Radioisotopes and Radiation in Entomology

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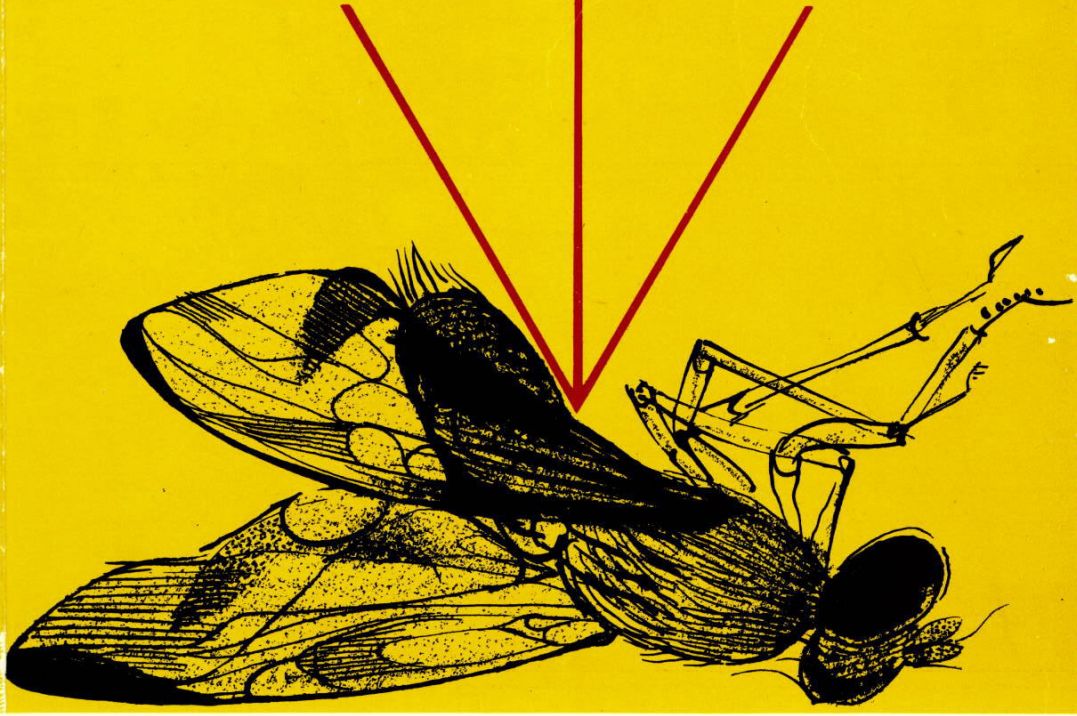

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA 1962

RADIOISOTOPES AND RADIATION IN ENTOMOLOGY

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FOREWORD

One of the most serious problems affecting the world's food supply is the enormous direct and indirect damage done by insects and other pests to agricultural crops and livestock. Each year the losses to agriculture caused by insects alone amount to thousands of millions of dollars; but in many regions of the world the true losses must be measured, not in money, but in terms of the misery, disease and loss of productivity that a serious food shortage entails. These losses can be diminished only by controlling or eliminating insects and other pests.

For some considerable time man has been fighting constantly against the innumerable insects which attack crops, livestock and health. Millions of tons of chemical poisons are used in this battle each year. But the use of these weapons has introduced new problems. Insects have, for instance, shown a capacity for developing resistance to these toxic substances so that new insecticides constantly have to be developed. Furthermore, the chemicals used are often toxic to animals and even to man himself. Long-lasting insecticide residues on consumable crops therefore present a potential hazard to public health.

Although radioisotopes are not a universal panacea, they play a unique role in opening several new avenues for research and an approach to new control methods. Research with radioisotopes improves understanding of the physiology and behaviour of insects and of their biochemical processes. This knowledge is essential for the development of better control techniques and more effective insecticides.

In radiation man has found a new and additional weapon for eliminating insect populations. The direct killing effect of radiation can, for instance, be used in the disinfestation of stored products. Another effect of radiation is the induction of sterility or lethal mutations which has already proved to be of extreme practical value, especially when used on insect populations already reduced to small numbers by insecticides.

The Proceedings now published give the record of the Symposium on Radioisotopes and Radiation in Entomology, held in Bombay at the invitation of the Indian Government. It was the first meeting organized by the IAEA in Asia. It discussed the above-mentioned problems and showed that radioisotopes and radiation are tools of real proved value of even greater potential than had previously been realized. It is hoped that the proceedings will be a valuable source of information to agricultural scientists and authorities.

The Agency's sincere thanks are due to the Indian Government, who so generously provided facilities and thus greatly assisted the Agency in the organization and conduct of the Symposium.

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ON THE ROLE OF LETHAL MUTANTS IN THE CONTROL OF POPULATIONS

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Abstract — Résumé — Аннотация — Resumen

On the role of lethal mutants in the control of populations. Population control by release of irradiated males requires that the sperm must be damaged by radiation. The type of damage induced by radiation imposes a restriction on which species may be controlled because if the sperm are functionally damaged by radiation, then for effective control, the females must be monogamous. If dominant lethality is induced in sperm then either polygamy or monogamy may prevail.

It is generally accepted that dominant lethal events are induced in sperm at doses much lower than those required to hamper sperm function or cause sperm inactivation. With *Drosophila* it is possible to test directly the effect of releasing irradiated males into an artificial population where polygamy is the rule.

Preliminary experiments have been performed under conditions of unlimited production of offspring.

It appears that radiation induces dominant lethality in sperm, and the sperm that bear dominant lethals are able to compete successfully with normal sperm. A series of tests are currently under way to ascertain the degree of induced dominant lethality and sperm inactivation at different X-ray dosages.

A series of experiments are outlined in a general discussion of the possible use of dominant and recessive lethals for bringing about collapse of artificial and natural populations.

Le rôle des mutants létaux dans la lutte contre les insectes. Pour que le lâcher de mâles irradiés puisse contribuer à la lutte contre la prolifération des insectes, il faut que le sperme ait été endommagé par les rayonnements. Les espèces contre lesquelles on peut lutter par ce moyen dépendent du genre de dommages radioinduits. En effet, si seule la fonction spermatique est atteinte, l'irradiation ne sera un moyen de lutte efficace que si les femelles sont monogames; si au contraire on provoque une létalité dominante dans le sperme, les femelles peuvent être polygames ou monogames.

On admet généralement que les phénomènes de létalité dominante interviennent dans le sperme à des doses sensiblement inférieures aux doses nécessaires pour modifier la fonction spermatique ou rendre le sperme inactif. La drosophile permet de constater directement les effets du lâcher de mâles irradiés dans une population artificielle dans laquelle la polygamie est la règle.

Les auteurs ont fait des essais préliminaires dans des conditions de reproduction illimitée.

Il est apparu que les rayonnements provoquent une létalité dominante dans le sperme, et que les spermes à mutants létaux dominants ne le cèdent en rien aux spermes normaux. Les auteurs font actuellement des essais pour déterminer le degré de létalité dominante radioinduite et le degré d'inactivation du sperme, selon la dose de rayons X.

Le mémoire présente les résultats des expériences faites et donne un exposé général des diverses façons dont on peut tirer parti des mutants létaux dominants ou récessifs pour décimer des populations artificielles ou naturelles.

О роли летальных мутантов в контроле над видами. При осуществлении контроля над видами при помощи выпуска облученных мужских особей необходимо, чтобы сперма была повреждена радиацией. Тип повреждения, вызванного радиацией, налагает ограничение в отношении возможности контроля над видами, поскольку, если радиация вызвала нару-

шение функций спермы, для обеспечения эффективности контроля необходимо, чтобы женские особи были моногамны. Если в сперме образована доминирующая летальность, то возможно преобладание либо полигамии, либо моногамии.

Общепризнано, что случаи доминирующей летальности вызываются в сперме дозами значительно меньшими, чем дозы, требуемые для нарушения функций спермы или для прекращения ее жизнедеятельности. При использовании дрозофилы возможно непосредственно исследовать влияние выпуска облученных мужских особей в искусственный рой, где полигамия является правилом.

Предварительные опыты были проведены в условиях неограниченного воспроизводства потомства.

Оказалось, что радиация вызывает в спермах доминирующую летальность, и спермы, несущие дозы доминирующей летальности, способны успешно конкурировать с нормальными спермами. В настоящее время проводится серия опытов с целью установления степени обрузуемой доминирующей летальности и прекращения жизнедеятельности спермы при различных дозах облучения рентгеновскими лучами.

Результаты наших опытов будут представлены вместе с общим обзором возможного использования доз доминирующей и уменьшающейся летальности для уничтожения искусственных и естественных роев.

Función de las mutaciones letales en la lucha contra los insectos. Para que la suelta de machos irradiados pueda contribuir a la lucha contra la proliferación de los insectos, es preciso que el esperma sufra daños como consecuencia de la irradiación. El tipo de daño radioinducido limita el número de las especies que pueden combatirse, porque en los casos en que la irradiación causa daños funcionales en el esperma, las hembras han de ser monógamas para que la lucha tenga eficacia. Si se inducen en el esperma mutaciones letales dominantes, puede predominar la poligamia o la monogamia.

Se admite generalmente que las mutaciones letales dominantes son inducidas en el esperma mediante dosis muy inferiores a las necesarias para entorpecer la formación del esperma o producir su inactivación. Empleando *Drosophila*, es posible comprobar directamente la influencia de la suelta de machos irradiados sobre una población artificial en la que predomina la poligamia.

Se han efectuado experimentos preliminares en condiciones que favorecían la producción ilimitada de progenie.

Se ha comprobado que la irradiación induce mutaciones letales dominantes en el esperma, y que los espermas que han sufrido esas mutaciones son capaces de competir con éxito con los espermas normales. Se está llevando a cabo una serie de experimentos para averiguar el grado de letalidad dominante inducida y de inactivación del esperma con diferentes dosis de rayos X.

Se presentan los resultados de estos experimentos, junto con un estudio general de la posible utilización de las mutaciones letales dominantes y recesivas destinadas a provocar el exterminio de las poblaciones naturales y artificiales.

I. Introduction

KNIPLING [1] has discussed the possibility of controlling insect and other animal populations through application of the following principle: introduction of a number of sexually vigorous, sterile males into the natural population will have a greater influence in reducing its biotic potential than if the same number were eliminated by destruction or removal. He has also discussed the need for studying physical and chemical means which may produce sterility in various types of animals. The eradication of the screwworm from Curaçao and Florida serves to underscore the essential correctness of his principle.

One of the present authors [2] has pointed out that where males are irradiated and released in the field, the restriction of monogamy in females of a species is not a requirement for controlling population-size, since sterility of the males (*sensu stricto*) is not necessarily the radiation effect which causes the population decline. KAUFMAN and WASSERMAN [3] have indeed shown that dominant lethals are induced by X-rays in the screwworm. Even

with multiple matings by every female, the population collapse would be as inevitable and rapid as when the females are monogamous.

II. Dominant lethals and sterility

Probably the most important effect of radiation is the induction of dominant lethal mutations in the sperm, not male sterility. For illustration, let us consider an insect population made up of ten males and ten virgin females. Ninety irradiated males are introduced into this population. The females mate only once. For this simple example, let us assume that nine of the females will mate with irradiated males and produce no viable offspring, and that one will mate with a normal male and produce normal offspring. 100% of the eggs from one female and 10% of the total batch of eggs will survive. In this case, it will not matter whether the sperm contain dominant lethals or the males are made sterile.

Now consider the same conditions, but let every female mate ten times. Of her ten mates, assume nine have been irradiated and have sperm containing dominant lethals and one has normal sperm. 10% of the eggs from each female and therefore 10% of the batch will survive; this result is identical to the outcome predicted from strict monogamy, even though polygamy is the case here. On the other hand, in the polygamous case, if the irradiated males are sterile, 100% of the total batch of eggs survive.

It is obvious that, if radiation primarily induces dominant lethal mutations in the sperm, the results are identical whether female monogamy or promiscuity obtains. In practice, one can, of course, imagine circumstances whereby monogamy or polygamy could influence the rate of decline, and according to the circumstances, polygamy actually could be a necessary requirement for population collapse. For instance, if selective mating of brothers and sisters occurs when adults from one clutch emerge simultaneously (as with certain wasp species), then further matings to irradiated non-brothers would be required for successful population-control by the irradiation-of-male method.

It is generally known that at levels of radiation of about 10 kr, dominant lethal events are induced in over 99% of the sperm, both in the fly *Drosophila* and the wasp *Habrobracon*. However, to obtain complete killing of the sperm, radiation levels of about 200 kr are required [4] [5]. It has been observed in *Drosophila* that dominant lethals are induced in mature sperm and spermatocytes in later stages of spermatogenesis, and that after these cells are exhausted a period of sterility sets in, from which, at doses of about 10 kr, the flies never recover [6]. The process of sperm exhaustion following irradiation requires about a week of continuous multiple matings, but *Drosophila* males that have not been mated for 19 d after irradiation still have sperm reserves containing dominant lethals [7]. With the simple cytological procedures now available for determining, at different doses of radiation the components of dominant lethality [8] and sterility [6], there should be little difficulty in determining dose-effect relations for any insect.

Furthermore, it seems likely that collapse of populations from chronic or acute irradiation is principally a reflection of the sensitivity of the dominant lethality component of the mutation spectrum. The general problem of eradication of populations by introduction of irradiated males is, therefore, closely related to the problem of population collapse from induction (by radiation) of dominant lethality *within* a population.

III. Experimental plan

Since the analytical and experimental procedures we are using with *Drosophila* populations are applicable to almost every other insect that can be reared in the laboratory, these methods are briefly described even though they have not yet been implemented in detail.

It is expected that a number of parameters affecting population size could be determined and that on this basis equations could be derived that show the most efficient radiation dose for males, the most effective time interval for the introduction of new males, and the optimal number of males introduced. Accordingly, experiments are under way to assess the optimal ratio of normal males to males which carry different amounts of dominant lethals or are sterile. Other parameters, such as the sensitivity of the gametes to radiations (dominant and recessive lethal mutability), the effective lifetime of females, and the lifetime of unirradiated and irradiated males, are already known or can be easily determined.

The second phase of the programme will be instituted on completion of data-processing to find how closely the observed results fit the expected results. If unforeseen parameters are uncovered (if population decline is slower or more rapid than expected), these will be studied with the second series of experiments.

The second series will consist in adjusting the parameters (radiation dose, number of males released, and intervals of release) so that the number of fertile flies will become constant and remain stabilized.

From this study it is expected that formal equations can be established from which one could learn many basic genetic features of natural populations on the verge of extinction; these equations also could serve as a basis for estimating parameters necessary for the eradication of any insect pest.

The third phase of the programme will be to use the data revealed by eradication from irradiation and release of males to estimate the effects of acute and chronic radiation directly upon populations of *D. melanogaster* without the introduction of irradiated males.

This programme could then fit in neatly with a separate programme being initiated to determine the effects of the high doses of radiation necessary for exterminating populations without the addition of individuals from outside. The components of the mutation spectrum that are effective in such an extermination (radiation-induced dominant lethality, recessive lethality, and subvital mutations) will be computed. These data will be used to aid in estimating radiation hazards to human populations.

IV. Recessive lethality and female sterility

It is of interest to consider briefly the possible consequences of introducing recessive lethal genes or female sterility genes into a natural population. With the successive introduction of large numbers of males, heterozygous at several loci for these genes or types of similar genetic character, it is inevitable that the reproducing population size would be depressed. Elimination of the population by this type of control-measure would proceed more slowly than after the introduction of males containing dominant lethals, and would reach equilibrium after each group of genetic defectives had been introduced. Final collapse of the population could not result directly from interference with its genetic structure but would depend upon extraneous factors such as the inability of mating pairs to find one another after the population declines to a certain critical size.

V. Distortion of segregation ratios

Several genetic and physiological situations are known which have one characteristic in common, namely that of distorting the normal, mendelizing, 1:1 ratio of gametes or of altering zygotic ratios. Two examples will be discussed which might possibly be used for the control and eradication of natural populations, once their characteristics become fully understood.

The first example concerns the existence of aberrant sex ratios in the genus *Drosophila*, caused by factors inherited in the cytoplasm [9] [10] or nucleus [11] [12]. Disproportionate segregation should eventually result in the population becoming either all male or all female; extermination would follow. Of such cases discovered in nature, small micro-environmental differences [13] or genetic factors working in the opposite direction [12] [14] tend to keep the sex-ratio factors from obliterating a population. For a population to survive, and thus for the condition to have been discovered in the first place, the sex-ratio factors must, of course, be mild and subject to selection pressures.

It is possible that sex-ratio distorters coming by chance into a genotype have been powerful enough to cause the extinction of a species. When better understood, sex-ratio distorters could become a useful weapon for the control of economically undesirable species that could not otherwise be touched.

The second example is that of distortion of segregation, which does not necessarily involve the production of unequal numbers of gametes deriving from nuclei at the opposite ends of the spindle (meiotic-drive chromosomes [15]). This provides a mechanism whereby a few individuals introduced into a population will have their chromosomes pass into the genetic make-up of the entire population without necessarily harming it. If, on the other hand, recessive genes for female sterility were on the chromosome exhibiting meiotic drive, then, as this chromosome sweeps through the population, the homozygous females would be useless for further propagation, but the males would still be produced in disproportionate numbers and eventually every female would become sterile. At present not enough is known about the characteristics of the meiotic-drive chromosomes to construct them at will, but meiotic-drive phenomena are being investigated vigorously [16]. It is possible that meiotic drive has been a potent evolutionary force [15] which could be controlled in order to exterminate economically undesirable populations.

VI. Summary

Radiation induces dominant lethal mutations in sperm. It therefore can be shown that monogamy is not requisite for eradicating a population through the introduction of irradiated males.

An outline is presented for experimental analysis of population collapse by the irradiation-of-male method where females mate more than once.

Possible effects on populations of release of males containing recessive lethal mutations or mutations for female sterility are briefly discussed. The possibility of genetic induction of population extinction is explored.

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