T_b was 10 h for new adults but only 7.5 h for year-old ones. This difference is probably a reflection of the metabolic status of the beetles. New adults were accumulating materials to prepare for hibernation, whereas year-old ones were mating, seeking oviposition sites and laying eggs. The radioisotope equilibration technique would have estimated different feeding rates for the two groups of adults, other things being constant. Laboratory information was not sufficient to show whether feeding rates really differed for new and year-old adults.

Multiple-species populations

Areas with natural vegetation may easily support several hundreds of different species of insects, and ecological measurements become infinitely more complicated in such communities. Direct measurement of food consumption becomes difficult if not impossible. Indirect estimates must be made if generalizations are to be applied to the entire insect community, and approximate techiques become particularly attractive. The radioisotope equilibration method of Eq. (3) can be applied to multiple-species populations as well as to single-species populations. The rate of plant consumption by the insect community living on the above-mentioned White Oak Lake bed has been estimated through this radioisotope technique by CROSSLEY and HOWDEN [6], and the theoretical validity has been discussed by CROSSLEY [9]. Additional samples of the flora and fauna of White Oak Lake bed, taken during the summer of 1961, form the basic data for the present discussion. The results are similar to the estimates reported previously [6, 9] but some differences noted in the discussions can be ascribed to unusually cool weather in the summer of 1961.

An appropriate value must be selected for the elimination constant k of Eq. (3). Ideally this value would be some sort of weighted average for all of the insect species feeding on vegetation on the White Oak Lake bed. Clearly it is impractical to measure radioisotope elimination rates for each

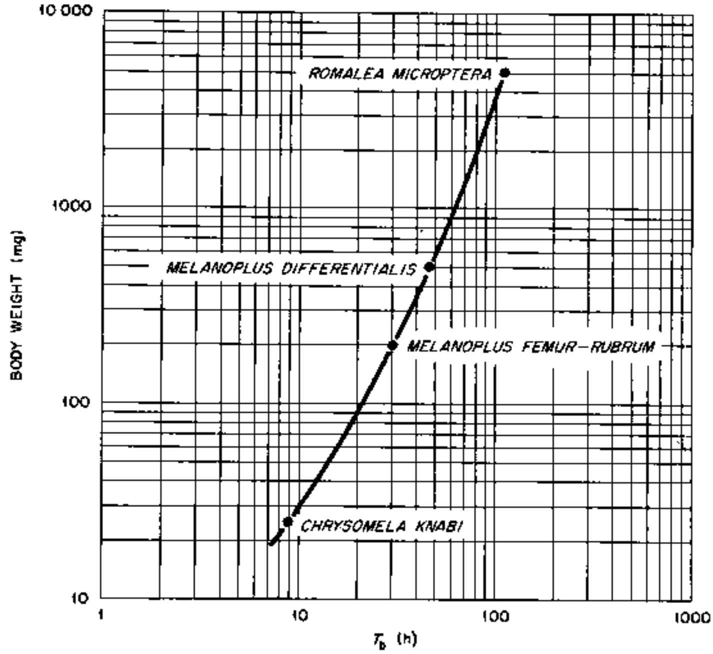


Fig. 3

Relation of insect weight to biological half-life of caesium.
(After CROSSLEY [9])

of the several hundreds of species. For caesium, however, the biological half-life has been found to be related to the size of the insect [9]. Fig. 3 shows the biological half-life of caesium in insect species plotted as a function of fresh weight (mg) of the insects. Biological half-lives of caesium in mammal species, as reported by LANGHAM and ANDERSON [10], fall close to a projection of the line in Fig. 3. Weights of insect species can thus be made the basis for selection of an average k for the entire community. For the present discussion we shall use a weighted average weight per insect collected in the 1961 samplings, which was approximately 20 mg fresh wt. The line in Fig. 3 suggests a biological half-life for caesium of about 7.5 h for this weight. This is 0.313 d, and the average elimination constant k is estimated as 2.21 d^{-1}. This is larger than the elimination constant of 0.98 d^{-1} estimated for the 1958 samplings [9] but evidently there was a real difference between the average size of insect in 1958 and that in 1961.

TABLE II

CONCENTRATIONS OF CAESIUM-137 AND BIOMASSES FOR SOIL AND TROPHIC LEVELS OF THE PLANT-INSECT FOOD CHAIN ON WHITE OAK LAKE BED, SUMMER 1961

Mean ± standard error; number of samples in parentheses.

	Caesium-137 concentration (pc/g dry wt.)	Biomass (g/m^2)
Soil	7300 [a]	-- [b]
Plants (leaves)	160 ± 32 (5)	317 ± 42.4 (4)
Herbivorous insects	78 ± 4.8 (5)	0.111 ± 0.0192 (8)
Predaceous insects	73 (8 samples lumped)	0.0117 ± 0.0015 (8)

[a] Soil value based on grand mean from core samples.

[b] No samples taken.

The basic data obtained in the 1961 samplings are given in Table II. Concentrations of Cs^{137} in plants (leaves) and in herbivorous and predaceous insects are essentially the same as those reported for 1958 [6]. Biomasses, however, are lower, evidently as a result of cooler weather in the summer of 1961. Plant biomass dropped from about 600 to about 300 g/m^2; the average herbivorous insect biomass dropped from approximately 250 to approximately 100 mg/m^2. Final biomass samples at the end of the summer approached 250 mg/m^2, however.

Using the data in Table II, one can estimate the average rate of feeding r for the herbivorous insects as follows: (2.21) (78) = 172 pc/g insect per day.

Dividing by plant concentration (160 pc/g), one obtains $r = 1.08$ g plant/g insect per day. This is 0.120 g plant/m^2 d for a biomass of 111 mg/m^2, or 12 g/m^2 in a 100-d growing season. The proportion of the plant biomass consumed by insects would thus be 3.8%, which compares favourably with the 4% estimate reported for the 1958 data [9].

This amount of plant consumption is the amount necessary to maintain the steady-state equilibrium concentration in the herbivorous insects. A certain amount of the insect biomass is lost through mortality during the season, and is offset (or more than offset) by insect production. This production represents an additional consumption of plants by insects, for which we have no measure in this mixed insect community. However, estimates of feeding for production in various communities are usually about 25% of the amount of feeding required for maintenance [11]. If this is true for the insect community on White Oak Lake bed vegetation, the total average daily consumption of plants would be about 0.150 g, or 15 g per season, or 4.7% of the plant biomass consumed during the entire growing season.

Similar calculations can be made for predaceous insects from the data in Table II. If all of the predators are assumed to feed on herbivores, and thus secondary predation is ignored, r can be estimated as 2.06 g herbivore consumed per g predator per day, or 0.024 g herbivore consumed/m^2 d, or 2.4 g herbivore consumed/m^2 per growing season. This value is 15% of the amount of plant material consumed by the herbivorous insects, but 80% of the total insect production estimated above. These estimates of herbivore consumption by predators seem high. However, TEAL [12], in discussing the predator-prey balance in a temperate cold spring, estimated non-predatory mortality of herbivores at 19.4%, which is the converse of the 80% predatory mortality estimated here. The relative paucity of predaceous insects makes sampling more difficult for both Cs^{137} content and biomass estimates. Further sampling and analysis of the predator trophic level will be required to improve estimates of food consumption by predators.

DISCUSSIONS AND CONCLUSIONS

The field aspects of the two examples given here were handled under conditions unfavourable for radioactive tracer work. White Oak Lake bed contains a mixture of heterogeneously distributed radioisotope species, at levels of radioactivity generally lower than is desirable for tracer studies. These difficulties have been overcome in newly created tagged field sites. Insect consumption of foliage in a forest site tagged with Cs^{137} is being measured in a manner similar to the studies performed on White Oak Lake bed, except that control over distribution and concentration of caesium-137 in the forest system has greatly eased the problems of measurement. The accumulation of various radioisotopes by forest-floor arthropods also is being studied through steady-state concentrations and biological half-lives. In this case tagging has been accomplished by placing tagged tree leaves in small fenced (1-m^2) plots. The arthropod food chain which has as its base leaf litter is being studied in this manner.

Preliminary results from these additional studies have confirmed the value of measurements of biological half-lives for the interpretation of food-chain transfer of radioisotopes. These radioisotope techniques are fulfilling their initial promise as an uncomplicated technique for specifying rates of movement of materials along food chains, and thus quantifying the relationships of herbivorous insect populations to plants and of predators to prey.

REFERENCES

[1] SMITH, F.F., Bull. Entomol. Soc. America 8 (1962) 188-189.
[2] RINGS, R.W. and LAYNE, G.W., J. Econ. Entomol. 46 (1953) 473-477.
[3] JENKINS, D.W., in Radioisotopes and Radiation in Entomology, IAEA, Vienna (1962) 3-20.
[4] DAVIS, J.J and FOSTER, R.F., Ecol. 39 (1958) 530-535.
[5] ROBERTSON, J.S., Physiological Rev. 37 (1957) 133-154.
[6] CROSSLEY, D.A., Jr. and HOWDEN, H.F., Ecol. 42 (1961) 302.
[7] BROWN, W.J., Canad. Entomol. 88 (suppl.3) (1956) 10-11.
[8] AUERBACH, S.I. and CROSSLEY, D.A., Jr., Proc. 2nd UN Int. Conf. Peaceful Uses of Atomic Energy, 18, Geneva (1958) 494.
[9] CROSSLEY, D.A., Jr., in Radioecology, Reinhold, New York (1963).
[10] LANGHAM, W.H. and ANDERSON, E.C., Health Physics 2 (1959) 38.
[11] TEAL, J.M., Ecol. 43 (1962) 614-624.
[12] TEAL, J.M., Ecol. Mono. 27 (1957) 296-297.

DISCUSSION

H. HUQUE: What was your technique for measuring radioactivity in vivo?

D. A. CROSSLEY: We used three methods: well-type scintillation counting for single insects; similar instruments for groups, so that we could measure and follow individuals and groups of insects through time; and total-faeces collections from cultures through time.

W. KLOFT: Did you include ants among predatory insects in your ecological analysis? Your method is excellent for estimating the predatory effect of ant colonies, but some complex problems arise here, such as social food exchanges within colonies.

D. A. CROSSLEY: The classification of certain insects as predators or as herbivores is bound to be an arbitrary one, since some insects will of course change their habits with changes in life history. Our method, therefore, was to consider the stage of the life-history. Larval forms which are predaceous were included as predators. A herbivorous adult was counted as a herbivore, even if the larval stage was predaceous. I believe we counted ants as herbivores.

R. von BORSTEL: Did you carry out your feeding experiments at different temperatures?

D. A. CROSSLEY: Yes, and we found that elimination rates follow approximately a Q_{10} law, i.e. for doubled metabolic activity twice the elimination rate. This suggests the use of elimination rates for radioisotopes to measure metabolic rates, which I believe to be feasible with the proper combination of isotope and insect. By selecting the proper isotope, in other words, you may be able to estimate metabolism in invertebrate populations through changes in the elimination rate.

M. FRIED: If I understood correctly, you have estimated the leaf consumption per unit of body-weight of the insect, using steady-state values of Cs^{137}. You also had the necessary data for the same calculation using steady-state values of Sr^{90}. Did these calculations give you similar results, and if not, why not?

D.A. CROSSLEY:. No, strontium concentrations did not yield identical results. The discrepancy is due to less complete absorption of Sr^{90} from the intestinal tract. We made the assumption of complete absorption in these studies. That is almost true for caesium, but definitely not true for strontium.