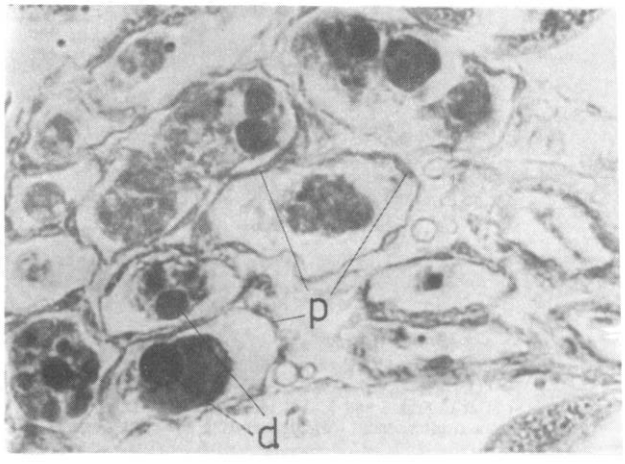

Fig. 6

Cross-section of a clump of ovarioles in an adult Dacus oleae Gmel. female irradiated at the pupal stage with 20 kr.
In the inner part of the peritoneal membranes (p) which are intact, a small number of cells can be seen, all of them degenerating (d), with a pyknotic nucleus and highly pyroninophile cytoplasm. Brachet's reagent, 560 diam.

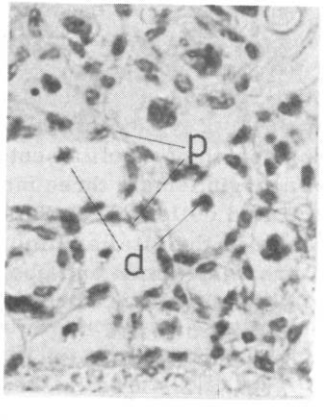

Fig. 7

Similar to Fig. 6, but with the nuclear structure more clearly brought out by Feulgen's reagent
In the inner part of the peritoneal membrane (p), very few nuclei, all degenerating (d), are visible.
Feulgen's reagent, 560 diam.

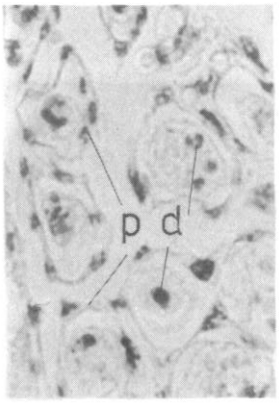

Fig. 8

Similar to Figs. 6 and 7, but after dose of 30 kr
(p), peritoneal membrane; (d) degenerating nuclei of oogonia.

BACCETTI [24] described in the nervous system of other Diptera. On the basis of our present knowledge of the connective tissue of insects, they may be assumed to be collagen.

Each ovariole is surrounded by a robust peritoneal membrane, nucleated and highly PAS-positive. The electron microscope shows it to consist of very broad, flattened cells (Figs. 9, 14 and 15) lying between two basal membranes which are homogeneously granulated and about 1000 Å thick.

These cells have small nuclei, with intact karyotheca having few pores, very abundant cytoplasm containing small ergastoplasmic formations, small mitochondria, well-marked Golgi fields and myofibrillae with well-marked myofilaments, clearly of the striated type. The cytoplasm is also furrowed by tracheoles and by minute tubules of sarcoplasmic reticulum: it shows, in other words, all the features of typical muscular tissue, with the special characteristic of a striking dearth of myofilaments.

Inside the ovariole of a virgin female three main types of cell are found: the oogonia, non-differentiated cells concentrated in the germarium, the oocytes, and the nurse-cells in the vitellarium.

The oogonia (Fig. 3) are very small and poor in cytoplasm, which is always weakly pyroninophile: they have spheroidal nuclei about 6 μm in diameter and frequently go into mitosis.

Under the electron microscope (Fig. 13) the oogonia with resting nucleus seem to be rather poor in organelles; one observes a few vesicular mitochondria with low internal cristae, spheroidal ergastoplasmic cysts rich in ribosomes, and some scattered Palade granules.

The Golgi apparatus is not evident; the cell boundaries are very tortuous; they almost always appear to be intact and show no signs of exchanges between adjacent cells. The nucleus seems to be filled with a coarse granular substance, very homogeneously diffused and rather thick, and contains only one rather small nucleolus. The karyotheca has few pores; its two osmiophilic layers are in many cases hollowed out to a considerable

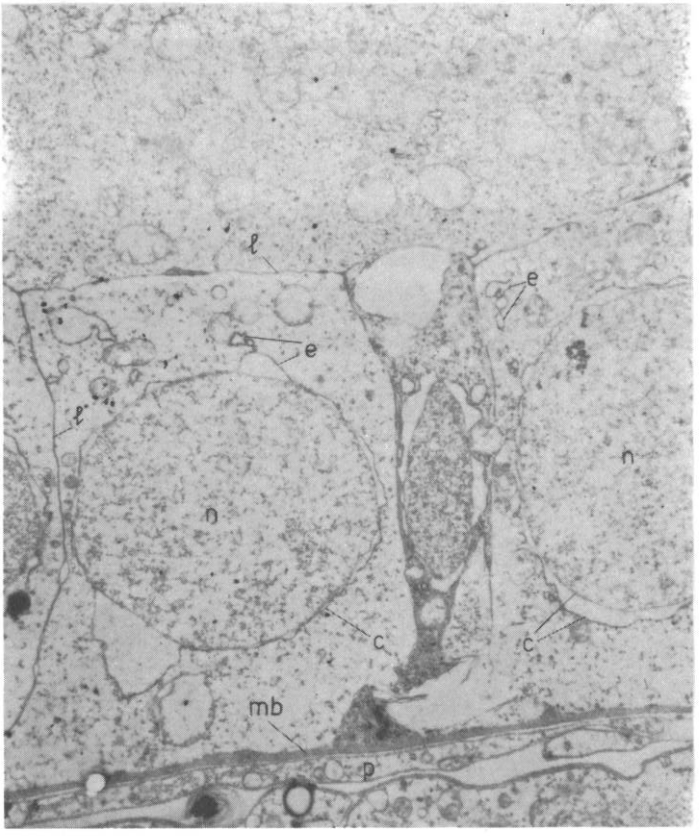

Fig. 9

Electron micrograph of the cortical zone of the ovariole in a normal adult Dacus oleae Gmel. female The section was made on the level of the vitellarium. In the upper part of the figure a portion on a nurse-cell can be seen. In the lower part, grouped together, there are some follicular cells, whose richness in ergastoplasm is evident. The ergastoplasm clearly originates in the outer layer of the karyotheca. (c), karyotheca; (l), cell membranes; (e), ergastoplasm; (m) mitochondria; (mb) basal membrane; (n) nuclei of the follicular cells, (p) peritoneal membrane.
Hitachi HS-6, 11 200 diam.

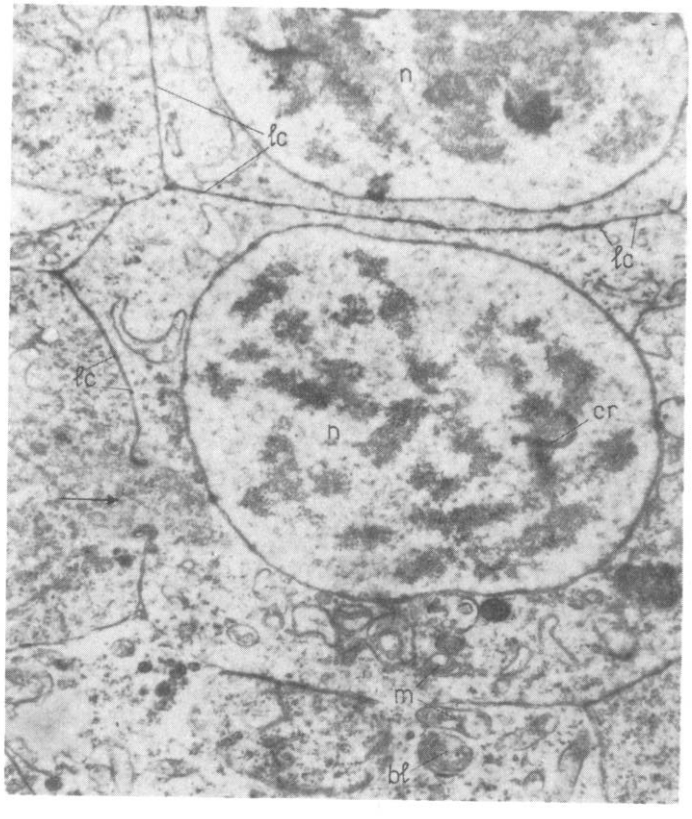

Fig. 10

Electron micrograph of some adjacent nurse-cells in the vitellarium of an ovariole in a normal adult Dacus oleae Gmel. female

Note the presence in the nuclei (n) of segments of polytene chromosomes (cr), and the richness of the cytoplasm in mitochondria (m) and lamellar bodies (bl). The cell membrane (lc) is very straight, but seems to be interrupted at the points indicated by an arrow, where an intense migration of substances can be seen. Hitachi HS-6, 11 200 diam.

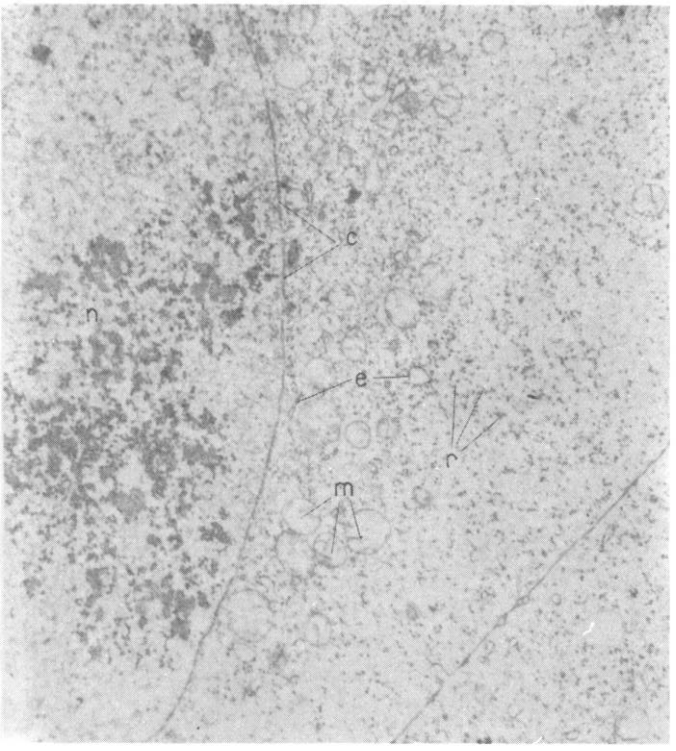

Fig. 11

Electron micrograph of a group of nurse-cells which have attained complete growth in the same subject as Fig. 10.

Note the large size of the nucleus (n) whose karyotheca (c) has no relation of continuity with the ergastoplasmic cysts (e) surrounding it. Besides these, many mitochondria (m) and free ribosomes (r) can be seen in the cytoplasm.

Hitachi HS-6, 11 200 diam.

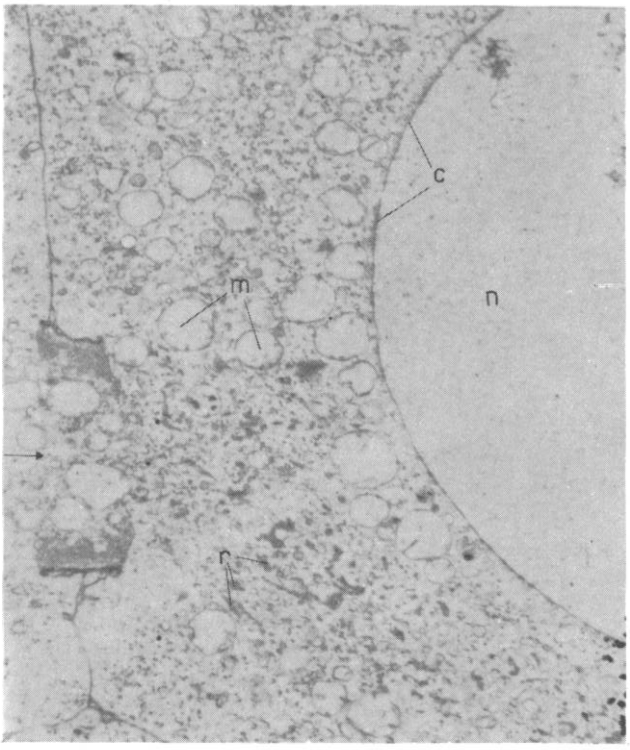

Fig. 12

Electron micrograph of a portion of an oocyte in the same subject as Fig. 10
Note the nucleus (n), very poor in chromatin and with intact karyotheca (c). In the cytoplasm there are many mitochondria (m) and free ribosomes (r), organelles from the neighbouring nurse-cell are passing through a wide opening (indicated by an arrow) in the cell membrane.
Hitachi HS-6, 11 200 diam.

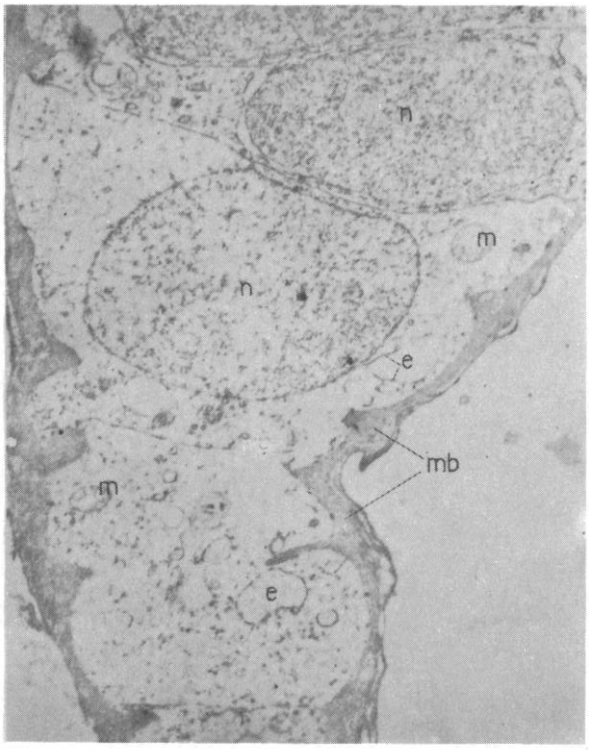

Fig. 13

Electron micrograph of a longitudinal section of the extremity of the germarium in an ovariole belonging to the subject of Fig. 10.

Note the thick basal membrane (mb) which surrounds the oogonia sideways. The oogonia are densely grouped, with large nuclei (n) and cytoplasm rather poor in organelles, limited to some mitochondria (m) and a small amount of ergastoplasm (e).

Hitachi HS-6, 11 200 diam.

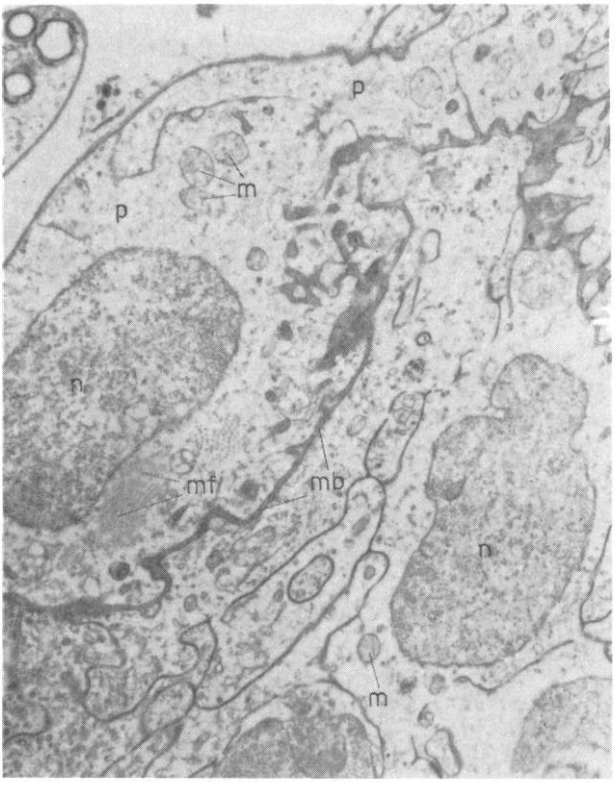

Fig. 14

Electron micrograph of the region corresponding to the vitellarium in an ovariole of adult Dacus oleae Gmel. female irradiated at the pupal stage with 5 kr

Note the perfect preservation of the peritoneal tunica (p) in which a nucleus (n), some mitochondria (m) and myofilaments (mf) can be seen. In the inner part of the basal membrane (mb) the cells are very small and resemble oogonia. Their nuclei (n) and mitochondria (m) are both well-preserved.

Hitachi HS-6, 11 200 diam.

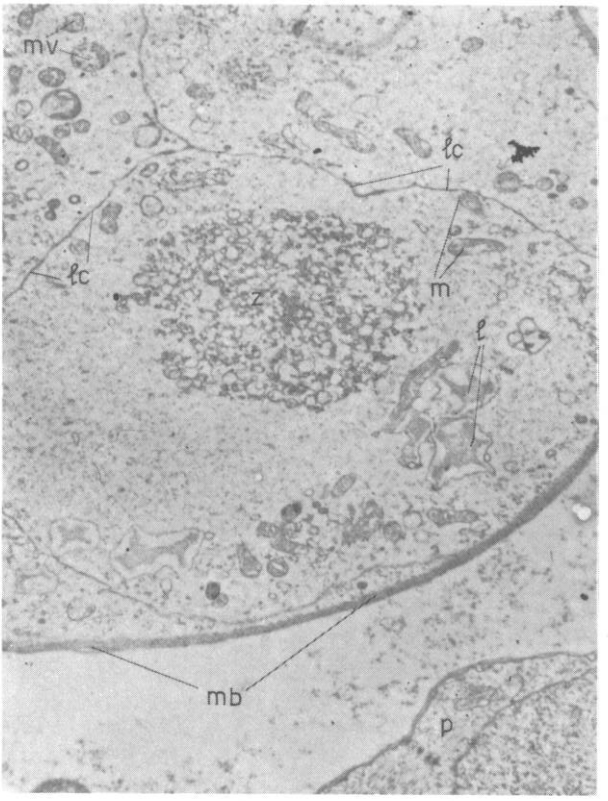

Fig. 15

Electron micrograph of a more distal level of the ovariole in the subject of Fig. 14. Inside the peritoneal membrane (p) and basal membrane (mb), some oogonia can be seen in which the cellular membranes (lc), some mitochondria (m) and multivesicular bodies (mv) seem to be perfectly preserved, whereas many other organelles are grouped in necrotic clumps (z) and vacuoles full of lipids (l) appear. Hitachi HS-6, 11 200 diam.

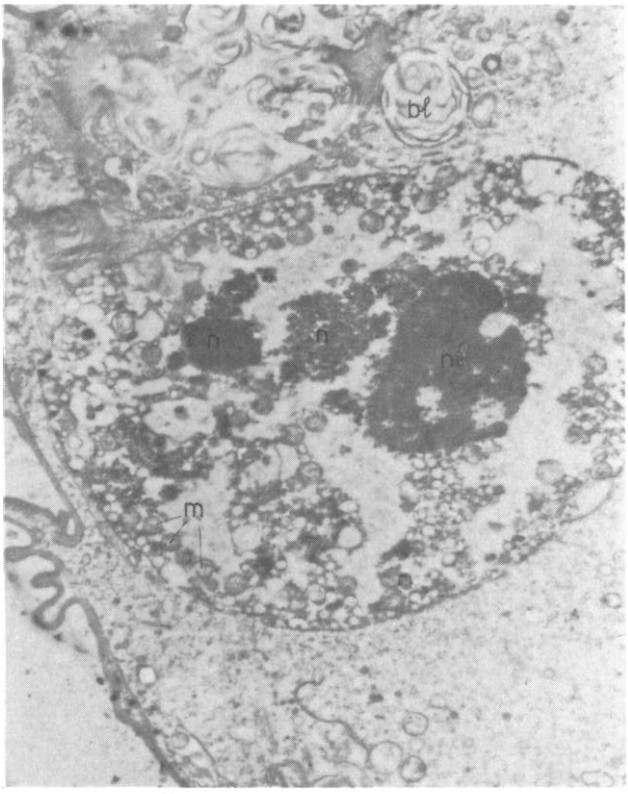

Fig. 16

Region of heavy damage at another level of the ovariole in the subject of Fig. 14 The cellular membranes (lc) seem to be often interrupted, while all the cytoplasmic organelles are accumulated in necrotic clumps.
Hitachi HS-6, 11 200 diam.

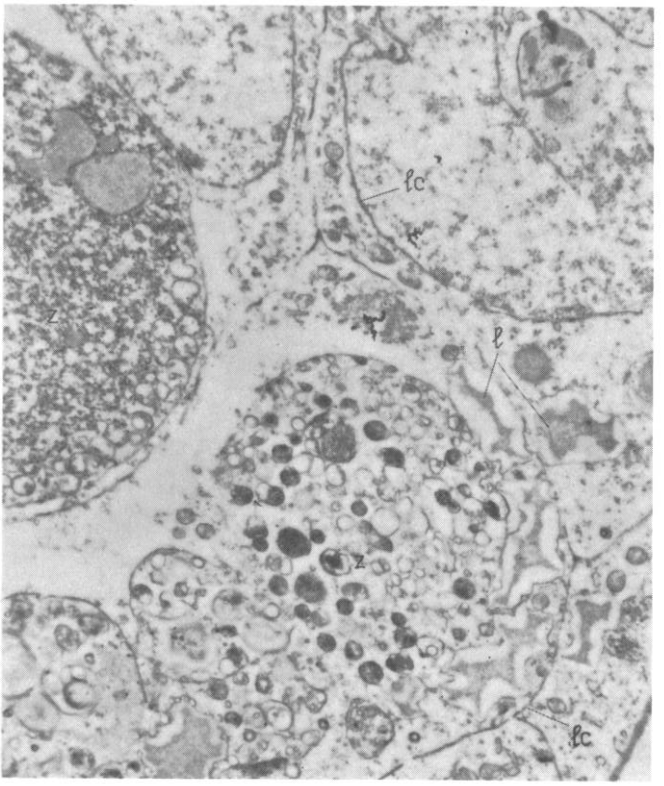

Fig. 17

Cell in karyorrhexis in the ovariole of an adult Dacus oleae Gmel. female, irradiated with 5 kr at the pupal stage

The mitochondria (m) and the other cytoplasmic organelles are accumulated in clumps, while the nuclear material (n) seems to have gathered into thick osmiophile clots. In the surrounding cells many organelles, including some lamellar bodies (bl), appear to be well-preserved.

Hitachi HS-6, 11 200 diam.

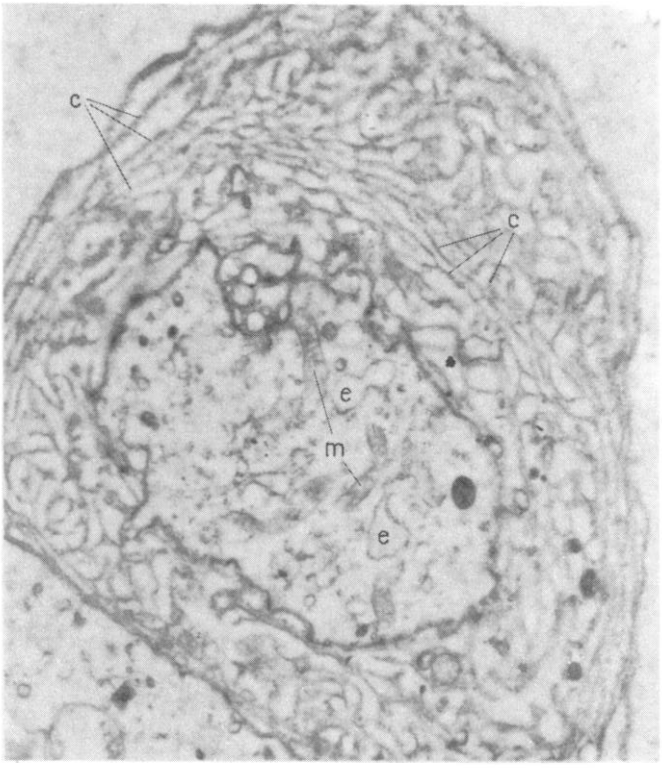

Fig. 18

Apex of an ovariole of the subject of Fig. 17
The only visible cell in which the organelles (mitochondria (m) and ergastoplasm (e)) seem to be well-preserved is surrounded by a thick involucrum of collagen fibrillae (c), with distinct regular markings.
Hitachi HS-6, 11 200 diam.

Fig. 19

Apex of the ovariole in an adult Dacus oleae Gmel. female irradiated at the pupal stage with 12 kr The cells all show signs of morbidity in the nuclei (n) and in the cytoplasm, where the mitochondria (m) seem rather dense and where frequent lipidic deposits (l) appear. The cells are isolated from each other and surrounded by a thick texture of collagen fibrillae (c).
Hitachi HS-6, 11 200 diam.

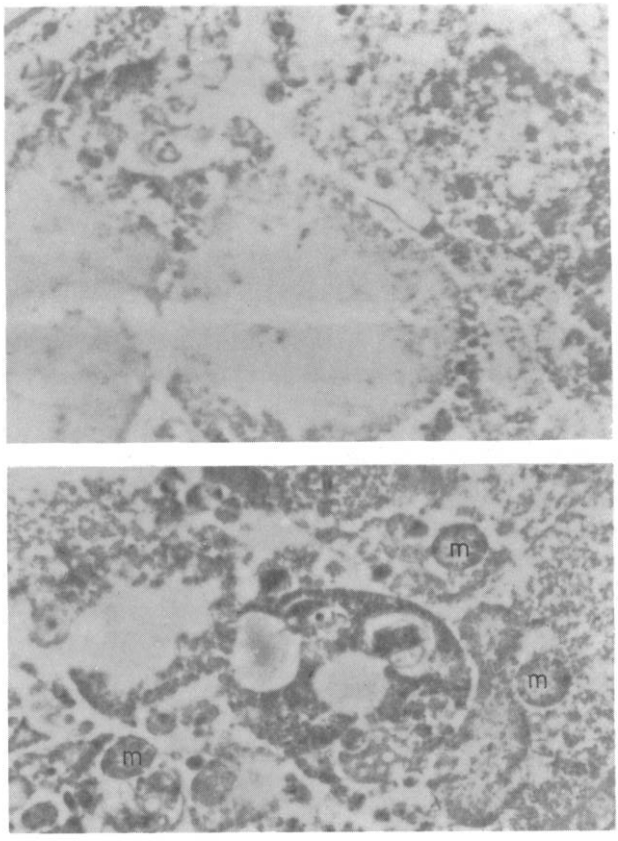

Fig. 20

Portions of cytoplasm in the ovarioles of an adult Dacus oleae Gmel. female irradiated with 20 kr at the pupal stage
Some degenerating mitochondria (m), can be seen, but the other organelles are unidentifiable.
Hitachi HS-6, 11 200 diam.

extent and show frequent vesiculations, leading to the formation of ergastoplasmic cysts from the outer layer, which is constantly very rich in ribosomes.

Oocytes and nurse-cells form groups of 16 units in each ovarian chamber. Of the 16 cells, only one evolves into a mature oocyte ready for fertilization; the remainder have a trophic function and stay between the oocytes themselves, conditioning the meroistic polytrophic type of the ovariole.

The size of the oocyte varies according to the phase of development of the egg. The oocyte (Fig. 2) is characterized, during the prophase, by a large (about 15 μm in diameter), distinctly achromatic, spheroidal nucleus, which is obviously diploid; this nucleus is clearly distinguishable from the polyploid nuclei of the nurse-cells (Fig. 1). The cytoplasm is seen under the optical microscope to be basophile and distinctly granular. During the development of the eggs it becomes charged with yolk spherules and its size increases enormously, while in the nucleus the compact group of chromosomes of the meiotic metaphase is blocked to await the continuation of meiosis that is caused by fertilization.

Under the electron microscope (Fig. 12) the oocyte in prophase is also well characterized by the achromatic appearance of the nucleus, which is transparent to the electrons in almost the whole of its surface, apart from a few accumulations of nuclear material.

The nuclear envelope seems to be intact throughout its length, to have very few pores and to be unconnected with the ergastoplasmic cysts.

The surrounding cytoplasm is very rich in organelles, especially large spheroidal mitochondria with moderately distended internal cristae. One also observes some lamellar bodies, lysosomes and masses of ribosomes, but ergastoplasm in its typical appearance is not found. The cell-boundaries of the oocytes are well squared and often have comparatively wide openings (as much as 2-3 μm) letting accumulations of substance (mitochondria and ribosomes) pass through from the surrounding nurse-cells. The cell membrane around the openings is perpendicular to the cell wall and looks like large rings (Fig. 12).

The nurse cells in full development have a very large spheroidal nucleus (reaching 10-12 μm in diameter) quite rich in DNA. In earlier studies [25] it was demonstrated that this value would correspond to 16-ploidy, although the structure of the chromosomes (Fig. 1) suggests that these cells are polytene. There is a single very large nucleolus, and the cytoplasm, seen under the light microscope, is homogeneous and rather rich in RNA.

Under the electron microscope (Fig. 10) the nurse-cells are seen to be bounded by straight membranes, almost completely without bends, often with quite wide openings at several points and communicating, either directly or by vesiculation and membrane flow effects, with neighbouring cells (trophocytes, oocytes or follicular cells). The richness of organelles (Fig. 11), consisting of large spheroidal mitochondria with few internal cristae, multivesicular and multilamellar bodies and small Golgi fields, is considerable. Sometimes large ergastoplasmic cristae are present, but more often the ribosomes are free and always extremely plentiful. These, together with mitochondria, pour into the oocytes through the large openings of the plasma membrane. The nucleus is filled with granular chromatin,

often in clumps; also the polytene, strongly osmiophilic chromosomes, looking like thick strings, can sometimes be seen, always in pairs. The nucleus is spheroidal and homogeneous. The nuclear envelope communicates with the outside through numerous pores with annuli; from its outside layer small chains of ribosomes originate which then separate into the cytoplasm, while the vesiculations with formation of ergastoplasmic cysts, which are characteristic of follicular cells, seem to be absent.

(B) Structure of the irradiated ovary

Females irradiated at the pupal stage with the lowest doses of irradiation (2, 4 and 5 kr) are found on emergence from the puparium to have very small ovaries bearing no eggs. At the lowest dose, a few mature oocytes may be found in the oviducts, but they are always malformed and have very little yolk. The ovarioles (Fig. 4) have a peculiar appearance: they show, throughout their length, inside the peritoneal sheath, an assemblage of non-differentiated cells, heaped up in disorder, that call to mind the oogonia in the germarium. These cells have diploid nuclei, with DNA accumulated in short, thick sticks; the cytoplasm is very sparse and poor in RNA. Mitosis is completely absent. In many cases the DNA is accumulated in one homogeneous drop, and later comes out by rupture of the nuclear envelope. One has, in other words, frequent pyknosis followed by karyorrhexis.

Less usually, small groups of cells tend towards a kind a maturation, i.e., the cytoplasm is slightly enriched in RNA, whereas the nucleus has not undergone any variation, either by becoming richer in DNA (as in the case of nurse and follicular cells), or by undergoing meiosis (as in the case of the oocytes).

Even under the optical microscope, these cells, and particularly those at the pointed end of the germarium, are seen to be sparsely distributed and separated from each other, and that compact mass of oogonia which characterizes the distal zone of normal ovarioles is no longer identifiable.

Investigation under the electron microscope confirms this picture.

In the whole ovary the greatest part of the tissue is constituted by peritoneal membranes, which preserve a regular structure (Fig. 14) and which, since they contain a much smaller organ than the normal one, are convoluted. Of the cells forming the ovarioles, the better-preserved ones show, as we have already seen under the light microscope, a structure quite similar to that of the oogonia: that is, they contain (Figs. 14 and 15) small mitochondria rich in cristae and small cysts of ergastoplasm, but very few free ribosomes. The nucleus shows chromatin normally distributed in granules, but it constantly shows signs of damage in the karyotheca, which is frequently seen to be interrupted for long stretches and to have lost the power of producing ergastoplasmic cysts.

Sometimes, though rarely, homogeneous, osmiophilic drops appear in the cytoplasm; they seem to be of a lipidic nature. In many cases (Figs. 16 and 17), the damage is more accentuated: the mitochondria, always slightly more opaque than in normal cases, contract and degenerate into homogeneous vesicles which are intensely osmiophilic; the lipidic drops increase in number and size; the free ribosomes tend to coalesce into granu-

lar, opaque clumps; the ergastoplasmic cysts and the Golgi fields dilate into spherical, empty vesicles.

The whole of the morbid organelles come together in degenerating masses displaced into the middle part of the cell(Fig. 15), while the remaining part of the cytoplasm contains only the submicroscopic trabecolatum. For its part, the nucleus degenerates with similar phenomena. Nuclear and nucleolar material gather separately and form large drops on the inside of the remains of the karyotheca, in which the normal exchanges with the cytoplasm are suspended; meanwhile breaks become more and more frequent. Mixed with these cells are some richer in ribosomes and ergastoplasm, but always with a small, diseased nucleus. Along the entire length of each ovariole, the basal membranes seem more compact and also slightly thicker than in normal subjects. However, the distal part (i.e., that of the germarium) presents a quite special picture (Fig. 18); it is surrounded by an enormous accumulation of collagen fibrillae identical with those which constitute the normal basal membrane, twisted up in several layers all round the cells and intruding between them in such a way as to separate them. The distal area of the ovariole is thus held together only by the accumulation of collagen fibrillae, which in normal subjects is much sparser and constitutes only the basal membrane. The direct inter-cell relations seem to be lost, but the extent of morbidity in the cells of this area is rather limited; they fall into the category of moderately damaged oogonia.

With doses of 8, 10 and 12 kr the damage to the ovaries is greatly increased. These are now tiny (they measure on the average $35 \times 15 \times 15\ \mu m$), are pyriform in appearance and have a very limited amount of cells, almost all degenerating. The peritoneal membrane appears, as usual, intact and constitutes the greatest part of each ovary; the ovarioles (Fig. 5) contain only a few cells (all of them can be regarded as degenerating oogonia) with sparse, Brachet-negative cytoplasm, and nuclei often in pyknosis or in karyorrhexis.

Under the electron microscope (Fig. 19), the integrity of the peritoneal membrane is confirmed, and one observes lesions of the oogonia of the same type as those described for the smallest doses, but becoming progressively more acute and serious as one proceeds from 8 to 12 kr. The characteristic increase of collagen fibrillae around the ovarioles is even more marked than in the cases described above, and the cells contained in the collagen mass seem to be small and degenerating. The cell boundaries and the nuclear envelope are often broken for considerable stretches and show no signs of exchange of substances; the mitochondria are small, dense and sometimes unidentifiable; ergastoplasm is almost entirely absent, whereas coarse lipidic inclusions in large vacuoles are abundant. The nuclear material is diffused in minute granules, often against the karyotheca, and shows no signs of a nucleolus.

At a dose of 20 kr, the damage to the gonads is even more accentuated. The ovaries are even smaller than in the cases just described and are reduced almost to nothing but empty peritoneal membranes (which appear, even under the electron microscope, perfectly preserved) whereas the oogonia have almost all degenerated. In fact one observes (Figs. 6 and 7) very few groups of cells along each ovariole, with almost always a nucleus in pyknosis

or karyorrhexis and very poor in cytoplasm, which seems to be stained by pyronin only in small, extremely concentrated drops.

Under the electron microscope (Fig. 20) the various cellular constituents are unidentifiable; the nuclear envelopes are dissolved and the nuclear content comes out in drops; the mitochondria are dense, granular and broken, tightly packed, by a homogeneous and granular substance which is difficult to identify, near the cell membranes. One recognizes some multilamellar bodies, quite altered, but on the whole the cells seem to be in necrosis.

At 30 kr the effect is even more accentuated. The ovaries are tiny (about $18 \times 12 \times 12$ μm) and very poor in cells (Fig. 8) on the inside of the peritoneal membranes, which are still intact. All of the oogonia present seem, without exception, to be in pyknosis or in karyorrhexis and present a cytoplasm which, under the optical microscope, seems concentrated in thick, strongly basophile drops, and under the electron microscope none of the cellular components is identifiable.

DISCUSSION

The results presented here lead us to the conclusion that the damage caused by ionizing radiation to the ovarian structures of Dacus oleae Gmel. irradiated at various doses at the pupal stage is qualitatively similar but variable in intensity.

The ovarioles always become extremely small, without mitosis and poor in cells, which are constantly blocked at the oogonial stage and are thus not permitted to differentiate into oocytes, nurse-cells and follicle cells. Thus there remains only one type of germinal element, the oogonia, which undergo alterations of all the cellular elements. The nucleus shows considerable pyknosis and karyorrhexis effects, nucleolar thickening and rupture of the karyotheca, which loses its contacts with the ergastoplasm; the cytoplasm is poor in ergastoplasm and shows few, small and thickened mitochondria but abundant lipidic inclusions, while the exchange with neighbouring cells becomes extremely poor or almost absent; in the case of extreme damage, large areas of cytoplasm seem to be necrotic, and the organelles, which are condensed into compact clumps, are unidentifiable. The single ovarioles are surrounded by a stroma of enormously thickened collagen fibrillae. The peritoneal membrane cells, on the other hand, do not show any alteration, even at the most powerful doses tested.

On the whole the alterations we have described generally agree with what has been reported on this subject in the literature, on both optical microscope studies in mammals and insects and electron-microscope investigation in mammals; thus, the nuclear lesions in the mouse described by Parsons are perfectly comparable with those that we have observed, even to the alterations of the mitochondria, the decrease of ribosomes, the accumulation of lipids, etc.

One particularly interesting observation that has emerged from our research is the blocking of the expulsion of ergastoplasmic vesicles from the outer layer of the nuclear envelope; this alteration had been observed earlier in the cells of the intestinal epithelium of irradiated Dacus oleae [23],

and the reason it is not reported in the descriptions of other authors (e. g. PARSONS [20]) is probably that a smaller amount of ergastoplasmic substance was contained in the cells they studied in comparison with the cells we have examined.

Another interesting observation is the accumulation of collagen fibrillae round each ovariole in such quantity as to cause the separation of whole groups of cells or of single cells.

This increase of collagen fibrillae, which is commonly observed in irradiated tissues, has already been described less fully in relation to the ovaries of mammals, but we have not found any reference to it in relation to the study of the ovaries of irradiated insects by optical microscope. Thus the electron-microscope observations represent completely new information.

REFERENCES

[1] HALBERSTAEDTER, L., Klin. Wochenschr. 42 (1905) 21. 6.
[2] BERGONIÉ, J., TRIBONDEAU, L. and RECAMIER, D., Compt. Rend. Soc. biol. 58 (1905) 284.
[3] OKINSHITS, L. L., Ž. Akuš. i Ženšk. Boljez., 20 (1906) 979.
[4] LACASSAGNE, A. et GRICOUROFF, G., Action des radiations sur les tiddus 9 Masson, Paris (1941) 92.
[5] WARREN, S., Amer. J. Roentgenol. 38 (1937) 899.
[6] BLOOM, W., Histopathology of Irradiation from External and Internal Sources 13 McGraw-Hill Book Co., Inc., New York (1948) 598.
[7] HOLLAENDER, A., Radiation Biology 17 McGraw-Hill Book Co., Inc., New York (1954) 1117.
[8] MAGNUSSEN, G., Acta Obstet. Gynaec. scand. 32 (1953) 1.
[9] TILLOTSON, F. W., ROSE, R. G. and WARREN, S., Amer. J. Roentgenol. 70 (1953) 599.
[10] ODEBLAD, E., Acta Radiol. 38 (1952) 33 and 375.
[11] REIHER, K. H. and LANG, G., Strahlentherapie 98 (1955) 453.
[12] WARREN, S., Arch. Path. 34 (1942) 749.
[14] LACASSAGNE, A., Lésions provoquées par les radiations ionisantes, Masson, Paris (1960) 2.
[15] AMY, R. L., Rad. Res. 3 (1955) 166.
[16] GROSCH, D. S., SULLIVAN, R. L. and LaCHANCE, L. E., Rad. Res. 5 (1956) 281.
[17] WHITING, A. R., J. exptl. Zool. 83 (1940) 249.
[18] ERDMAN, G. E., Int. J. Rad. Biol. 3 (1961) 183.
[19] KESSEL, R. G. and KEMP, N. E., J. Ultrastructure Research 6 (1962) 57.
[20] PARSONS, D. F., J. Cell Biology 14 (1962) 31.
[21] MELIS, A., and BACCETTI, B., Redia 45 (1960) 193.
[22] BACCETTI, B. and CAPPELLINI, M., Numtius Rad. 7 (1961) 600.
[23] BACCETTI, B., DE DOMINICIS, R. and CAPPELLINI, M., Redia 48 (1961) 205.
[24] BACCETTI, B., Redia 46 (1961) 1.
[13] ROUGH, R., in ERRERA-FORSSBERG, Mechanisms in Radiobiology 2 New York (1960) 1.
CLEMEDSON, C. J., NELSON, A., ibidem 95.

DISCUSSION

Mrs. M.-H. BINGGELI: In one of the slides you showed during your oral presentation I observed a number of well-defined patches in the cytoplasm, as seen in section in the electron microscope. You showed us some drops which you referred to as lipid, and I think you referred to some mitochondrial changes, whereas I am thinking of certain rather diffuse patches in the cytoplasm. You said they were due to radiation damage, and I was wondering whether there were any structural components as well?

B. BACCETTI: Yes, in certain cases, though not for the first irradiations, you do get, especially in the case of damage, certain substances diffused in the cytoplasm, owing, I think, to a rupture, shall we say, of the vessels or cellular membranes. These substances showed up in my illustrations as necrotic areas in the central part of the cytoplasm, and this is what you saw. I would stress that this is something quite different from the mitochondrial and lipidic degeneration, which is the most classical of all known lesions, not only in radiobiology but also in the general pathology of mammals and of man.

W. KLOFT: Having had personal experience in the pathology of holometabolic insects, I should like to ask whether the degeneration of the ovaries that was described could not be direct as well as indirect radiation damage. As I was able to show, the developing ovary of a pupa which is damaged in any way is, so to speak, the insect's weakest point. It degenerates easily since it is not an organ necessary to survival. One should therefore always consider also the more indirect radiation effects of ovaries in disturbing any other systems within the irradiated insect.

B. BACCETTI: I was referring to a previous study I carried out on the middle intestine of the same insect at the same irradiation doses. I always make observations on other somatic tissues besides the peritoneal membranes, and it can be said that for the higher irradiation doses, 20 or 30 kr, total damage does occur. For 10 kr, i.e. in almost all the slides I showed you, there is no total damage to the most sensitive tissues. The major part of the lesions shown are directly on ovaries and it is only at higher irradiation doses that there may be indirect damage, which is not very important, however, since it is the same type of damage as we saw in connection with the first dose.

P. NARDON: When you irradiate pupae, do you ever observe in the adults any decrease in the number of ovarioles, or ovarioles with a double germarium? I ask this because I have observed such phenomena in the progeny of the grain weevil which I irradiated with X-rays.

B. BACCETTI: I have never observed any difference in the number of ovarioles, but I must say that I did not look for it. I simply made peripheral observations, since the methods of fixation I had to use are expensive and I could not undertake a microscopic study. From what I saw I think there is no great difference in the number of ovarioles, especially as the peritoneal membranes are absolutely normal and consequently the envelope, shall we say, of each ovariole is completely preserved. Their number did not, I think, diminish in any way, mainly because irradiation took place with the number already established at the pupal stage.

STERILIZATION OF DACUS OLEAE BY GAMMA RADIATION

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Abstract — Résumé — Аннотация — Resumen

STERILIZATION OF DACUS OLEAE BY GAMMA RADIATION. Three developmental stages of the olive fly were exposed to gamma radiation from a Co^{60} source and the sterilization dose was determined. For the fourth-instar larvae the sterilizing dose was 2000 ± 300 rad, for the pupae 11 000-15 000 rad and for the adults 15 000-18 000 rad. Mating studies showed that the sterility lasted throughout the life of the adults.

The effect of dose on emergence was studied with fourth-instar larvae and on the pupae at daily intervals. The larvae were exposed to 300-3000 rad at 300-rad intervals, while the pupae were exposed to 3000-15 000 rad at 3000-rad increments. Maximum emergence was obtained when eight-day-old pupae were irradiated at the above-mentioned dose-rates. Significant fluctuations occurred at all dose-rates.

STERILISATION DE LA DACUS OLEAE PAR IRRADIATION GAMMA. On a exposé aux rayons gamma d'une source au ^{60}Co des mouches de l'olive se trouvant à trois stades de développement, et on a déterminé la dose nécessaire à la stérilisation. Au quatrième stade larvaire, la dose de stérilisation était de 2000 ± 300 rad; pour les pupes, elle était de 11 000 à 15 000 rad; pour les adultes, elle se situait entre 15 000 et 18 000 rad. Des études sur la reproduction ont montré que la stérilité persistait pendant toute la durée de vie des adultes.

On a aussi étudié l'effet de la dose au quatrième stade larvaire et sur les pupes, à des intervalles de un jour. Les larves ont été exposées à des rayonnements de 300 à 3000 rad, la dose augmentant de 300 rad par jour, tandis que les pupes recevaient des doses de 3000 à 15 000 rad, l'augmentation étant de 3000 rad chaque fois. On a obtenu l'éclosion maximum en irradiant des pupes âgées de huit jours avec les doses mentionnées plus haut. Toutes les doses ont entraîné des fluctuations sensibles.

СТЕРИЛИЗАЦИЯ DACUS OLEAE ГАММА-ОБЛУЧЕНИЕМ. Три группы оливковых мух в различных стадиях развития подвергали гамма-облучению Co^{60} и определяли дозу стерилизации. Для четвертой возрастной стадии личинок доза стерилизации оказалась равной 2000 ± 300 рад, для куколок 11 000 - 15 000 рад и для взрослых особей 15 000 - 18 000 рад. Изучение мужских особей показало, что у взрослых особей стерильность сохраняется в течение всей жизни.

Изучалось влияние доз на выплод для четвертой возрастной стадии личинок и куколок с интервалом в один день. Личинки подвергались облучению в 300 - 3000 рад с интервалами в 300 рад, тогда как куколки облучались при 3000 - 15 000 рад с инкрементом в 3000 рад. Максимальный выплод был получен, когда восьмидневные куколки подвергались облучению при вышеупомянутых мощностях доз.

Значительные колебания наблюдались при всех мощностях доз.

ESTERILIZACIÓN DE DACUS OLEAE POR IRRADIACIÓN GAMMA. El autor ha sometido tres fases de desarrollo de la mosca del olivo a las radiaciones gamma de una fuente de ^{60}Co, determinando la dosis esterilizadora que fue de 2000 ± 300 rad para las larvas del cuarto estadio, de 11 000 a 15 000 rad para las ninfas y de 15 000 a 18 000 rad para los adultos. Al estudiar las relaciones intersexuales se observó que la esterilidad perdura durante toda la vida de los adultos.

Todos los días se estudiaron los efectos de la dosis sobre la transformación de larvas del cuarto estadio y de ninfas. Las larvas fueron expuestas a dosis de 300 rad que se fueron aumentando de 300 en 300 rad hasta llegar a 3000, y las ninfas a dosis de 3000 rad que se fueron aumentando de 3000 en 3000 rad hasta llegar a 15 000. El porcentaje máximo de transformación se obtuvo irradiando ninfas de ocho días de vida con las dosis mencionadas. Para todas las intensidades de dosis se observaron fluctuaciones importantes.

INTRODUCTION

The control of harmful insects has occupied many a scientist's time. In some cases, efforts to control some of them with insecticides have proved unsatisfactory. However, research on the control of some harmful insects by irradiating them with Co^{60} has been promising and in a few cases, such as with Callitroga hominivorax [1, 2, 3] in Curaçao and Florida [4, 5], the results have been quite satisfactory.

This success induced the Greek Atomic Energy Commission to study the possibility of the control of Dacus oleae by Co^{60} irradiation. This study is of supreme importance in the economy of Greece.

The control of Dacus oleae Gmel. presents more problems than that of Callitroga, because it is a polygamous insect and has four to five annual generations. To alleviate these difficulties it has been suggested that a large number of sterilized males be released, a successful method which has brought positive results in laboratory and field experiments conducted by the Hawaii Fruit Fly Laboratory of the United States Department of Agriculture, on Dacus dorsalis Hendel, Ceratitis capitata Wied and Dacus cucurbitae Coq.

The aim of the present investigation was to determine the correct sterilizing dose and its effect on the emergence, vitality and fertility, and also on the resistance, of Dacus oleae to Co^{60} irradiation with the advancement of age. A similar experiment has been carried out in Italy [6].

The life-cycle of Dacus oleae consists of the three stages larva, pupa and adult. The female insects oviposit in the olive fruit, where the larva develops, feeding on the mesocarp. Pupation takes place either in the larval gallery in the fruit or, especially in the late-Autumn generations, in the soil. The duration of the larval and pupal stages varies, depending, among other factors, on temperature. In our laboratory, at 25°C, the larval life was about 12 d and the pupal life also about 12 d.

MATERIALS AND METHODS

The insect material used was taken from infested olive fruit sent to us from several areas of the country, mainly Patras and Chania, which we held at a temperature of 26 ± 1°C and a relative humidity of 70 ± 5%.

Larvae were irradiated during the period between their exit from the fruit and the formation of the puparium. Each group of irradiated larvae consisted of 20-30 individuals. There were three replications of each test in each season. The tests were repeated in three different seasons. Thus, 180-200 larvae were used in determining the effects of each radiation dose.

Pupae were irradiated 1 to 9 days after pupation. Each group of irradiated pupae consisted of 100 individuals. Repetitions were conducted in the same way as in the larvae. About 900 insects were used in determining the effects of each radiation dose.

Adults were irradiated only on the day of emergence, after they had fed on an adult liquid food kindly given to us by Dr. K. S. Hagen. Each group of irradiated insects consisted of 40 individuals. Repetitions were conducted in the same way as in the larvae and pupae. About 360 insects were used in determining the effects of each radiation dose.

The irradiation was done at a 550-c Co^{60} source. The total dose varied between 330 and 18 000 rad, depending on the developmental stage of the insect, at dose-rates of 330 rad/min and 600 rad/min, with 2% accuracy. Groups of 30 or more insects were irradiated in small test tubes stoppered with cotton-wool at approximately 20°C. The irradiated insects were maintained at 25°C and 70% relative humidity in Plexiglass insect cages.

After irradiation, all the adults, including those originating from irradiated larvae or pupae, were offered the above-mentioned liquid food.

Two days after emergence, the separation of males from females took place. The irradiated males were put with normal females and vice versa. In each cage, paraffin domes were placed for oviposition. A water-soaked cotton-wool wad was inserted in each dome to prevent the eggs from drying out.

The fertility and fecundity were determined by counting the eggs laid and finding the percentage of hatching. It was not possible to follow up the further development of the newly hatched larvae and subsequent stages because of lack of a satisfactory artificial larval food medium.

The eggs collected each day from the paraffin domes were placed on absorbent paper soaked in distilled water in a Petri dish.

At 25°C hatching occurred within the first two days after the eggs had been put in the Petri dish. The mating ability of the irradiated males or females was determined by ascertaining the presence of spermatozoa in the spermatheca of the females.

The number of adults emerged in each case was recorded as a percentage of the number emerged in the controls.

PRELIMINARY RESULTS

Irradiation of larvae

The results are shown in Table I and Fig. 1. The figures on the percentage of adult emergence from irradiating larvae is the average of three groups, each including approximately 25 individuals. Doses above 1650 rad caused sterility in both sexes, without obviously affecting the mating habits or the longevity of the adults, although on an average irradiated individuals had a somewhat shorter adult life than normal ones.

In females from irradiated larvae, the ovaries were atrophied: there was a complete absence of ova. Insects placed with normal males showed a very small number of spermatozoa in their spermathecae.

In males from irradiated larvae, live spermatozoa were observed in their testes; however, their premating period was extended to 10 or more days on an average against 1-2 days in the normal males.

Irradiated pupae

The results are shown in Table II and Figs. 2 and 3. Figs. 4-9 show the tolerance of pupae to radiation with increasing age.

TABLE I

EFFECT OF GAMMA IRRADIATION ON FULL-GROWN LARVAE OF DACUS OLEAE IN RELATION TO ADULT EMERGENCE
(Larvae fed on olive fruit)

Total dosage (rad)	Adults emerged (corrected) (%)	Fertile females (%)
330	95	60
660	74	48
990	82	30
1320	83	15
1650	36	3
1980	29	0
2310	23	0
2640	17	0
2970	14	0
3300	12	0

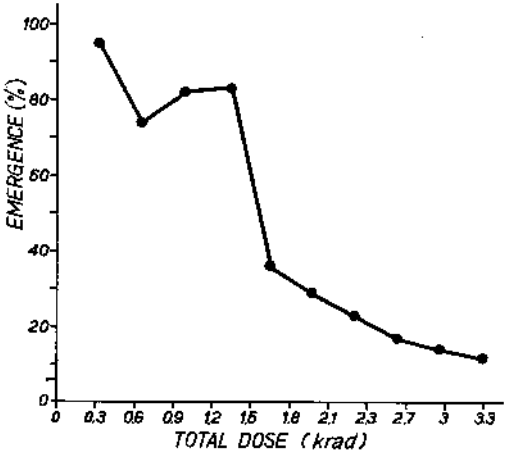

Fig. 1

Irradiation of larvae

Females (Observations December 1961 - February 1962)

Their mating ability was not impaired. The females which had emerged from pupae irradiated with 9, 12 and 15 krad showed, from the tenth day after their emergence up to their natural death:

(a) Spermatozoa in their spermatheca, like those of the control group.
(b) Completely formed eggs, of about the same number as those of the control group.
(c) A greatly prolonged preoviposition period as compared with that of the control group.

TABLE II

EFFECT OF GAMMA IRRADIATION ON PUPAE OF DACUS OLEAE OF VARIOUS AGES IN RELATION TO ADULT EMERGENCE
Samples from different parts of the country
(December 1961 - February 1962)

Total dosage	Adult emergence (corrected) (%) after irradiation of pupae at (d)					
(krad)	4	5	6	7	8	9
3	30	36	69	83	85	107
4.5	8	9	51	70	100	86
6	0	0	72	99	83	86
9	17	12	81	76	73	86
12	9	80	79	55	67	40
15	0	50	89	118	109	101

TABLE III

EFFECT OF GAMMA IRRADIATION ON VARIOUSLY AGED PUPAE OF DACUS OLEAE IN RELATION TO ADULT EMERGENCE
Samples from one part of the country
(May-June 1962 and October - December 1962)

Total dosage	Adult emergence (corrected (%) after irradiation of pupae at (d)			
(krad)	5	6	7	8
3	42	44	69	72
4.5	11	33	58	85
6	10	71	84	75
9	11	77	64	66
12	44	50	64	60
15	44	52	76	82

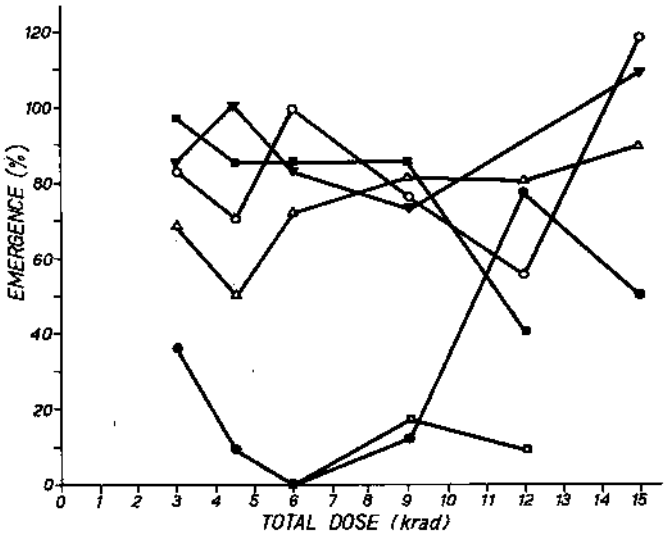

Fig. 2

Irradiation of pupae

□ 4 d old ○ 7 d old
● 5 d old ▼ 8 d old
△ 6 d old ■ 9 d old

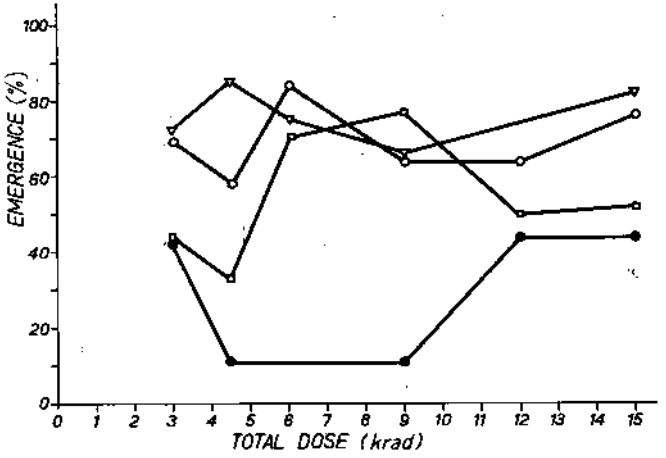

Fig. 3

Irradiation of pupae

● 5 d old ○ 7 d old
□ 6 d old ▽ 8 d old

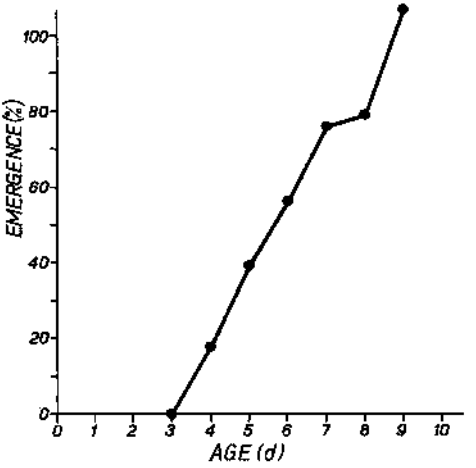

Fig. 4

Tolerance of pupae to 3 krad

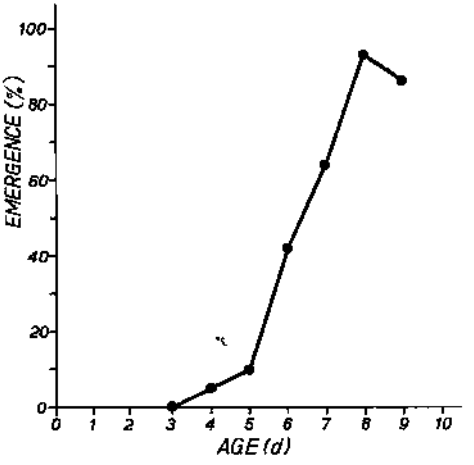

Fig. 5

Tolerance of pupae to 4.5 krad

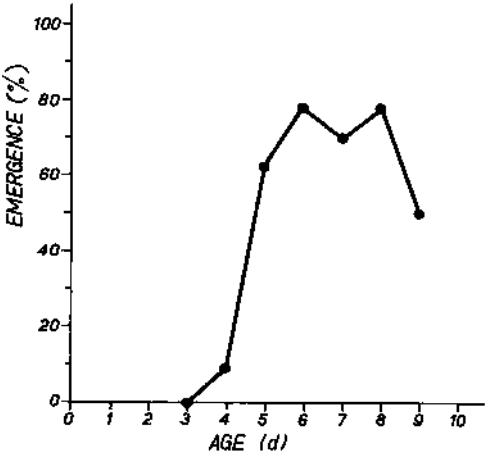

Fig. 6

Tolerance of pupae to 6 krad

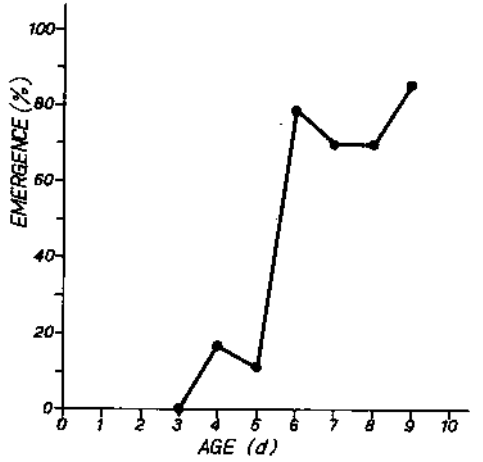

Fig. 7

Tolerance of pupae to 9 krad

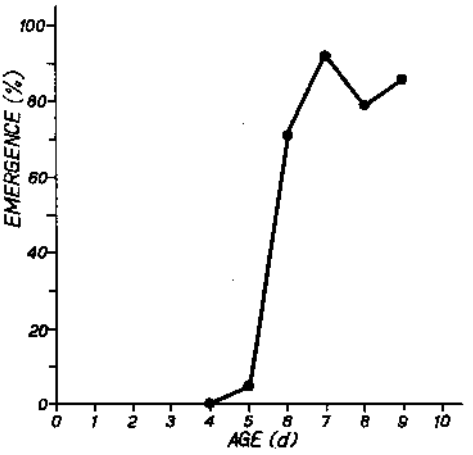

Fig. 8

Tolerance of pupae to 12 krad

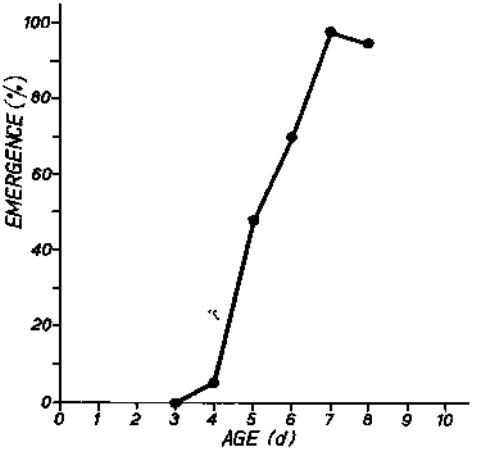

Fig. 9

Tolerance of pupae to 15 krad

Females (Observations May-June 1962 and October-December 1962)

Females from pupae irradiated at doses of 12 and 15 krad showed a great number of spermatozoa in the spermathecàe but atrophied ovaries with no eggs 25, 30 and 62 d after emergence.

Males (Observations December 1961-December 1962)

The mating ability was not impaired after pupal doses of 12-15 krad but mating occurs 5-10 d* later, depending on the strength of the dose. The normal mating time is 1-2 d* after emergence.

Male fertility was 0-5%, as compared with 70% for the control group (Fig. 10). The density and motility of spermatozoa in vitro was the same as for the control group.

Adults of both sexes emerging from pupae irradiated with 12 and 15 krad lay upside-down in their cages for the first four days after emergence. They subsequently recovered and behaved approximately the same way as irradiated adults.

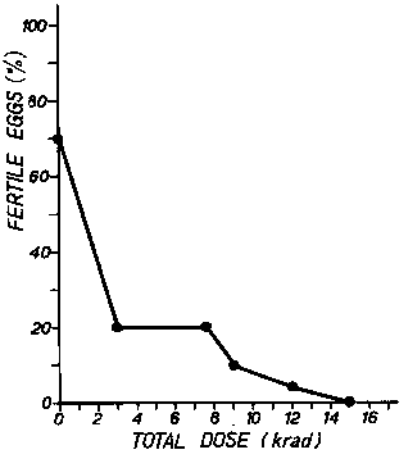

Fig. 10

Male fertility
(Doses 12-15 krad)

Irradiated adults

The results are shown in Table IV. In females, the mating ability was about the same as that of the control group. Fecundity was nil, as no eggs were laid or observed in the ovaries, after doses of 15-18 krad.

In males, mating ability did not seem impaired but mating was delayed by about 10 days. Sterility was complete after doses of 15-18 krad.

*The premating period figures should be increased by 5 to give the actual days on which mating occurred. It was noticed that the females would not accept the males for the first five days after emergence.

TABLE IV

EFFECT OF GAMMA IRRADIATION ON ADULT DACUS OLEAE IN RELATION TO SURVIVAL

Total dosage (krad)	Surviving 3 d after irradiation (%)	
	Males	Females
6	65	70
9	75	86
12	93	97
15	80	90
18	72	80

The length of the adult life after small doses (3-6 krad) was not different from that of normal adults. However, with increasing dosage the adult life was considerably shortened [7].

CONCLUSIONS

Larvae

The adults (male and female) which emerged from larvae irradiated with 2000 ± 300 rad remained sterile throughout their adult life (85-90 d). This dose seems to be satisfactory. Larvae irradiated with 2970-3300 rad developed into pupae which died as such before adult emergence.

Pupae

This stage is the most suitable and easy to handle for irradiation purposes. Adults from pupae irradiated with 12-15 krad remained sterile throughout their life. This stage was selected because the testes can be distinguished and the progress of spermatogenesis can be satisfactorily observed. In the testes of normal pupae, all stages of spermatogenesis except the mature spermatozoa are apparent on the fourth day after pupation and on the fifth day all stages, including the mature one, are apparent. On the sixth, seventh and eighth days the number of mature spermatozoa increases steadily.

Adults

It seems desirable to feed adults before irradiation, since those irradiated before they were fed had a high mortality (about 50%). Adults irradiated with 15-18 krad remained sterile throughout their life.

Sensitivity to radiation differed between insects from different parts of the country.

ACKNOWLEDGEMENTS

I wish to express my deepest appreciation to Professor G. Pantazis for the suggestion of this problem and his advice during the course of this experiment.

REFERENCES

[1] BUSHLAND, R.C. and HOPKINS, D.E., J. Econ. Entomol. 46 (1953) 648.
[2] KNIPLING, E.F., Scient. Monthly, 85 (1957) 195.
[3] KNIPLING, E.F., J. Econ. Entomol. 48 (1955) 459.
[4] BUSHLAND, R.C., KNIPLING, E.F. and LINDQUIST, D.A., Proc. UN Int. Conf. Peaceful Uses Atomic Energy, 12 (1956) 216.
[5] BAUMHOVER, A.H., GRAHAM, A.J., BITTER, B.A., HOPKINS, E.D., et al., J. Econ. Entomol. 48 (1955) 462.
[6] MELIS, A. and BACCETTI, B., Redia XLV (1960) 193-218.
[7] CLARK, A. Radiation Research, 17 (1961) 515-19.

DISCUSSION

B. BACCETTI: I am happy to learn that my Greek colleagues have studied the problem of possible Dacus control by the method of sterilized males. These results confirm perfectly the findings I myself obtained in Italy, which were published two years ago. It has already been shown in Italy that it is possible to control the Dacus with irradiated males and we have also studied the vitality of the adults at the different irradiation doses, not only on the basis of adult emergence, but also using an electron microscope on particularly sensitive somatic tissue.

P.S. ORPHANIDIS: I would like to ask whether there was a difference in the duration of the pupal stage between pupae from sterilized, third-stage larvae and those from non-sterilized larvae.

Mlle. H. THOMOU: No, we did not observe any difference.

EFFECTS OF VARIABLE DOSE-RATES ON RADIATION DAMAGE IN THE RUST-RED FLOUR BEETLE, TRIBOLIUM CASTANEUM HERBST

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Abstract — Résumé — Аннотация — Resumen

EFFECTS OF VARIABLE DOSE-RATES ON RADIATION DAMAGE IN THE RUST-RED FLOUR BEETLE, TRIBOLIUM CASTANEUM HERBST. In order to find out whether a change in dose-rate would significantly alter a radiation response, eggs as well as the adults of Tribolium castaneum were tested for viability and fertility respectively after exposure to gamma radiation at different dose-rates. The doses employed were 2000 and 5000 rad and the dose-rates ranged from 126 rad/h to 140 000 rad/h. It was observed that with an increase in dose-rate there was a decline in the fertility of the adults. Similarly the viability of the eggs was considerably reduced as the dose-rate increased, but at very high dose-rates there was a significant increase in viability. The significance of these findings is discussed.

EFFETS DU DÉBIT DE DOSE SUR LES DOMMAGES RADIOINDUITS CHEZ LE TÉNÉBRION ROUX (TRIBOLIUM CASTANEUM HERBST). En vue de déterminer si une variation du débit de dose modifie de manière significative la réponse aux rayonnements, on a fait des essais sur des œufs et des adultes de Tribolium castaneum, dont on a étudié respectivement la viabilité et la fécondité après exposition à des doses de rayons gamma d'intensités différentes. On a administré des doses de 2000 et 5000 rad, le débit de dose variant de 126 à 140 000 rad/h. On a constaté que la fécondité des adultes diminuait à mesure que le débit de dose augmentait. De même, la viabilité des œufs était considérablement réduite à mesure que le débit de dose augmentait, mais pour des débits de dose très élevés la viabilité augmentait sensiblement. Les auteurs analysent les données obtenues.

ВЛИЯНИЕ РАЗЛИЧНЫХ МОЩНОСТЕЙ ДОЗ НА РАДИАЦИОННОЕ ПОВРЕЖДЕНИЕ КРАСНОГО МУЧНОГО ЖУКА, TRIBOLIUM CASTANEUM (HERBST). Для определения зависимости реакции на облучение от изменения мощности дозы изучались жизнеспособность и фертильность яиц, а также взрослых особей Tribolium castaneum после гамма-облучения при различных мощностях доз. Использовались дозы в 2000 и 5000 рад, а мощность доз изменялась от 126 до 140 000 рад в час. Было замечено, что с повышением мощности дозы понижалась фертильность взрослых особей. Подобным же образом с повышением мощности дозы значительно уменьшалась жизнеспособность яиц, однако при очень больших мощностях доз жизнеспособность заметно увеличивалась. Обсуждается значение полученных данных.

RADIOLESIONES QUE SUFRE EL TRIBOLIUM CASTANEUM HERBST CUANDO SE LE SOMETE A DOSIS DE RADIACIONES DE DIVERSAS INTENSIDADES. Para averiguar si un cambio en la intensidad de la dosis produce alteraciones fundamentales en la radiosensibilidad, se comprobó la viabilidad de los huevecillos y la fertilidad de los adultos de Tribolium castaneum después de exponerlos a dosis de radiaciones gamma de intensidad diferente. Las dosis empleadas fueron 2000 y 5000 rad, y las intensidades oscilaron entre 126 rad/h y 140 000 rad/h. Al aumentar la intensidad de la dosis se produce una disminución de la fertilidad de los adultos. De modo similar, la viabilidad de los huevecillos disminuyó considerablemente al aumentar la intensidad de dosis, aunque vuelve a aumentar de manera notable cuando las intensidades de dosis alcanzan valores muy elevados. La memoria discute el significado de estos resultados.

INTRODUCTION

It has been known for some time that the development of radiation injury is conditioned by a variety of physical and biological factors before, during

and after treatment. Recently, attention has been focused on the role of dose-rate in modifying the extent of radiation damage. RUSSEL et al. [5] and RUSSEL [6] reported that the frequency of mutations from spermatogonia irradiated at a dose-rate of 0.009 r/min was significantly lower than obtained when the dose-rate was 90 r/min. KALLMAN [4] observed that the LD50 of mice of two inbred strains increased with decreasing dose-rate. BANHAM [1] has mentioned that the survival of Tribolium confusum was very much lower at a dose-rate of 4000 rad/h than at 2000 rad/h. JEFFERIES [3] observed in Oryzaephilus surinamensis that by varying the dose-rate from 1600 to 4000 rad/h "doses for 50% reduction, both in survival and progeny, were significantly increased when the dose-rate was decreased".

The above-mentioned studies have indicated that dose-rate can modify the radiation damage in a number of biological systems. Since the minimum effective dose for radiation disinfestation of grain on a commercial scale will be largely conditioned by the intensity at which this dose is delivered, it is necessary that all future evaluation of this dose be determined in relation to the dose-rate of the source in any projected irradiator. The present study was therefore undertaken with some of the available dose-rates obtained in our gamma irradiation facility with a view to finding out the effects of dose-rate on the viability of eggs and also fertility in the adults of Tribolium castaneum. The findings reported here are of a preliminary nature and it is hoped to pursue this problem in greater detail.

MATERIAL AND METHODS

Adult beetles were placed on previously sieved and sterilized wheat flour for oviposition. The eggs collected after 12 h were irradiated on the second day, at which time their age was approximately between 36 and 48 h. The total dose used was 2000 rad, and this was delivered at different dose-rates, viz. 1020, 3520, 47 420, 81 550 and 140 000 rad/h. For each dose-rate 12 replications of 25 eggs each were irradiated in gelatin capsules. Both the irradiated and the control eggs were incubated at 30°C and hatching was scored on the eighth day after irradiation.

Five-day-old pupae were sexed before emergence. The emerged adults were irradiated on the seventh day, after which they were paired. The doses employed were 2000 and 5000 rad. The dose-rates used for 2000 rad were 126, 3050 and 14 000 rad/h and for 5000 rad 236, 3050, 3520 and 140 000 rad/h. Each mating combination consisted of a single pair and 15 such replications were used for each dose-rate. In all cases about 5 g of sterilized wheat flour with 2% yeast formed both the food and the ovipositing medium. Equal numbers of unirradiated insects served as controls. At intervals of 4 d, eggs laid were removed from the tubes and kept separately for hatching. The experiment was discontinued on the 32nd day after pairing. The average number of eggs laid as well as the percentage viability were recorded.

IRRADIATION FACILITY

The Co^{60} sources used in this study were lodged in a pool containing 15 ft of water. The low dose-rates were obtained by irradiating the samples

at different distances from the sources. The samples were irradiated through an aluminium tube 17 ft in length which ensured aeration during irradiation.

RESULTS AND DISCUSSION

An analysis of the data on the viability of the eggs and the induced sterility of the adults at different dose-rates are presented in Tables I and II.

TABLE I

VIABILITY OF TWO-DAY-OLD EGGS OF TRIBOLIUM CASTANEUM IRRADIATED AT DIFFERENT DOSE-RATES

Total dose (rad)	Dose-rate (rad/h)	Viability and S.E. (%)
0 (Control)	-	78.3 ± 1.9
2 000	1 020	55.3 ± 2.6
2 000	3 520	33.3 ± 4.2
2 000	47 420	7.0 ± 1.8
2 000	81 550	24.3 ± 3.1
2 000	140 000	35.6 ± 2.6

TABLE II

EFFECT OF DIFFERENT DOSE-RATES ON THE FECUNDITY AND FERTILITY OF TRIBOLIUM CASTANEUM

Total dose (rad)	Dose-rate (rad/h)	Number of eggs laid/female	Viability and S.E. (%)
0 (Control)	-	299.3	50.4 ± 2.0
2 000	126	235.1	31.4 ± 2.9
2 000	3 050	217.8	25.57 ± 2.002
2 000	140 000	233.0	21.7 ± 1.8
5 000	236	155.1	16.1 ± 3.23
5 000	3 050	105.8	1.054 ± 0.024
5 000	3 520	113.4	1.005 ± 0.028
5 000	140 000	67.5	0

At a high dose-rate of 47 420 rad/h, the viability of the eggs decreased considerably in comparison with what was obtained at dose-rates below this

level. On the other hand, when the dose-rate was further increased, the susceptibility of the eggs to radiation decreased, as is evidenced by the higher percentage of hatching obtained at 81 550 and 140 000 rad/h. (Fig. 1)

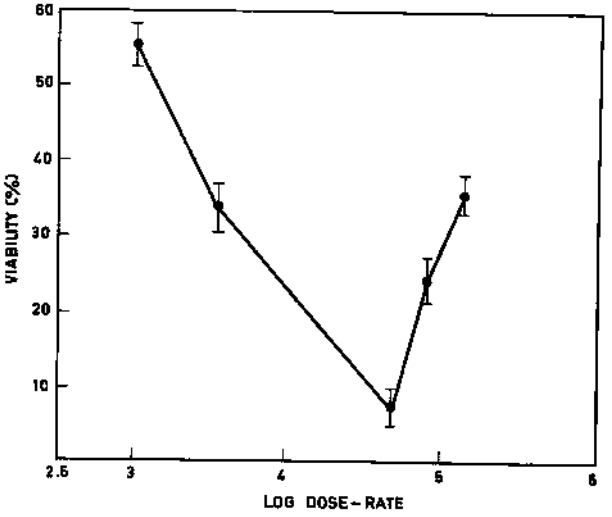

Fig. 1

The effect of different dose-rates on the viability of the eggs of T. castaneum

BULL et al. [2] found in their studies on the susceptibility of the grain weevils to electrons and gamma rays that the sterilizing dose was very much higher in the case of electrons than in the case of Co^{60} gamma rays. This they attributed to the very high dose-rate of the electron accelerator (5.76×10^9 rad/h). SCHWARTZ [7] has shown that at very high doses the extent of radiation damage in maize seeds was less than that observed at lower doses as measured by seedling height. Our results suggest that, as in the case of doses, an inverse relation exists even with dose-rates up to a point beyond which there is an increase in the viability of the eggs. It is apparent from the results obtained in our study that the factors which contribute to an increased viability at low dose-rates are different from those which also increase the viability at high dose-rate. The increased viability at low dose-rates is probably due to the phenomenon of repair taking place in the cells. That this is so has been confirmed by other workers on mice [4, 5].

The way in which very high dose-rates increase survival is at the present moment only a matter of conjecture; presumably it must be brought about by the interplay of several factors. If one assumes that for the manifestation of maximum radiation damage the irradiation period is a critical factor, then any irradiation time greater or less than this fraction might produce less damage in the system. Under these conditions the extent of damage at the above-mentioned high dose-rates can be expected to be less. It is also likely that at very high dose-rates relatively anoxic conditions might result, affording protection against radiation injury.

SUTTON and ROTBLAT [8] have shown in chemical reactions in aqueous systems exposed to 15-MeV electrons that the ratio G (radical reaction)/G (molecular reaction) falls when dose-rates of 10^9 r/s and above are used. This has been explained by the increased probability of track overlap and radical recombination. It is unlikely that the effects observed in this experiment could be ascribed to this phenomenon, since the dose-rates employed are far below the levels where this mechanism is likely to operate.

Though the sterility of the adults showed an increase with increase in dose-rate, no change in pattern of response, as seen in the eggs, was observed in the adults at high dose-rate. It is likely that the dose-rate needed to bring about a similar response in the adults must be of a higher order. No attempt could be made to test the validity of this assumption in view of the limitations of the present Co^{60} source.

REFERENCES

[1] BANHAM, E.J., AERE-R 3888 (1962).
[2] BULL, J.O., WOND, T. and CORNWELL, P.B., AERE-R 3890 (1961).
[3] JEFFERIES, D.J., AERE-R 3891 (1962).
[4] KALLMAN, R.F., Rad. Res. 16 (1962) 796-810.
[5] RUSSEL, L.B., STELZNER, K.F. and RUSSEL, W.L., Proc. Soc. Exptl. Biol. & Med., 102 (1959) 471-479.
[6] RUSSEL, W.L., J. Cell. Comp. Physiol., 58 (Suppl. 1) (1961) 183-187.
[7] SCHWARTZ, D., Science, 119 (1954) 3079, 45-56.
[8] SUTTON, H.C. and ROTBLAT, J., Nature, 180 (1957) 1332, cited by BACH, Z.M. and ALEXANDER, P., in Fundamentals of Radiobiology, Pergamon Press (1961) 146-147.

DISCUSSION

R.C. von BORSTEL: I think your results are extremely interesting. Is it possible that some of these dose-rate effects can be explained by changes in the cell cycle to radiation sensitivity during the period the embryos were irradiated? In Habrobracon, the cell cycle of the young embryo is only about 15 min long, and the sensitivity can vary over a twenty-fold range at least.

K.K. NAIR: Your suggestion is an excellent one. We have thought of this probability and it needs some ingenious experimentation to test its validity.

B.D. BAINES: With reference to Table I on the viability of eggs against dose-rate, is there a plateau at the minimum percentage viability shown at a dose-rate of 47 420 rad/h, or is this a point minimum? This is not clear, owing to the large difference between dose-rates of 3 520 and 81 550 rad/h on either side of the minimum shown. The exact nature of this minimum could be of great interest to irradiation plant designers.

K.K. NAIR: It is difficult to say whether or not this is a point minimum, as dose-rates immediately below 47 420 r and those above 81 550 r have not been listed for their effects. These studies are still in progress.

INFLUENCE DE L'IRRADIATION SUR LES ADULTES DE SITOPHILUS SASAKII TAKAHASHI (CURCULIONIDAE) ET LEURS DESCENDANTS

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Abstract — Résumé — Аннотация — Resumen

EFFECTS OF IRRADIATION ON THE ADULTS OF SITOPHILUS SASAKII TAKAHASHI (CURCULIONIDAE) AND THEIR OFFSPRING. Co^{60} gamma rays bring about a significant reduction in expectation of life as from 5000 r in the case of males and 7000 r in that of females. All insects die within about twelve days after a dose of 8000 r and within four days after 100 000 r.

Study of the pattern of mortality as a function of time shows that, up to 6000 r, irradiation induces a progressive heterogeneity in the population, the more resistant individuals being isolated.

Examination of the survival curves shows that death is always deferred. It occurs suddenly after a latent phase, the duration of which varies, according to dose, between twelve days (5000 r) and one day (100 000 r).

Study of the variability coefficient and of the average survival time according to dose suggests that two sensitive mechanisms are destroyed by irradiation.

Radiosensitivity appears to increase with age.

Fertility is reduced by half as from 2000 r. A period of temporary sterility appears at 5000 and 6000 r, and complete sterility sets in at 15 000 r. Sterilization by X-rays is more effective at 150 than at 80 kV.

Egg production is much more radioresistant than fertility, eggs being laid even at 16 000 r.

Irradiation of parents induces a decrease in both fertility and weight of offspring and shows the latter's rate of development. These effects are transmitted to succeeding generations without any effort at selection on the part of the experimenter. The first two effects tend to disappear, but the decrease in the rate of development remains constant for at least ten generations.

It would seem possible to use radiation for disinfestation of foodstuffs.

INFLUENCE DE L'IRRADIATION SUR LES ADULTES DE SITOPHILUS SASAKII TAKAHASHI (CURCULIONIDAE) ET LEURS DESCENDANTS. Le rayonnement gamma de ^{60}Co réduit significativement la moyenne de vie des mâles à partir de 5000 r, et celle des femelles à partir de 7000 r. Tous les insectes meurent en une douzaine de jours à 8000 r, en quatre jours à 100 000 r.

Jusqu'à 6000 r, l'étude de la répartition de la mortalité dans le temps montre que l'irradiation fait apparaître progressivement une hétérogénéité dans la population, isolant des individus plus résistants.

L'examen des courbes de survie révèle que la mort est toujours différée. Elle apparaît brusquement après une phase de latence plus ou moins longue selon la dose, variant de douze jours (5000 r) à un jour (100 000 r).

L'étude du coefficient de variabilité et de la moyenne de survie en fonction de la dose suggère la présence de deux mécanismes sensibles détruits par l'irradiation.

La sensibilité au rayonnement semble augmenter avec l'âge.

Dès 2000 r, la fertilité se trouve réduite de moitié. A 5000 r et 6000 r, on observe une phase de stérilité temporaire. La stérilisation totale est acquise dès 15 000 r. La stérilisation par les rayons X est plus efficace à 150 kV qu'à 80 kV.

La fécondité est beaucoup plus résistante que la fertilité au rayonnement, puisque même à 16 000 r des œufs sont pondus.

L'irradiation des parents a pour conséquence de diminuer la fertilité et le poids des descendants, et d'augmenter leur durée de développement. Ces effets se transmettent d'une génération à l'autre sans que l'expérimentateur procède à aucune sélection. Alors que les deux premiers tendent à disparaître, l'augmentation de la durée du développement reste stable pendant au moins dix générations.

Il est possible d'envisager l'emploi des rayonnements pour la désinsectisation des denrées.

ДЕЙСТВИЕ ОБЛУЧЕНИЯ НА ВЗРОСЛЫХ ОСОБЕЙ SITOPHILUS SASAKII TAKAHASHI (CURCULIONIDAE) И НА ПОСЛЕДУЮЩИЕ ПОКОЛЕНИЯ. Облучение гамма-лучами Co^{60} значительно уменьшает среднюю продолжительность жизни мужских особей, начиная с дозы 5000 р, и женских особей - с дозы 7000 р. Все насекомые погибают в течение 12 дней при дозе 8000 р и четырех дней при дозе 100 000 р.

Исследование распределения смертности в зависимости от времени при дозе до 6000 р показывает, что облучение последовательно выявляет гетерогенность популяции, изолируя при этом более стойкие особи.

Изучение кривых выживания показывает, что наступление смерти постоянно замедлено. Она наступает внезапно после более или менее продолжительной латентной фазы в зависимости от дозы. Колебания составляют от 12 дней (5000 р) до одного дня (100 000 р).

Исследование коэффициента вариабильности и среднего срока выживания в зависимости от дозы предполагает наличие двух чувствительных механизмов, нарушаемых облучением.

Чувствительность к облучению, по-видимому, возрастает с возрастом. С 2000 р плодовитость сокращается вдвое. При 5000 и 6000 р наблюдается фаза временного бесплодия. Полная стерилизация достигается при дозе 15 000 р и выше. Стерилизация рентгеновскими лучами более эффективна при 150 кв, чем при 80 кв.

Способность к оплодотворению значительно более стойка, чем плодовитость, по отношению к облучению, потому что даже при 16 000 р продолжается откладывание яиц.

Облучение родителей ведет к сокращению плодовитости и веса последующих поколений и увеличению срока их развития. Эти последствия передаются от одного поколения другому, причем экспериментатор не ведет никакой селекции. В то время как два первых поколения имеют тенденцию к исчезновению, увеличение срока развития продолжает оставаться стабильным, по крайней мере, для десяти поколений.

Представляется возможным применение радиации для дезинсекции продуктов питания.

INFLUENCIA DE LA IRRADIACIÓN SOBRE LOS ADULTOS DEL SITOPHILUS SASAKII TAKAHASHI (CURCULIONIDAE) Y SUS DESCENDIENTES. La radiación gamma del ^{60}Co reduce significativamente la vida media de los machos a partir de 5000 r, y la de las hembras a partir de 7000. Todos los insectos mueren en doce días si la dosis es de 8000 r, y en cuatro días si es de 100 000.

Hasta 6000 r, el estudio de la distribución de la mortalidad en el tiempo muestra que la irradiación hace aparecer una heterogeneidad progresiva en la población, aislando los individuos más resistentes.

El examen de las curvas de supervivencia revela que la muerte siempre es diferida. Sobreviene bruscamente después de una fase de latencia más o menos prolongada según la dosis y que varía entre 12 días (5000 r) y un día (100 000 r).

El estudio del coeficiente de variabilidad y de la supervivencia media en función de la dosis sugiere la existencia de dos mecanismos sensibles que son destruidos por la irradiación.

La sensibilidad a las radiaciones parece aumentar con la edad.

A 2000 roentgens, la fertilidad queda ya reducida a la mitad. A 5000 y 6000 roentgens se observa una fase de esterilidad temporal. La esterilización total se alcanza a 15 000 roentgens. La esterilización por rayos X es más eficaz a 150 kV que a 80 kV.

La fecundidad es mucho mas resistente a las radiaciones que la fertilidad, pues incluso a 16 000 roentgens se observa la puesta de huevos.

La irradiación de los padres tiene por consecuencia la disminución de la fertilidad y del peso de los descendientes y el aumento de la duración de la fase de desarrollo. Estos efectos se transmiten de una generación a otra sin que el experimentador proceda a selección alguna. Mientras los dos primeros tienden a desaparecer, el aumento de la duración de la fase de desarrollo permanece estable durante diez generaciones, por lo menos.

Cabe prever el empleo de las radiaciones para la desinsectación de los artículos alimenticios.

Les insectes utilisés sont élevés à la température de 27, 5° (± 0, 2°) et 75% d'humidité relative (± 5%). Ils sont placés dans des boîtes à couvercle et fond grillagés et disposent en moyenne de 15 à 20 grains de blé par individu et par jour. Chaque boîte renferme au maximum 25 insectes pour la reproduction. La source d'irradiation est une bombe au ^{60}CO. Quel-

ques traitements ont été faits toutefois aux rayons X, avec différents voltages (80, 100, 120 et 150 kV) et un filtre d'aluminium de 0,5 mm.

A. ACTION SUR LA MORTALITÉ

1. Réduction de la durée moyenne de vie

Le tableau I donne les résultats bruts. L'analyse statistique par le test de Student prouve que l'influence du rayonnement γ se manifeste seulement à partir de 5000 r pour les mâles et entre 6000 r et 8000 r pour les femelles. Celles-ci sont donc plus résistantes à l'irradiation, bien que vivant moins longtemps que les mâles chez les témoins.

L'analyse de la répartition des pourcentages de mortalité en fonction du temps après l'irradiation (fig. 1) montre que dès 4000 r (dose non létale), la mortalité apparaît plus rapidement que chez les témoins (44^{e} jour). A 5000 r une phase primaire de mortalité se déclenche dès le 12^{e} jour, et elle s'accentue à 6000 r. Cette première phase ne dure que quelques jours, et elle est nettement séparée dans le temps de la phase de mortalité normale, qui n'apparaît, chez les irradiés comme chez les témoins, que le 65^{e} jour.

Les deux maxima (12^{e} et 86^{e} jours) ne correspondent pas à la sensibilité différente des deux sexes, et l'on doit conclure que l'irradiation entraîne l'apparition progressive d'une hétérogénéité dans la population d'insectes, isolant des individus plus résistants qui meurent ensuite au même rythme que les témoins.

2. Courbes de survie

L'examen de ces courbes (fig. 2) montre que même pour des doses fortement létales, la mortalité ne se déclenche qu'après une phase de latence au cours de laquelle les insectes continuent à se nourrir.

Ce fait a été signalé par d'autres auteurs [2, 8] et paraît constant si l'on irradie de jeunes imagos. Il met en évidence que les DL 50 calculées par de nombreux auteurs au bout d'un ou deux jours seulement ne rendent que partiellement compte de la radiosensibilité véritable des insectes.

Pour établir des comparaisons valables, il faudrait déterminer la dose réduisant de moitié la durée moyenne de vie: la DV 50. Elle serait voisine de 7000 r pour S. sasakii.

3. Etude de la variabilité

Le coefficient de variabilité (tableau II) augmente régulièrement avec la dose jusqu'à 6000 r et le test de Snedecor indique que la variance de chacune des séries irradiées est significativement différente de celle des témoins. Le rayonnement modifie donc la sensibilité des insectes aux variations incontrôlables du milieu.

Mais on doit remarquer que cet effet est bivalent: à 8000 r la variance diminue brusquement de près de 3000 fois. Puis de nouveau le coefficient de variabilité augmente régulièrement. Il paraît donc exister entre 6000 r et 8000 r un seuil que sépare deux types d'action différente du rayonnement [7].

TABLEAU I

MOYENNES DE SURVIE DE SITOPHILUS SASAKII
ÂGÉS DE MOINS DE TROIS SEMAINES
APRÈS DIFFÉRENTES DOSES DE RAYONS GAMMA

Doses (r)	Sexe	Nombres d'insectes	Moyennes de survie (j)
Témoins	Mâle	91	123,71
	Femelle	88	74,28
4 000	Mâle	103	124,75
	Femelle	83	71,24
5 000	Mâle	105	88,78
	Femelle	85	77,90
6 000	Mâle	98	43,78
	Femelle	103	67,48
8 000	Mâle	249	8,75
	Femelle	246	8,75
15 000	Mâle et Femelle	343	9,01
30 000	-	364	8,30
60 000	-	187	5,26
100 000	-	194	2,13

On doit remarquer par ailleurs que la moyenne de survie décroît rapidement de 4000 r à 8000 r (de plus de 10 fois). Puis de 8000 r à 15 000 r on obtient un palier. Enfin, au delà de 15 000 r, elle diminue à nouveau, mais plus lentement.

Cette analyse suggère l'intervention de deux mécanismes sensibles, dont l'un serait totalement détruit dès 8000 r, et l'autre atteint au delà de 15 000 r seulement.

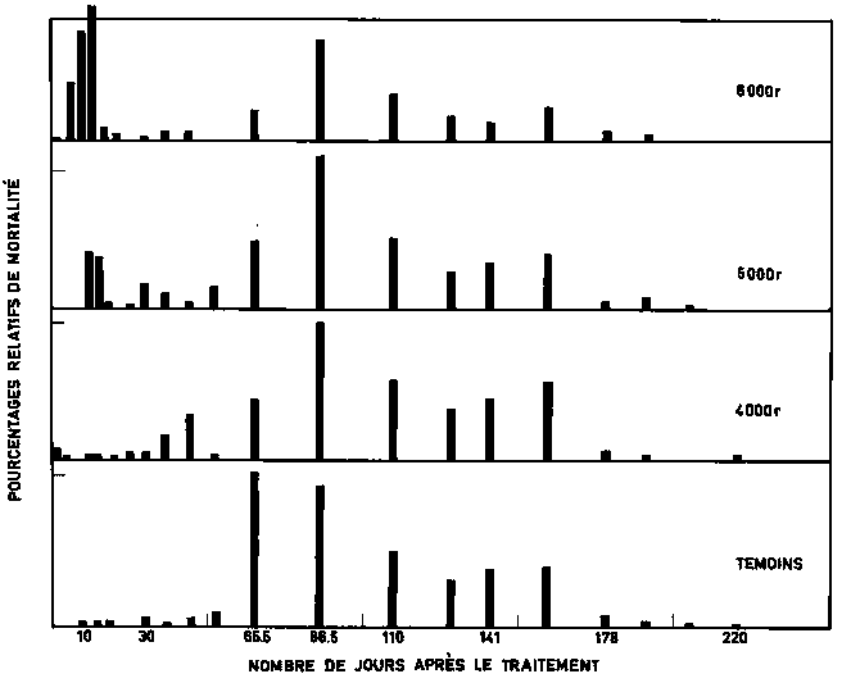

Figure 1

Répartition de la mortalité de Sitophilus sasakii dans le temps, après irradiation à 6000 r, 5000 r, 4000 r et chez les témoins.
En abscisses: nombre de jours après le traitement.
En ordonnées: pourcentages relatifs de mortalité.

4. Influence de l'âge sur la sensibilité au rayonnement

Les insectes irradiés étaient âgés de moins de trois semaines et leur radiosensibilité paraît très proche de celle des espèces voisines S. granarius et S. oryzae [2, 8], et également de celle de Anthonomus grandis [3]. Mais des expériences préliminaires montrent que la sensibilité au rayonnement augmente significativement avec l'âge dès que la différence dépasse un mois.

B. ACTION SUR LA REPRODUCTION

1. Etude de la fertilité

Nous entendons par fertilité le nombre d'adultes obtenus par femelle et par unité de temps.

- Le processus de reproduction est très sensible au rayonnement: alors qu'une femelle témoin produit en moyenne 249 descendants au cours de sa vie, une femelle irradiée à 2000 r n'en donne que 101, bien que cette dose n'ait aucune influence sur la longévité.

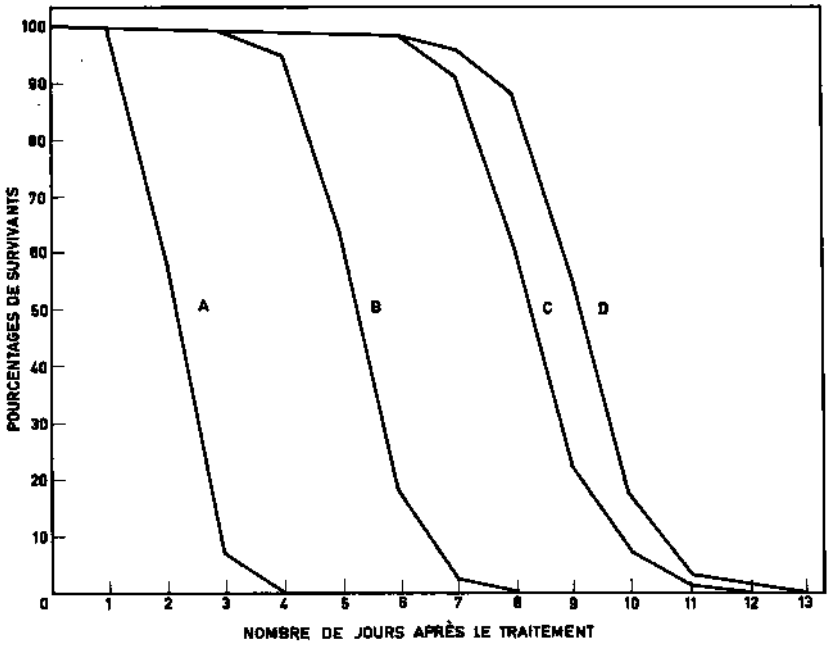

Figure 2

Courbes de mortalité de Sitophilus sasakii après irradiation à 15 000 r (D), 30 000 r (C), 60 000 r (B) et 100 000 r (A).
En abscisses: nombre de jours après le traitement.
En ordonnées: pourcentages de survivants.

- L'augmentation de la dose entraîne une inhibition supplémentaire de la fertilité. Ainsi, à 4000 r, elle est 25 fois plus faible chez les irradiés que chez les témoins pendant les 27 premiers jours. Au bout de deux mois, la stérilité est pratiquement acquise.
- A 5000 r apparaît une phase de stérilité temporaire du 7e au 12e jour après le traitement. Elle s'étend jusqu'au 19e jour à 6000 r. A 8000 r, 246 femelles n'ont donné que 18 descendants adultes.
- A 15 000 r la stérilisation est totale.
- Avec les rayons X, la stérilisation est plus efficace à 150 kV qu'à 80 kV: pour une dose de 8000 r, on obtient des descendants dans ce dernier cas, mais pas dans le premier.

2. Etude de la fécondité

La recherche des œufs pondus par les insectes irradiés [10] montre que le processus de ponte est beaucoup moins sensible au rayonnement. Ainsi, à 5000 r et 6000 r la ponte ne cesse pas pendant la phase de stérilité temporaire et des œufs sont encore pondus à 16 000 r. Ils n'éclosent pas, sans qu'on puisse dire à quel stade de développement s'est trouvé bloqué.

Ce phénomène paraît général. ERDMAN l'a fort bien démontré chez la guêpe Habrobracon [4] et chez Tribolium [5].

TABLEAU II

MODIFICATION DE LA VARIABILITÉ DE LA MOYENNE DE SURVIE EN FONCTION DE DIFFÉRENTES DOSES DE RAYONS GAMMA

Doses (r)	Témoins	4000	5000	6000	8000	10 000	15 000	30 000	60 000	100 000
Ecart type S	40, 19	45, 90	50, 59	53, 53	0, 98	0, 97	1, 39	1, 24	0, 87	0, 62
C = S/M	0, 40	0, 45	0, 59	0, 96	0, 11	0, 11	0, 15	0, 15	0, 17	0, 29

M = moyennes de survie C = Coefficient de variabilité

C. ACTION DU RAYONNEMENT GAMMA SUR LES DESCENDANTS

Nous avons constaté que, sans procéder à aucune sélection, l'irradiation des parents perturbe la physiologie des descendants pendant de nombreuses générations [9]. Ces effets sont résumés dans le tableau III.

TABLEAU III

INFLUENCE DU TRAITEMENT AUX RAYONS GAMMA DES PARENTS SUR LES DESCENDANTS

Caractères étudiés	Dose (r)	Moyennes au cours des générations successives					
		Parents	F_1	F_2	F_3	F_4	F_5
Poids (1/100 mg)	8000	-	-	125, 52	133, 00	-	143
	Témoins	-	-	144, 88	138, 18	-	144
Fertilité (nombre d'imagos par femelle et par jour)	5000	0, 08	2, 42	2, 62	3, 18	3, 07	-
	Témoins	4, 82	4, 37	4, 01	4, 19	3, 82	-
	8000	0, 01	0, 48	0, 67	1, 34	1, 36	-
	Témoins	3, 30	3, 60	3, 50	3, 92	3, 25	-
Durée de développement (j)	5000	-	32, 92	32, 99	32, 68	32, 78	32, 60
	Témoins	-	31, 48	31, 07	31, 35	31, 84	31, 79
	8000	-	34, 11	36, 41	35, 61	35, 59	36, 06
	Témoins	-	31, 14	31, 58	32, 81	32, 01	32, 03

1. Etude du poids

La diminution est très nette par rapport aux témoins en F_2 de la série irradiée à 8000 r ($p = 0,001$ pour les mâles et $p = 0,005$ pour les femelles). Elle tend à s'effacer en F_3 ($p = 0,05$) et s'annule en F_4. Il se produit donc une régulation. Par ailleurs, la dispersion des poids est augmentée chez les descendants d'irradiés, à la fois par l'apparition d'individus de poids plus faible et plus élevé.

2. Etude de la fertilité

La diminution des taux de fertilité par femelle reste significativement inférieure à ceux des témoins jusqu'en F_4 où l'on a $p = 0,05$ pour 5000 r et

p = 0,001 pour 8000 r. L'effet est statistiquement plus marqué dans cette dernière série.

Toutefois, on remarque que, là encore, une régulation se produit et la fertilité des descendants d'irradiés tend à reprendre la même valeur que chez les témoins.

3. Duré de développement

Elle est accrue dans les deux séries issues d'insectes irradiés et bien que la différence avec les témoins ne soit que de quelques jours, le test de Student indique qu'elle est très significative. Ici encore, l'effet de 8000 r est plus important; pour les cinq générations, on a $M_8 = 35,10$ j et $M_5 = 32,84$ j ($p = 0,001$).

Mais la variance intra-inter générations confirme qu'ici les moyennes restent stables au cours des cinq générations. Un calcul à la dixième génération indique encore la persistance de l'effet.

Pour l'instant, il n'est pas possible de donner une explication de ces phénomènes dont la nature paraît complexe. Des expériences sont en cours pour étudier dans le détail les modalités précises de la transmission.

D. POSSIBILITÉS D'EMPLOI DES RADIATIONS POUR LA DÉSINSECTISATION DES DENRÉES

D'après ces quelques résultats qui confirment ceux d'autres auteurs, il semble possible de détruire et stériliser facilement les charançons des grains. Une dose de 20 000 r paraît suffisante pour stériliser un quelconque insecte parasite des denrées entreposées [6].

Le fait que les stades immatures sont plus sensibles que les imagos aux radiations, que ces dernières ne laissent pas de résidus toxiques et permettent un traitement parfaitement homogène, milite en faveur de leur utilisation pour la désinsectisation des denrées. BROWNELL [1] et CORNWELL [2] ont déjà étudié des modalités d'application.

L'emploi des radiations comme insecticides doit cependant être envisagé comme une technique de complément plutôt qu'une technique de remplacement. En effet, en matière de lutte antiparasitaire, surtout en ce qui concerne les denrées entreposées, il est aussi important de prévenir que de stopper les infestations. Ainsi, l'emploi de poudre répulsive à long pouvoir d'action paraît-il présenter un intérêt majeur pour assurer la protection des denrées irradiées, dans tous les cas où le stockage lui-même (emballage ou ensilage) ne pourra garantir aucune nouvelle infestation.

RÉFÉRENCES

[1] BROWNELL, L.E. et al., USAEC Rpt. (1955), AECU 3050, 624-6.
[2] CORNWELL, P.B. et al., J. Sci. Food. Agric., London, 11 (1960) 754-68.
[3] DAVICH, T.B. et al., J. econ. Ent. 55 (1962) 164-7.
[4] ERDMAN, H.E., Nature 186 (1960) 254-5.
[5] ERDMAN, H.E., Nature 195 (1962) 1218.

[6] HILCHEY, J. D., Radiation preservation of food, PB - 151 493 (1957) 240-66.
[7] LAVIOLETTE, P. et al., (à paraître au Bulletin biologique).
[8] MORTREUIL, M., C.R. Soc. Biol. 153 (1959) 393-4 et 1165-6.
[9] NARDON, P., C.R. Acad. Sci. 254 (1962) 2454-6.
[10] NARDON, P., Phytoma 144 (1963) 7-12.

DISCUSSION

G. T. SCARASCIA: Do you think that the resistant specimens you found after irradiation are due to mutations or are they genotypes already present in the irradiated population?

P. NARDON: I have not studied the phenomenon in detail, but the resistance is not due to mutations, since these specimens are ones that have been irradiated - it is not the offspring. I think one must assume that some members of the population are more resistant than the rest. For that reason it is not unlikely that a certain degree of resistance to radiation can be acquired.

R. CAVALLORO: In irradiating the Sitophilus larvae, did you give single or fractionated doses?

P. NARDON: We administered single doses. Only adults were irradiated - not larvae. With an X-ray dose of 8000 r we got better sterilization at 150 kV than at 80 kV.

S. FUSEAU-BRAESCH: What exactly is meant by the "modifications" in sensitivity to uncontrollable environmental changes to which you refer in your paper?

P. NARDON: The insects were all irradiated at the same time and were then distributed into different boxes for statistical analysis. In view of the fact that the insects were all irradiated at the same time in an absolutely homogeneous part of the beam there can be no difference in the dose received. The variations observed in the insects in different boxes are therefore of the same type as those observed in the controls, which also show variations in fertility, weight, etc. In the case of the irradiated insects the variations are more marked and in my opinion they can only be due to uncontrollable environmental factors, such as temperature differences during handling of the insects caused by opening and closing of doors, small variations in relative humidity, etc.

S. FUZEAU-BRAESCH: This assumption of altered sensitivity to environmental factors is, of course, only a hypothesis, but personally I am not sure that it is satisfactory. The various biological disturbances, such as those caused by irradiation, do not normally have large-scale repercussions from the statistical point of view, but they do broaden the distribution spectra and this is something we encounter in many biological phenomena. I also wanted to ask you whether you obtain normal insect progeny or have you found abnormal specimens?

P. NARDON: We have found a few abnormalities of the legs and of the genital organs when using X-rays, but never with gamma radiation.

S. FUZEAU-BRAESCH: With regard to what is perhaps the most important part of your paper, namely the study of the offspring of your insects, I think such work has been neglected in the past and these modifications,

which are not strictly speaking chromosomal though they are found in several successive generations, should really be studied further. With regard to the loss of weight and fertility, the general reduction in vitality, do you think there is then a general regulatory process such that the vitality may exceed that of the controls? I think it was David in Lyons who got additional regulatory functions, but if I remember correctly he was not using radiation but certain toxic substances.

P. NARDON: The development period stays increased until the tenth generation, but I did not go beyond that. The effect on the weight disappears after the fifth generation, and we obtained a number of giant specimens. The fertility is also regulated, but I did not obtain any heightened fertility. Of course, I did not test the fertility during the whole lifetime because these grain weevils live for about a year and it would have required very complicated experimental arrangements. I only tested the fertility for a period of two weeks.

S. FUZEAU-BRAESCH: I think these modifications, which you have observed over ten generations, are highly interesting and they draw our attention to the general problem of the long-term phylogenetic effects of radiation such as might be caused by atomic explosions, fall-out, etc.

R. CAVALLORO: I believe that Cornwell, Crook, Bull and others reported that with Curculionidae, insects of the same family as those you refer to, a dose of 20 000 r gave only 6% sterilization. You stated that with a dose of 15 000 r you obtained total sterilization. Can you explain this difference?

P. NARDON: If I am not mistaken, Cornwell published some more recent work, in 1959, in which he got exactly the same results as those I quoted. I think these results also agree with those reported by several other authors, including, for example, Dr. von Borstel.

EFFECTS OF GAMMA RADIATION ON THREE SPECIES OF PHILIPPINE INSECT PESTS

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Abstract — Résumé — Аннотация — Resumen

EFFECTS OF GAMMA RADIATION ON THREE SPECIES OF PHILIPPINE INSECT PESTS. A study was conducted on the effects of gamma radiation from a Co^{60} source on three agricultural insect pests, namely (1) the tomato lady beetle, Epilachna philippinensis Dieke, a pest of tomato and eggplant; (2) the rice weevil, Sitophilus oryzae L.; and (3) the rust-red flour beetle, Tribolium castaneum Herbst. The last two species are the most common and most destructive pests of corn and other stored grains and grain products in the Philippines.

Eggs, larvae, pupae and adults of E. philippinensis on tomato and adults of S. oryzae and T. castaneum on corn were used.

Four doses were used on Epilachna, namely 1, 5, 10 and 20 kr. In the other two species, five doses were used, 10, 20, 40, 60 and 80 kr.

At 1 kr, the proportion of unhatched eggs in Epilachna was about 10%. There was no proportionate increase in mortality at 5 kr, but at 10 kr the mortality increased to more than three times that at 5 kr. Mortality at 20 kr was about 90%. The threshold dose seems to lie between 5 and 10 kr. The embryo of eggs that did not hatch failed to continue development. At 1 kr, over 20% of the larvae were killed, although some of these succeeded in pupating before they died. Most of the larvae attained the adult stage. All the larvae that survived the treatments above 1 kr, some for as long as four weeks, died without attaining the pupal stage. The pupae were more resistant than the larvae and the adults. Complete mortality was not attained even with 20 kr. Some adults which emerged in the 5-kr and 10-kr lots had abnormally developed wings and/or legs. In the adults, the 10 and 20 kr doses had an adverse effect, killing about 75% of the individuals subjected to the lower, and all subjected to the higher, dose three weeks after irradiation. No significant difference in susceptibility between the sexes was noted.

About 40% of the S. oryzae in the lots treated with 60 and 80 kr were killed one week after irradiation, but only about 10% were killed with 20 kr and about 20% with 40 kr. The mortality with 40, 60 and 80 kr increased to about 75% two weeks after the start of the test. The mortality with 10 kr corresponding to this period was less than 10% and that with 20 kr was about 25%. The threshold dose seems to lie between 20 and 40 kr.

The mortality trend in the T. castaneum was similar to that in the S. oryzae, except that the threshold dose seems to lie at about 40 kr.

EFFETS DES RAYONS GAMMA SUR TROIS ESPÈCES D'INSECTES NUISIBLES DES PHILIPPINES. On a étudié les effets des rayons gamma provenant d'une source au ^{60}Co sur trois espèces d'insectes nuisibles pour l'agriculture: 1. l'épilachne de la tomate (Epilachna philippinensis Dieke) parasite de la tomate et de l'aubergine; 2. le charençon du riz (Sitophilus oryzae L.); 3. le ténébrion roux (Tribolium castaneum Herbst). Les deux dernières espèces sont les plus répandues et les plus nuisibles des parasites du maïs et autres céréales engrangées ainsi que des denrées à base de céréales aux Philippines.

On a étudié les effets des œufs, des larves, des pupes et des adultes de E. philippinensis sur la tomate et les effets des adultes de S. oryzae et T. castaneum sur le maïs.

Pour l'épilachne, on a utilisé quatre doses: 1, 5, 10 et 20 kr. Pour les deux autres espèces, on a employé cinq doses: 10, 20, 40, 60 et 80 kr.

Pour 1 kr, le pourcentage des œufs non éclos d'épilachne était d'environ 10%. On n'a pas constaté une augmentation proportionnelle de la mortalité pour 5 kr; mais pour 10 kr, la mortalité était plus de trois fois supérieure à ce qu'elle était pour 5 kr. Avec 20 kr, elle était d'environ 90%. La dose seuil semble se situer entre 5 et 10 kr. L'embryon des œufs non éclos ne continuait pas à se développer. Pour 1 kr, plus de 20% des larves étaient anéanties, bien que certaines d'entre elles eussent réussi à se métamorphoser en pupes avant de mourir. La plupart des larves atteignaient le stade adulte. Toutes les larves qui ont survécu à des irradiations

de plus de 1 kr, certaines pendant des périodes allant jusqu'à quatre semaines, sont mortes sans avoir atteint le stade de la pupe. Les pupes se sont révélées plus résistantes que les larves et que les adultes. On n'est pas parvenu à une mortalité totale, même avec 20 kr. Certains adultes apparus dans des lots irradiés à 5 et 10 kr avaient des ailes et/ou des pattes anormalement développées. Chez les adultes, les doses de 10 et 20 kr avaient des effets nocifs puisqu'elles anéantissaient, trois semaines après l'irradiation, 75% environ des individus ayant été soumis à la première dose et tous ceux qui avaient reçu la seconde. On n'a pas constaté de différence notable de sensibilité entre les sexes.

Dans les lots ayant reçu 60 et 80 kr, près de 40% de S. oryzae mouraient une semaine après l'irradiation, contre 10% seulement pour 20 kr et environ 20% pour 40 kr. Avec 40, 60 et 80 kr, la mortalité atteignait 75% deux semaines après le début de l'expérience. Pour 10 kr, la mortalité au terme de la même période était inférieure à 10% et pour 20 kr elle était d'environ 25%. La dose de seuil semble se situer entre 20 et 40 kr.

La mortalité de T. castaneum était comparable à celle de S. oryzae si ce n'est que la dose seuil semble se situer aux environs de 40 kr.

ДЕЙСТВИЕ ГАММА-ИЗЛУЧЕНИЯ НА ТРИ РАЗНОВИДНОСТИ НАСЕКОМЫХ-ВРЕДИТЕЛЕЙ НА ФИЛИППИНАХ. Изучалось действие гамма-излучения от источника Co^{60} на трех сельскохозяйственных насекомых-вредителях, а именно: 1) томатного жука Epilachna philippinensis Dieke, вредителя томатов и баклажанов; 2) рисового амбарного долгоносика Sitophilus oryza (L) и 3) красного мучного жука Tribolium castaneum (Herbst.). Последние две разновидности являются наиболее распространенными и опасными вредителями зерна и других хранящихся зерновых продуктов на Филиппинах.

При проведении опытов на томатах использовались яйца, личинки, куколки и взрослые особи E. philippinensis, а на зерне - взрослые особи S. oryza и T. castaneum.

Четыре дозы были применены для Epilachna, а именно: 1, 5, 10 и 20 кр. При обработке других двух разновидностей использовались пять доз: 10, 20, 40, 60 и 80 кр.

При дозе в 1 кр количество невылупившихся из яиц личинок у Epilachna составляло приблизительно 10%. При увеличении дозы до 5 кр не наблюдалось пропорционального увеличения смертности, но при дозе в 10 кр смертность возросла более чем в три раза, по сравнению со смертностью при дозе в 5 кр. Смертность при дозе в 20 кр составляла приблизительно 90%. Пороговая доза находится, по-видимому, в диапазоне между 5 и 10 кр. Зародыши яиц, из которых не вывелись личинки, перестали развиваться. При дозе 1 кр более 20% личинок погибло, причем некоторые из них окуклились, прежде чем погибнуть. Большинство личинок развилось во взрослые особи. Все личинки, которые вышли после облучения дозой свыше 1 кр, а некоторые из них жили в течение 4 недель, погибли, не достигнув стадии окукливания. Куколки оказались более устойчивыми, чем личинки и взрослые особи. Стопроцентная смертность не была достигнута даже при облучении 20 кр. У некоторых взрослых особей, подвергшихся облучению дозами в 5 и 10 кр, наблюдалось неправильное развитие крыльев и/или конечностей. У взрослых особей, подвергшихся облучению дозами 10 и 20 кр, наблюдался противоположный эффект, выражавшийся в гибели через три недели после облучения приблизительно 75% особей, облученных дозой 10 кр, и всех особей, облученных дозой 20 кр.

Около 40% S. oryza в партиях, получивших 60 и 80 кр, погибли через неделю после облучения. Однако только около 10% всех особей погибли после облучения 20 кр и около 20% особей - после 40 кр. Смертность после облучения 40, 60 и 80 кр увеличилась приблизительно до 75% через две недели после начала эксперимента. Смертность при дозе в 10 кр, соответствующая этому периоду, составляла менее 10%, а при дозе в 20 кр около 25%. Пороговая доза, по-видимому, лежит в диапазоне между 20 и 40 кр.

Тенденция к гибели у T. castaneum была подобной тенденции у S. oryza, за исключением того факта, что пороговая доза лежит, по-видимому, около 40 кр.

EFECTOS DE LAS RADIACIONES GAMMA SOBRE TRES PLAGAS INSECTILES DE LAS FILIPINAS. Se han estudiado los efectos de las radiaciones gamma de una fuente de ^{60}Co sobre tres insectos nocivos para la agricultura: 1) el escarabajo del tomate Epilachna philippinensis Dieke, que ataca al tomate y a la berenjena; 2) el gorgojo del arroz Sitophilus oryzae L., y 3) el escarabajo de la harina Tribolium castaneum Herbst. Las dos últimas especies son las plagas más corrientes y destructivas del maíz y de otros granos y derivados de granos ensilados en las Filipinas.

Los autores emplearon los huevos, larvas, ninfas e imagos del E. philippinensis en tomates, e imagos de S. oryzae y T. castaneum en el maíz.

Con el Epilachna se ensayaron cuatro dosis de 1, 5, 10 y 20 kr. Con las otras dos especies se ensayaron cinco dosis de 10, 20, 40, 60 y 80 kr.

Tras una dosis de 1 kr, el porcentaje de huevos hueros de Epilachna fue aproximadamente del 10%. La dosis de 5 kr no provocó un aumento proporcional de la mortalidad, pero con 10 kr la letalidad fue el triple que la correspondiente a 5 kr. Con una dosis de 20 kr, el índice de mortalidad alcanzó aproximadamente el 90%. Al parecer, la dosis umbral está comprendida entre 5 y 10 kr. En los huevos sin abrir, el embrión dejó de desarrollarse. Con 1 kr, murieron más del 20% de las larvas, aunque algunas de ellas llegaron a formar ninfas. La mayor parte de las larvas alcanzaron el estado adulto. Las larvas que sobrevinieron a dosis de más de 1 kr, algunas durante hasta cuatro semanas, murieron sin llegar a formar ninfas. Las ninfas resultaron más resistentes que las larvas y los insectos adultos, pues la mortalidad completa no se alcanzó ni siquiera con dosis de 20 kr. Algunos de los adultos que surgieron de los grupos que habían sido expuestos a dosis de 5 y 10 kr tenían alas y/o patas anormales. Las dosis de 10 kr matan aproximadamente al 75% de los adultos a las tres semanas de haberlos irradiado, y 20 kr bastan para matar a todos al cabo del mismo tiempo. No se registraron diferencias importantes entre los sexos en cuanto a la sensibilidad.

Aproximadamente el 40% de los S. oryzae que habían recibido dosis de 60 y 80 kr murieron una semana después de la irradiación; en cambio, las dosis de 20 kr sólo mataron a un 10% y las de 40 kr al 20%. La mortalidad con dosis de 40, 60 y 80 kr aumentó hasta un 75% a las dos semanas de haber comenzado el ensayo. La mortalidad con 10 kr correspondiente a este período resultó inferior al 10%, y con 20 kr alcanzó aproximadamente el 25%. Al parecer, la dosis umbral parece encontrarse entre 20 y 40 kr.

La mortalidad en el caso del T. castaneum resultó similar que para el S. oryzae, con la salvedad de que la dosis umbral parece encontrarse hacia los 40 kr.

The use of gamma radiation for the control of various insect pests has been the subject of various investigations abroad in a number of countries. In the Philippines, no work has been done in this field of research.

Of major concern in this country is the control of agricultural insect pests, which have been undermining the economy of the country for decades. Insects are responsible for millions of pesos' worth of damage to Philippine agriculture. A case in point is that of the pests of stored grains and copra. Maize, which is the staple food in some regions of the country, could not be stored for more than two to three months without extensive damage by insect pests. As a result, this foodstuff cannot be stocked to provide a continuous supply of the commodity throughout the year. Another example is the case of the copra beetle, Necrobia rufipes De G. One of the major causes of the poor quality of Philippine copra in the world market is the damage inflicted by this insect on the stored product. Various insecticides have been found very effective against these pests, but as the products are processed or manufactured into food the use of poisons is ruled out, for obvious reasons.

The need for basic information on the effects of radiation on Philippine insect pests of economic importance has long been felt. With the reported success, at least in some instances, of the utilization of gamma radiation in the field of pest control abroad, considerable interest has been shown in this field of investigation by local entomologists and food processors. A programme of studies along this line has been initiated at the Philippine Atomic Research Centre. Radiosensitivity studies of some of our important insect pests are now being undertaken. It is expected that with the completion of the Philippine research reactor and the Co^{60} facility of the Philippine Atomic Research Centre of the Philippine Atomic Energy Commission in a few months from now, research along this line will be stepped up.

The present study is confined to the gross effects of gamma radiation on the insects. A more detailed study has recently been initiated. Part of the material used in the present investigation will be studied for microscopic effects of radiation.

MATERIALS AND METHODS

In the present investigation, a study was conducted on the effects of gamma radiation on three species of agricultural insect pests, namely, the tomato lady-beetle, Epilachna philippinensis Dieke, the rice-weevil, Sitophilus oryzae L. and the rust-red flour beetle, Tribolium castaneum Herbst. The first species is the most common pest of tomato and the other two are the most common and most destructive pests of stored cereals.

The radiation source used in this investigation was a Co^{60} teletherapy machine giving 39 r/min at a distance of 70.5 cm from the source at the time of the tests. The source had a strength of 1996 c on 18 September 1958, when it started operation.

The irradiation dose rates used on E. philippinensis were 1, 5, 10 and 20 kr. In S. oryzae and T. castaneum, the dose rates were 10, 20, 40, 60 and 80 kr. These rates were determined after a preliminary test. The specimens for irradiation were placed in short plastic vials with loose caps. The insects were transferred to rearing containers with feed soon after treatment.

Eggs, fifth-instar larvae, pupae and adults of E. philippinensis varying in age from one to two days were used in the tests. In order to provide a steady supply of the various metamorphic stages, the insect was mass-reared on tomato plants grown on plots in a screenhouse and in wire-screen cages in the laboratory.

The mortality of the irradiated eggs and the larvae from the hatched eggs in all treatments were determined. In addition, the percentage of living larvae that attained the adult stage, the fertility of the eggs laid by these adults and any abnormality in the larvae and adults were also recorded. In the irradiated larvae, the percentage mortality of the larvae and the pupae and other pertinent observations were taken. Similar observations were conducted on the irradiated pupae and adults, but the mortality of the adults in both cases was taken four weeks from the date of irradiation.

Approximately one-week-old adults of S. oryzae and T. castaneum were irradiated at 10, 20, 40, 60 and 80 kr. The percentage mortality of the adults was taken 30 d after irradiation. Observations were continued beyond 30 d to determine the reproductive potential of the irradiated insects.

RESULTS AND DISCUSSION

Epilachna philippinensis Dieke

Egg. The results obtained with the irradiation of the eggs are shown in Table I. The lethal effect of the gamma radiation on the eggs is clearly evident from the percentage of eggs that failed to hatch. Microscopic exami-

TABLE I

EFFECTS OF GAMMA RADIATION ON EGGS OF EPILACHNA PHILIPPINENSIS DIEKE

Treatment (kr)	Mortality (%)		Living larvae that attained adult stage (%)	Remarks
	Eggs	Larvae		
0 (Control)	3.1	4.8	95.2	All adults normal. Fertility of eggs laid about 93%.
1	10.2	37.3	70.2	All adults apparently normal. Fertility of eggs laid about 95%.
5	25.7	60.7	39.3	About 6% of adults had abnormal legs and/ or wings. Fertility of eggs laid about 92%.
10	81.8	92.1	5.3	About 10% of adults had abnormal legs and/ or wings. Adults incapable of reproduction. No eggs laid.
20	90.5	96.8	2.4	About 25% of adults had abnormal legs and/ or wings. Adults incapable of reproduction. No eggs laid.

nation of the unhatched eggs showed that the embryo failed to continue development. The mortality is dose-dependent – the higher the dose the more lethal the effect. This effect was carried to the larval and subsequent developmental stages. Beyond 10 kr, however, the increase in mortality was not commensurate with the increase in dose. An investigation on the lethal and sterilizing effects of gamma radiation on insects infesting cereal commodities by some workers showed that beyond 6 000 and up to 50 000 rep there was only a slight reduction in the time required to obtain a 100% kill [4]. The adults which developed from the eggs irradiated with 1 kr were all apparently normal and laid eggs with a fertility of about 95%. In the lot treated with 5 kr, some of the adults that developed had abnormal legs and/or wings, but the eggs they laid were fertile. More adults with abnormal appendages appeared in the lot treated with 10 kr. All the adults which emerged from this treatment were incapable of reproduction and no eggs were laid. The same was observed in the eggs irradiated with 20 kr. In addition, about one-fourth of the adults that emerged from this lot had abnormal appendages. The existence of a threshold dose between 5 and 10 kr is evident. The eggs of this species seem more resistant than those of T. castaneum, which require a dose of only 11 100 rad for the development of adults from irradiated eggs to be completely prevented [6].

Fifth-instar larvae. Table II shows the effect of the various dose rates of radiation on the fifth-instar larvae. The larvae were much more sensitive to radiation than the eggs. At 1 kr, about one-fourth of the larvae were killed, the rest attaining the pupal stage. Only 85.7% of the pupae reached the adult stage but about 35% of the adults had abnormal wings and/or legs. The eggs laid, however, had about the same fertility as those of the control. The higher dose-rates (5, 10 and 20 kr) had very deleterious effects on the larvae. About one week after irradiation with 5 kr, all the larvae became inactive and stopped feeding. The larvae turned yellow to orange and all died in about a month without attaining the pupal stage. The larvae treated with 10 kr exhibited practically identical conditions. At 20 kr the larvae stopped feeding and became inactive about 24 h after irradiation. All turned yellow and died within two weeks after irradiation without attaining the pupal stage. There was a protracted delay in the pupation of the larvae. Some of the larvae treated with 5 and 10 kr lived for about a month without attaining the pupal stage. It is quite possible, as in the case of Drosophila, that the delay in pupation is caused by hormonal disturbance in the larva [3]. The larvae of this species are much more susceptible to gamma radiation than those of the rust-red flour beetle, T. castaneum, as reported by CROOK [6].

Pupa. (Table III) The pupae were more resistant to gamma radiation than the larvae. At 1 kr, about 5% of the pupae were killed and about 15% of the adults that emerged died within four weeks after irradiation. The remaining adults died within six weeks. The fertility of the eggs laid by the adults was about 90%. All the adults which emerged from the lots treated with 5 and 10 kr died within four weeks after irradiation. About 10% of the adults which emerged in the lot treated with 5 kr had abnormal appendages. The fertility of the eggs laid was only about 40%. In the pupae treated with 10 kr, only about 5% of the adults had abnormal appendages. All the adults died within four weeks after irradiation without laying eggs. About one-fourth of the adults that emerged in the lot treated with 20 kr had abnormal

TABLE II

EFFECTS OF GAMMA RADIATION ON FIFTH-INSTAR LARVAE OF EPILACHNA PHILIPPINENSIS DIEKE

Treatment (kr)	Mortality (%)	Pupae that attained adult stage (%)	Remarks
0 (Control)	0	100	All adults normal. Fertility of eggs laid about 95%.
1	22.2	85.7	About 34.5% of adults had abnormal wings and/or legs. Fertility of eggs laid about 91%.
5	100	-	Larvae became inactive and stopped feeding about one week after irradiation; turned bright yellow or orange. All died in about a month from irradiation without attaining pupal stage.
10	100	-	As above.
20	100	-	Larvae stopped feeding and became inactive about 24 h after irradiation. All turned yellow and died within two weeks from date of irradiation without attaining pupal stage.

appendages. All adults died within two weeks after irradiation without laying eggs.

Adult. (Table IV) The adults treated with 1 kr were active and appeared unaffected by the radiation. Like those in the control lot, they all died within seven weeks after treatment and the fertility of the eggs laid was about 94%. In the lot treated with 5 kr, the fertility of the eggs laid was about 72% and about 63% of the larvae attained the adult stage. At 10 kr some adults became inactive after two or three days and all the adults died within five weeks after irradiation. The fertility of the eggs laid was about 60% and approximately 50% of the larvae attained the adult stage. The adults treated with 20 kr became inactive soon after irradiation and died within three weeks. The fertility of the eggs laid was only about 30%. Of the total larvae that emerged, close to 15% attained the adult stage.

The data obtained on this species indicate differences in the susceptibility of the various metamorphic forms of the insect to gamma radiation. The eggs showed marked resistance to radiation, about 10% of those treated with 20 kr attaining the adult stage. The fifth-instar larva was the most susceptible of the various metamorphic forms of the insect tested. There was no significant difference in susceptibility between sexes. In stored-grain insects, it has been reported that graded resistance within a species was exhibited, increasing with the development stage from egg to adult [8]. On the basis of the data obtained in this investigation it appears that E. philippinensis is more susceptible to gamma radiation than S. oryzae and T. castaneum. The data obtained on the last two species seems to agree with those found by other workers [2, 5, 6, 8, 11].

Sitophilus oryza and Tribolium castaneum

The results on the irradiation of the stored grain insects are shown in Table V. Of the two species studied, S. oryzae appeared more susceptible to gamma radiation than T. castaneum. The mortality in the lot treated with 10 kr two weeks after irradiation was 17.3% in the case of S. oryzae and 5.8% in the case of T. castaneum. The figures corresponding to these species in the 20-kr treatment were 46.4 and 16.7%. At 40 kr the mortality in S. oryzae was 86.3% as against 64.1% in the T. castaneum. In S. oryzae the increase in mortality from 40 to 60 kr was only 0.8%, and from 60 to 80 kr 9.6%. In T. castaneum the corresponding figures were 9.3 and 3.3%. The mortality at 80 kr was 96.7% in the former species and 76.7% in the latter. In both species there was only a slight increase in mortality from dose to dose above 20 kr compared with the dose-to-dose increase below 20 kr.

Thirty days after irradiation, about one-third of the S. oryzae and about one-ninth of the T. castaneum were killed. The 20-Kr treatment killed about 70.7% of the former and about 38.6% of the latter. The corresponding figures in the 40 kr treatment were 100 and 92.3% respectively. At 60 kr, all the T. castaneum were killed within 30 d. The existence of a threshold dose, particularly in the case of T. castaneum, is very evident.

It has been reported [7] that a dose of 8 400 r prevented T. confusum from reproducing. The possibility of grain-weevils providing a biological dosimeter for radiation disinfestation of grains had been considered. The results of other investigators [4], have, however, shown that this method

TABLE III

EFFECTS OF GAMMA RADIATION ON PUPAE OF EPILACHNA PHILIPPINENSIS DIEKE

Treatment (kr)	Pupae that attained adult stage (%)	Mortality of adults four weeks after irradiation (%)	Remarks
0 (Control)	100	0.3	All adults normal. Fertility of eggs laid about 96%. About 15% of adults died within six weeks from start of tests.
1	94.7	15.3	All adults apparently normal. All adults died within six weeks from time of irradiation. Fertility of eggs laid about 90%.
5	61.5	100	About 10% of adults with abnormal wings and/or legs. All adults died within four weeks from time of irradiation. Fertility of eggs laid about 40%.
10	43.8	100	About 5% of adults with abnormal wings and/or legs. All died in four weeks after irradiation. No eggs laid.
20	16.7	100	About 25% of adults with abnormal wings and/or legs. All died within two weeks after irradiation. No eggs laid.

TABLE IV

EFFECTS OF GAMMA RADIATION ON ADULTS OF EPILACHNA PHILIPPINENSIS DIEKE

Treatment (kr)	Mortality four weeks after irradiation (%)	Remarks
0 (Control)	1.3	All adults active; about 25% died within seven weeks from start of tests. Fertility of eggs laid about 91%.
1	11.6	All adults active like those in control; all died within seven weeks after irradiation. Fertility of eggs laid about 94%.
5	60.4	Adults active; all died within seven weeks after irradiation. Fertility of eggs laid about 72%. About 63% of larvae attained the adult stage.
10	96.7	Some adults became inactive 2 to 3 d after irradiation. All adults died within five weeks from time of irradiation. Fertility of eggs laid about 60%. About 50% of larvae attained adult stage.
20	100	Some adults became inactive soon after irradiation. All adults died within three weeks from time of irradiation. Fertility of eggs laid about 30%. About 15% of larvae attained adult stage.

TABLE V

MEAN MORTALITY OF SITOPHILUS AND TRIBOLIUM ADULTS AT VARIOUS DOSE-RATES 30 d AFTER IRRADIATION (%)

Species	Control	At dose-rates (kr): 10	20	40	60	80
Sitophilus oryza	1.8	32.3	70.7	100	100	100
Tribolium castaneum	2.6	11.2	38.6	92.3	100	100

holds little promise. A more detailed study on the effects of gamma radiation on all species of stored product was recently started at the Philippine Atomic Research Centre.

REFERENCES

[1] AMY, R.L., Radiation Research, 3 (1955) 166-18.
[2] BANHAM, E.J., AERE-R3888 Atomic Energy Research Establishment, Harwell, Berks (1962) 1-32.
[3] BOURGIN, ROSE C. et al., Radiation Research 5 (1956) 657-673.
[4] BULI, J.O. et al., AERE-R3890 Atomic Energy Research Establishment, Harwell, Berks. (1961) 1-45.
[5] CORNWELL, P.B. et al., Nature 179 (1957) 670-672.
[6] CROOK, L. J. AERE-R3889 Atomic Energy Research Establishment, Harwell, Berks. (1962) 1-19.
[7] DENNIS, N.M., J. Econ. Entomol. 54 (1961) 211-213.
[8] HASSETT, C.C., Pest Control 25 (1957) 13-14.
[9] NICHOLAS, R.C. and WIANT, D.E., Food Tech. 13 (1959) 58-62.
[10] PENDLEBURY, I.B. et al., AERE-R4003 Atomic Energy Research Establishment, Harwell, Berks. (1962) 1-23.
[11] PROCTOR, B.E. et al., Food Tech. 8 (1954) 536.

DISCUSSION

C. P. PAPADOPOULOU: Did you observe any recoveries during this period of one month?

G.B. VIADO: No, there was no instance in which the insect completely recovered.

P. NARDON: With regard to the phenomena you observed following the irradiation of Epilachna larvae in the fifth stage you referred to the possibility of a hormonal disturbance, and I think that it is indeed a possibility which one can hardly ignore in many cases; the grain-weevil, as I mentioned earlier, experiences changes in growth period, fertility and weight, and such disturbances are also obtained with insecticides, which indicates that there may be some action on the neuro-secretion system.

G.B. VIADO: Yes; we have not made a detailed study of the hormonal aspects but it does look as though there is something of that nature.

PRELIMINARY STUDIES ON IRRADIATION OF SOME COMMON STORED-GRAIN INSECTS IN PAKISTAN

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Abstract — Résumé — Аннотация — Resumen

PRELIMINARY STUDIES ON IRRADIATION OF SOME COMMON STORED-GRAIN INSECTS IN PAKISTAN. The response of some major insect pests of stored grains to various radiation dosages from a Co^{60} source made available through the courtesy of the Pakistan Atomic Energy Commission has been studied. A dosage of 20 000 r produced 100% mortality of the adults of Rhizopertha dominica and Tribolium castaneum within a period of 11 d. A lower dose (10 000 r) was, however, effective in 23 d. The adults of Sitophilus granarius evinced comparatively high susceptibility and when they were exposed to 10 000 r, total mortality was attained in 7 d. The larvae of Trogoderma granarium, however, proved to be the most resistant; they were not responsive to the lower dosages, and 100% control was attained only with 25 000 r in 26 d. The maximum dosage of 250 000 r gave an instant kill in all these cases, but apart from being too high it also adversely affected the viability of the grains. Since, however, their nutritive value remained unaffected, this treatment might be of use where immediate disinfestation of non-seeding edible grains was desired. Eggs of Bruchus quadrumaculatus failed to hatch when exposed to 2500 r. Studies on other stages and dosages are in progress.

ÉTUDES PRÉLIMINAIRES SUR L'IRRADIATION DE CERTAINES ESPÈCES COMMUNES D'INSECTES NUISIBLES AUX CÉRÉALES ENGRANGÉES AU PAKISTAN. On a étudié la réponse à différentes doses de rayonnements de quelques espèces d'insectes particulièrement nuisibles aux céréales engrangées; on a utilisé à cet effet une source au ^{60}Co fournie par la Commission pakistanaise de l'énergie atomique. Une dose de 20 000 r a provoqué en l'espace de 11 j une mortalité de 100% chez les adultes de la Rhizopertha dominica et du Tribolium castaneum. Une dose de 10 000 r a donné le même résultat en 23 j. Les adultes du Sitophilus granarius ont montré une sensibilité beaucoup plus élevée; une dose de 10 000 r a entraîné une mortalité de 100% en 7 j. Ce sont les larves de la Trogoderma granarium qui se sont révélées les plus résistantes; elles ont été insensibles aux doses de faible intensité et la mortalité de 100% n'a été obtenue qu'avec 25 000 r, en 26 j. Une dose maximum de 250 000 r a entraîné une mort instantanée dans tous les cas; mais, outre le fait qu'elle est trop élevée, cette dose nuit à la conservation des céréales. Toutefois, la valeur nutritive des céréales ne s'en trouvant pas affectée, on pourrait appliquer des doses de cette intensité lorsqu'on veut rapidement désinsectiser des grains comestibles qui ne sont pas destinés à la semence. Des œufs de Bruchus quadrumaculatus exposés à une dose de 2500 r ne sont pas parvenus à éclosion. On poursuit les études sur des insectes à d'autres stades de leur développement et pour des doses différentes.

ПРЕДВАРИТЕЛЬНОЕ ИЗУЧЕНИЕ РЕЗУЛЬТАТОВ ОБЛУЧЕНИЯ НЕКОТОРЫХ ОБЫЧНЫХ АМБАРНЫХ НАСЕКОМЫХ В ПАКИСТАНЕ. Были изучены ответные реакции некоторых основных амбарных насекомых-вредителей на облучение различными дозами от источника с C^{60}, любезно предоставленного Пакистанской комиссией по атомной энергии. Доза 20 000 р вызвала 100-процентную смертность взрослых особей Rhizopertha dominica и Tribolium castaneum в течение 11 суток. Доза 10 000 р оказалась эффективной только через 23 суток. Взрослые особи Sitophilus granarius проявляли относительно большую чувствительность, и при облучении их 10 000 р полная смертность наступала через 7 суток. Однако личинки Trogoderma granarium оказались наиболее стойкими; на более низкие дозы они вовсе не реагировали, а 100-процентная смертность была достигнута только через 26 суток после облучения 25 000 р. Максимальная доза 250 000 р приводила к мгновенной смерти во всех случаях, но эта доза слишком высока, и приводит к нарушению прорастаемости семян. Однако ввиду того, что питательные свойства не страдают, эта доза может быть использована там, где желательно немедленное истребление насекомых в непредназначенных для посева продовольственных зерновых. Bruchus quadrumaculatus не вылупливается при облучении дозой в 2500 р. В настоящее время продолжается изучение других стадий и дозировок.

ESTUDIOS PRELIMINARES SOBRE LOS EFECTOS DE LA IRRADIACIÓN EN ALGUNAS DE LAS PLAGAS INSECTILES DE LOS SILOS MÁS COMUNES EN EL PAKISTAN. El autor ha estudiado los efectos que en algunas de las principales plagas insectiles de los silos ejercen diversas dosis de irradiación aplicadas con una fuente de ^{60}Co que puso a su disposición la Comisión de Energía Atómica del Pakistán. Una dosis de 20 000 r causó al cabo de 11 d una mortalidad del 100% entre los Rhizopertha dominica y Tribolium castaneum adultos. Una dosis de 10 000 r produjo los mismos resultados al cabo de 23 d. El Sitophilus granarius adulto acusó una sensibilidad relativamente mucho más elevada, pues con una dosis de 10 000 r se obtuvo una mortalidad del 100% en sólo 7 d. Las larvas de Trogoderma granarium demostraron ser las más resistentes; con dosis inferiores no acusaron efecto alguno y su exterminio total exigió 25 000 r y 26 d. Una dosis máxima de 250 000 r produjo inmediatamente efectos letales en todos los insectos, pero aparte de ser demasiado elevada resultó perjudicial para la vitalidad de los granos. Ahora bien, como no altera su poder nutritivo, se puede emplear cuando se quiera lograr la desinsectación inmediata de granos comestibles que no sean para la siembra. Los huevecillos de Bruchus quadrumaculatus dejan de desarrollarse cuando sufren una irradiación de 2500 r. El autor está estudiando los efectos de otras dosis en otras fases de la metamorfosis.

INTRODUCTION

Of the several factors limiting crop production, insect pests occupy a place of great economic significance. They ravage the crops not only from the seedling to the growing stage but in addition continue to steal their share from the field to the storage bins. All the world over, insects constitute one of the most important hazards to the safe storage of grains. They have been estimated to take a toll of at least 5% of the world production of cereal grains. A survey conducted by the Food and Agriculture Organization in 1947 indicated that, in 29 countries, of the total loss of cereal grains of 12.5 million tons about 50% was caused by insect pests alone [1]. For a predominantly agricultural country like Pakistan, the problem of the safe and clean storage of grain and grain products was a problem of its very economic viability. The tropical and sub-tropical climate of the country is highly favourable to the growth and multiplication of stored-grain insects. Of the total of about 50 insect species recorded as infesting grains, about 10 are reckoned as pests of major importance that account for at least 3-5% of the country's much-needed staple grains. On a conservative estimate, the annual insect damage in State storage alone is of the order of one million rupees.

The conventional methods of controlling these insects have so far varied between the age-old sun treatment and the modern insecticidal or fumigation methods. Fumigation, though by far the most effective method of grain disinfestation, has its own serious limitations. The efficiency and efficacy of application are governed by the physical factors of distribution and penetration and are subject to human error as well as to several indeterminable factors; then there are hazards of application as well as the toxicity of residues left behind. The development of immunity or resistance amongst the insects through persistent application of chemical insecticides is yet another incentive to devise and develop a better method of grain disinfestation which will take care of the basic control problem without leaving any toxic or poisonous residue.

With the discovery of radioisotopes and the phenomenon of radiation, there have recently been a great many studies on their exploitation and utilization towards the solution of many entomological problems. Apart from the use of radioisotopes as tagging or labelling material for the determination of

various ecological and physiological complexes and manifestations of insect life, studies on achieving population control through direct irradiation have acquired great impetus during the recent past. Though the first known application of ionizing radiation for the control of the cigarette beetle (Lasioderma serricorne) dates back as far as 1912 [3], it has only been since the early fifties that the problem has been studied in greater and more practical detail. Considerable work on the radiation disinfestation of cosmopolitan stored-grain insects has since been carried out in different countries, particularly the United Kingdom, Australia, the United States and the Soviet Union. The effect of radiation on the various life stages, the doses required, and the sequence of their effect on grains have been under constant investigation. HASSETT and JENKINS irradiated six species of stored-product insects with X-rays [5]. They concluded that heavy infestations could be quickly killed with a dose of 65 kr and that lesser doses might prevent reproduction and control higher infestation levels. CORNWELL and BULL, while working on adults of Sitophilus granarius and Sitophilus oryzae, derived a very high dose, above 300 kr, for killing these insects in less than 24 hours [2]. Lower doses of 15 kr and 25 kr gave complete control within about a week and no mortality was observed to occur below 2 kr. A dose of 0.5 Mrad was found by HORNE and BROWNELL to cause reproductive sterilization and death within 24 hours [4]. About 0.1 Mrad induced a radiation lethargy such that subsequent feeding and damage were minimized, leading finally to death within a week. Lower doses, in the range of 15-20 kr, also caused sterilization, but total mortality was delayed.

The effects of gamma irradiation of Rhizopertha dominica, Cadra (Ephestia) cautella, Plodia interpunctella and Lasioderma serricorne carried out by PENDLEBURY et al. [6] showed that the emergence of adult Rhizopertha dominica was not affected by doses of up to 20 000 rad. The emergence of Cadra cautella males was reduced by 50% at 28 400 rad and that of females at 11 600 rad. In Plodia interpunctella a 60-70% reduction in emergence was brought about by 36 000 rad, there being no difference in susceptibility between the sexes. Emergence in Lasioderma serricorne was unaffected by doses of up to 36 000 rad. Pupae and adults of Rhizopertha dominica were equally susceptible to gamma radiation.

These studies have in large measure demonstrated the economic as well as the technical feasibility of irradiation as a practical method of grain-insect control. In order, therefore, to determine the specific response of the local species and strains to the various radiation dosages and to establish its optimum rate, preliminary studies on the irradiation control of some of the important grain pests have been conducted in Pakistan. On the basis of these laboratory studies, which are being continued, it is proposed to launch a project on the large-scale control of stored-grain pests. A brief résumé of the material studied, methods adopted and results obtained is presented in the following lines.

METHOD AND MATERIAL

There are two potential methods of insect control through irradiation, indirect and direct. The indirect method consists in releasing sterile males

into the infested hold or habitat. On account of certain biological and ecological limitations, however, success so far has been confined to a limited number of species; the eradication of the screw-worm (Callitroga hominivorax) in the south-eastern United States is the only outstanding example of the success of the sterile-male technique up till now. The present series of experiments relate to direct irradiation, i.e. exposure of test insects to gamma radiation from a Co^{60} source in a "Gamma-Cell-220". This is a commercial irradiator designed for laboratory trials by Atomic Energy of Canada limited. The source is heavily shielded and is almost completely foolproof and safe for the applicators and research workers. The field in the gamma cell is provided by a Co^{60} source set up in the form of a squirrel cage. The chamber provided therein holds samples for irradiation when lowered into the source cage. The whole process of introducing the material for irradiation and removing it afterwards is designed to operate in an automatic, "push-button" manner.

The insects selected for the present study were Sitophilus granarius, Rhizopertha dominica, Tribolium castaneum, Trogoderma granarium (khapra) and Callosobruchus subinnotatus, which have been found to be the major pests of rice, wheat, wheat products and pulses during storage. Stock cultures of these insects were reared and maintained under their respective optimum conditions of temperature and humidity. 50 adults, each 1-2 weeks old, of Sitophilus granarius, Rhizopertha dominica and Tribolium castaneum, and third-instar larvae of Trogoderma granarium were placed in 3-in polyethylene tubes. Each tube contained 10 g of grain (gram in the case of Callosobruchus and wheat for the others) and was covered with muslin. All the batches of adults and larvae were then subjected to gamma irradiation from the Co^{60} source described above. Several doses ranging between 2.5 and 250 kr at a dose-rate of 8.0×10^5 r per hour were tried, each dose being given three replications. After irradiation, the larvae and the adults were returned to the controlled optimum conditions of temperature and humidity. Controls were also separately kept for each species and each dose.

RESULTS AND CONCLUSIONS

From the results obtained (Tables I-IV) it will be seen that while in all the four cases of Rhizopertha dominica, Tribolium castaneum, Sitophilus granarius and Trogoderma granarium 100% mortality immediately after exposure was obtained with the maximum radiation dosage of 250 kr, the minimum lethal dosages varied in each case. The minimum dose of 10 kr produced a 100% kill in 23 d amongst adults of both Rhizopertha dominica and Tribolium castaneum. Although the complete kill was very much delayed, the insect ceased to evince feeding activity after irradiation, so that further damage was precluded. Increased dosages of 20, 50, 80, 100, 120 and 150 kr against Rhizopertha dominica were effective in producing a total kill in 11, 9, 8, 6, 5 and 3 d respectively. The rate of control therefore more or less directly varied with the dosage rate. Similarly, in the case of Tribolium castaneum the higher dosages of 20, 50, 80, 100, 120 and 150 kr were effective in 11, 9, 7, 6, 5 and 3 d respectively. The responses of

TABLE I

EFFECT OF GAMMA RADIATION ON RHIZOPERTHA DOMINICA AND TRIBOLIUM CASTANEUM
(50 insects per treatment)

Insect and stage	Dose (kr)	Mortality (mean of 3 replications) (%) after (d)											
		0 (Immediately after exposure)	2	3	5	6	7	8	9	10	11	14	23
Rhizopertha dominica (adults)	10	-	-	-	-	-	70	-	90	-	-	-	100
	20	-	-	30	-	50	60	-	90	-	100	-	-
	50	-	-	40	40	-	50	60	100	-	-	-	-
	80	-	-	50	70	-	80	100	-	-	-	-	-
	100	-	50	80	90	100	-	-	-	-	-	-	-
	120	-	80	-	100	-	-	-	-	-	-	-	-
	150	-	80	100	-	-	-	-	-	-	-	-	-
	250	100	-	-	-	-	-	-	-	-	-	-	-
	Nil (control)	-	-	-	-	-	-	-	-	-	-	-	-
Tribolium castaneum (adults)	10	-	-	-	70	-	-	80	-	-	90	-	100
	20	-	-	30	50	60	-	90	-	100	-	-	-
	50	-	-	40	40	-	50	60	100	-	-	-	-
	80	-	50	70	-	80	100	-	-	-	-	-	-
	100	-	50	80	90	100	-	-	-	-	-	-	-
	120	-	80	100	-	-	-	-	-	-	-	-	-
	150	-	80	100	-	-	-	-	-	-	-	-	-
	250	100	-	-	-	-	-	-	-	-	-	-	-
	Nil (control)	-	-	-	-	-	-	-	-	-	-	-	-

TABLE II

EFFECT OF GAMMA RADIATION ON SITOPHILUS GRANARIUS (ADULTS)
(50 insects per treatment)

Dose (kr)	Mortality (mean of 3 replications) (%) after (d)				
	0 (Immediately after exposure)	2	3	5	7
10	-	25	55	75	100
20	-	50	90	90	100
50	-	50	80	100	-
100	100	-	-	-	-
Nil (control)	-	-	-	-	-

Rhizopertha dominica and Tribolium castaneum adults to the various dosages of gamma rays were, therefore, identical.

Sitophilus granarius, however, proved to be the most susceptible when exposed to 10 kr and 20 kr; a 100% kill was attained within 7 d. The next higher dose of 50 kr was effective in giving total control in 5 d. Larvae of Trogoderma granarium turned out to be most resistant of all the test insects. Lower doses of the order of 2.5 kr and 5 kr did not produce any adverse effect. Radiation dosages of 10, 15 and 25 kr also proved practically ineffective, because total kill was obtained only after 30, 28 and 26 d respectively. Complete mortality immediately after irradiation was obtainable only with the maximum dosage of 250 kr. However, 3-d-old khapra eggs exposed to 10 kr gave only 50% hatching. The emerged larvae also died within 3 d, and there was no emergence at all with increased dosages of 15 and 25 kr. The 1-d-old eggs of Callosobruchus subinnotatus when exposed to 3 and 5 kr failed to give any emergence, while at 2.5 kr the life cycle was extended to 48 d from 39 d for the control. Similarly the 12-d-old grubs did not moult and died after 18, 16, 16 and 10 d when exposed to 5, 10, 15 and 20 kr.

The behaviour of the adults of Rhizopertha dominica, Tribolium castaneum and Sitophilus granarius to various radiation dosages as discussed above indicated that they were capable of being controlled within the dose range 10 – 50 kr. The maximum dosage of 250 kr, though giving instant kill, was considered high and thus unsuitable for commercial operation, as the cost involved would not be economical.

The observations on khapra larvae, which have so far defied economical control, are considered inconclusive. Further studies on khapra and other insects are being continued.

TABLE III

EFFECT OF GAMMA RADIATION ON TROGODERMA GRANARIUM

Stage	No. of larvae or eggs	Dose (kr)	Mortality (mean of 3 replications) (%) after (d)																			
			0 (Immediately after exposure)	6	8	9	10	11	12	14	16	18	20	22	23	24	26	28	30	32	34	36	40
Third-instar larvae	50	5	-	12	-	-	-	-	20	-	30	-	44	50	54	-	62	-	75	-	85	95	100
		10	-	20	-	-	40	-	-	50	-	-	55	60	-	-	75	95	100	-	-	-	-
		15	-	20	-	-	37	-	50	-	60	-	75	-	-	80	90	100	-	-	-	-	-
		20	-	25	-	-	45	-	-	65	-	72	85	-	-	88	100	-	-	-	-	-	-
		25	-	35	40	-	55	-	60	-	77	-	85	86	-	90	100	-	-	-	-	-	-
		250	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Nil (control)[a]		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Stage	No. of larvae or eggs	Dose (kr)	Emergence (%)
3-d-old eggs	100	10	50
		15	Nil
		25	Nil
		Nil (control)[b]	87

[a] Pupation began after the 18th day of the experiment.
[b] But no larva survived more than 3 d.

TABLE IV

EFFECTS OF GAMMA RADIATION ON EGGS AND GRUBS OF CALLOSOBRUCHUS SUBINNOTATUS
(100 eggs or grubs per treatment)

Stage	Dose (kr)	Results (mean of 3 replications)
24-h-old eggs	2	Emergence delayed by 9 d
	3	No emergence
	5	No emergence
	Nil (control)	95% usual hatching took place after 8 d
Second-stage grubs (12 d old)	5	No moulting and all died on the 18th day
	10	No moulting and all died on the 16th day
	15	No moulting and all died on the 16th day
	20	No moulting and all died on the 10th day
	Nil(control)	Normal moulting took place after 6-8 d.

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REFERENCES

[1] COTTON, R.T. and ASHBY, Wallace, "Insects", The Year Book of Agriculture (1952) 629.
[2] CORNWELL, P.B. and BULL, J.O., J.Sci.Food Agric. 11 12 (1960) 754.
[3] HILCHEY, J.D., "Action of ionizing radiation on insects" in Radiation Preservation of Food, (Bailey, S.D. et al. eds.) (1957) 240-266.
[4] HORNE, T. and BROWNELL, L.E., Radioisompes and Radiation in Entomology, IAEA, Vienna (1962) 233.
[5] HASSETT, C.C. and JENKINS, D.W., Nucleonics 10 12 (1952) 42.
[6] PENDLEBURY, J.B., JEFFERIES, D.J., BANHAM, E.J. and BULL, J.O., Some effects of gamma radiation on the Lesser Grain Borer, Tropical Warehouse Moth, Indian Meal Moth and Cigarette Beetle, AERE-4003, Harwell (1962).

DISCUSSION

E.E. TURTLE: You stated that Cornwell and Bull had reported a very high dose for grain disinfestation. I think the dose they indicated was one

which would have an immediate killing effect. In countries where the grain may be stored for some time an immediate kill is not necessary and consequently a lower dose can be used.

H. HUQUE: That is correct. Sometimes we have to export rice to other Far Eastern countries, such as Thailand, and in that case we need to know the dose which will kill the insects within a certain period of time.

STUDIES ON THE EFFECTS OF GAMMA RADIATION ON THE DIFFERENT DEVELOPMENTAL STAGES OF THE KHAPRA BEETLE, TROGODERMA GRANARIUM EVERTS

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Abstract — Résumé — Аннотация — Resumen

STUDIES ON THE EFFECTS OF GAMMA RADIATION ON THE DIFFERENT DEVELOPMENTAL STAGES OF THE KHAPRA BEETLE, TROGODERMA GRANARIUM EVERTS. Different stages in the development of the Khapra beetle were exposed to various doses of gamma radiation and their effects were recorded. It was observed that the females were more susceptible to gamma radiation than the males. A dose of 6000 rad induced sterility in the females, whereas the males required 16 000 rad for complete sterility. The reasons for such differences are discussed.

In addition the influence of post-irradiation temperature on radiation damage has been investigated. The response of the larvae to gamma radiation appeared to be considerably influenced by temperature following irradiation. Thus the LD50 (8 d) at temperatures of 30°C, 34°C and 38°C was 59 980, 26 360 and 24 100 rad respectively.

Evidence is presented to show that, within certain limits, the stage at which the radiation damage manifests itself is largely dependent on the ambient temperature. In the irradiated larvae maintained at 30°C, pupation was arrested above a dose of 8000 rad. In those maintained at 34°C and 38°C doses above 10 000 and 12 000 rad respectively were required to bring about the same effect. It is well known that a reduction in ambient temperature prolongs the larval stage. This is reflected in this study by the fact that at low temperature mortality occurred in the larval stage itself, whereas at higher temperature it was seen in the pupal stage.

EFFETS DES RAYONS GAMMA AUX DIVERS STADES DU DÉVELOPPEMENT DU DERMESTIDÉ KHAPRA, TROGODERMA GRANARIUM (EVERTS). Le dermestidé Khapra a été exposé, à divers stades de son développement, à des doses variables de rayons gamma dont on a noté les effets. On a observé que les femelles étaient plus sensibles que les mâles aux rayons gamma. Une dose de 6000 rad a provoqué la stérilité chez les femelles, alors qu'il en fallait 16 000 pour provoquer la stérilité complète chez les mâles. Les auteurs discutent les raisons de ces différences.

En outre, les auteurs ont étudié l'influence de la température après irradiation sur les dommages radioinduits. La sensibilité des larves aux rayons gamma semble être fortement influencée par la température après l'irradiation. Par exemple, la DL_{50} (8 j) à des températures de 30°C, 34°C et 38°C était respectivement de 59 980, 26 360 et 24 100 rad.

Les auteurs indiquent des faits qui portent à croire que, dans certaines limites, le stade auquel les dommages radioinduits se manifestent dépend dans une large mesure de la température ambiante. Chez les larves irradiées maintenues à 30°C, la pupation était arrêtée pour une dose supérieure à 8000 rad. Chez les larves maintenues à 34 et 38°C, il fallait, pour obtenir le même effet, des doses de 10 000 et 12 000 rad respectivement. Il est bien connu que la diminution de la température ambiante prolonge le stade larvaire. Cette prolongation se traduit, dans cette étude, par le fait qu'aux basses températures la mortalité est apparue au stade larvaire, tandis qu'aux températures plus élevées elle a été constatée pendant la pupation.

ИССЛЕДОВАНИЕ ВЛИЯНИЯ ГАММА-ОБЛУЧЕНИЯ НА РАЗЛИЧНЫЕ ФАЗЫ РАЗВИТИЯ ЖУКОВ КАПРА, TROGODERMA GRANARIUM (ЭВЕРТСА). Жуки Капра на разных фазах развития подвергались гамма-облучению в различных дозах с регистрацией последствий облучения. Было замечено, что женские особи более чувствительны к гамма-облучению, чем мужские. Доза 6000 рад приводит к стерильности у женской особи, тогда как для полной стерилизации мужской особи требуется 16 000 рад. Обсуждаются причины такого различия.

Кроме того, исследовалось влияние на течение радиационного повреждения температуры окружающей среды после облучения. Реакция личинок на гамма-облучение, как кажется, в значительной степени зависит от температуры, при которой они содержатся после облучения. Таким образом, LD_{80} (8дней) при температурах 30°C, 34°C и 38°C соответственно равнялась 59 980, 26 360 и 24 100 рад.

Приводятся доказательства того, что с некоторыми ограничениями стадия, на которой проявляется радиационное повреждение, в значительной степени зависит от температуры окружающей среды. Окукливание личинок, содержащихся при температуре 30°C, прекращалось при дозе свыше 8000 рад. Для получения такого же эффекта прекращения окукливания личинок, содержащихся при температурах 34°C и 38°C, требовались дозы сверх 10 000 и 12 000 рад соответственно. Хорошо известно, что снижение температуры окружающей среды продлевает стадию личинки. Это подтверждается в данном исследовании тем, что при низкой температуре смертность наблюдается в самой стадии личинки, тогда как при более высокой температуре она наблюдается в стадии окукливания.

ESTUDIO DE LOS EFECTOS DE LAS RADIACIONES GAMMA SOBRE LAS DIVERSAS FASES DE DESARROLLO DEL COLEÓPTERO TROGODERMA GRANARIUM (EVERTS). Los autores expusieron T. granarium en diversas fases de desarrollo a dosis variadas de radiaciones gamma y registraron los efectos observados. Las hembras se mostraron más sensibles a las radiaciones gamma que los machos, pues quedaron estériles con una dosis de 6000 rad mientras que los machos precisaron 16 000 rad para llegar a la esterilidad completa. La memoria discute los motivos de esta diferencia.

Además, se ha investigado la influencia que ejerce sobre las radiolesiones la temperatura a que se mantiene al insecto después de la irradiación. Se encontró que la reacción de las larvas a las radiaciones gamma varía bastante con dicha temperatura. Las DL_{50} (8 d) a temperaturas de 30°C, 34°C y 38°C fueron 59 980, 26 360 y 24 100 rad respectivamente.

La memoria demuestra que, dentro de ciertos límites, la fase en que se manifiestan los daños radioinducidos depende en gran parte de la temperatura ambiente. Manteniendo a 30°C las larvas irradiadas, la formación de ninfas se interrumpió con dosis superiores a 8000 rad. Para lograr el mismo efecto con larvas mantenidas a 34°C y 38°C se precisaron, respectivamente, dosis superiores a 10 000 y a 12 000 rad. Como se sabe, al reducir la temperatura ambiente se prolonga la duración del estado larval. El presente estudio refleja esto en el hecho de que la mortalidad a temperaturas bajas se produjo en el propio estado larval, mientras que a temperaturas más elevadas se produjo en las ninfas.

INTRODUCTION

For the efficient utilization of radiation in the disinfestation of grain it is imperative that the minimum dose suggested for commercial treatment must be effective against all the known insect pests in the grain. Extensive studies carried out by CORNWELL, P.B. and MORRIS, J.A. [6] on the susceptibility of Sitophilus granarius and S. oryzae to gamma radiation have shown that a dose of 16 krad was sufficient for complete control of these insects in the grain. MARTIN, V.J. et al. [11] have indicated that changes in population densities will not affect the susceptibility of grain weevils to sterilizing doses of gamma radiation and that it is unlikely to reduce the efficacy of 16 krad for the control of this insect. PENDLEBURRY, J.B. and BANHAM, E.J. [12] studied the influence of culture temperature in modifying the radiation damage in the grain weevil and concluded that, though changes in both the pre- and the post-irradiation temperatures modify radiation response at certain dose levels, the efficacy of the recommended dose will not be reduced by the "fluctuating and variable temperatures such as are found in commercial practice".

It is well known that changes in environmental factors, such as low temperature and scarcity of food, can induce "diapause" or quiescence in the larvae of the Khapra beetle, Trogoderma granarium [3]. It is therefore

reasonable to expect that under such conditions of suspended animation the response of the larvae to gamma radiation will be different from what it would be when they are irradiated during a period of high physiological activity. The present study was undertaken with a view to assessing the role of "diapause" in modifying the radiation damage in the larvae, and also the susceptibility of the various stages of development of the Khapra beetle to different doses of gamma radiation.

MATERIAL AND METHODS

The developmental stages of the Khapra beetle used in these studies were taken from cultures maintained in the laboratory at 38 ± 1°C. Irradiation was carried out in a cobalt-60 source at a dose-rate of 1.5×10^5 rad/h.

In order to evaluate the sterilizing dose for both the sexes, male and female pupae were collected from the culture over a period of four days at one-day intervals and pooled to give random distribution of all the age groups. Requisite numbers were then taken and irradiated with doses ranging from 5 to 18 krad. After irradiation the male and female pupae were kept separately for adult emergence along with a set of controls. The emerged adults were then paired as follows:- (a) Irradiated male × normal female, (b) Normal male × irradiated female, (c) Irradiated male × irradiated female and (d) Normal male × normal female. For each mating combination three replications of fifteen pairs were used. The insects thus paired were kept in specimen tubes (8 cm × 2.5 cm) along with crushed wheat to serve both as food and as a medium for oviposition. The food was sieved for the eggs at the end of four days and on alternate days thereafter till all the females died. Observations on the viability of the eggs were made five days after oviposition.

For studies on the lethal effects of radiation, two-day-old eggs were irradiated with doses ranging from 2 to 6 krad. In each replication 50 eggs were taken and each dose treatment was replicated three times. The viability of the irradiated and control eggs was recorded at the end of five days. Wherever hatching occurred the larvae were provided with food and allowed to complete development.

Freshly hatched larvae were irradiated with doses ranging from 5 to 16 krad. For each dose three replications of 100 larvae each were taken. After irradiation they were released over crushed wheat in Petri dishes kept at 38°C. Mortality as well as pupation rates were recorded till all the larvae had either pupated or died. In addition, larvae in the late instar and prepupal stage were also irradiated with doses ranging from 40 to 150 krad. Mortality counts were taken at the end of 48 h and the data were subjected to "probit" analysis for the estimation of LD 50.

In order to determine whether post-irradiation temperature would modify radiation response, larvae collected at random from the culture maintained at 34 ± 1°C and which consisted of instars after the third, were irradiated with doses ranging from 5 to 18 krad. After irradiation they were separated into three groups. Of these, one was transferred to a temperature of 30°C, while the second and the third were kept at 34 and 38°C respectively. The data obtained on mortality at the end of the eighth day were subjected to "probit" analysis for the calculation of LD50.

RESULTS

It is seen from Table I that the eggs when exposed to a dose of 4 krad and above failed to hatch. At 2 krad the viability, pupation and adult emergence were 19.3%, 7% and 5.4% respectively, while in the control the corresponding figures were 88.6%, 74.7% and 65.4% respectively.

TABLE I

EFFECT OF GAMMA RADIATION ON THE EGGS OF TROGODERMA GRANARIUM EVERTS

Dose (krad)	Viability (%)	Pupation (%)	Adult emergence (%)
0 (Control)	88.6	74.7	65.4
2	19.3	7.0	5.4
4	0	0	0
5	0	0	0
6	0	0	0

The results obtained on the response of the freshly hatched larvae to gamma radiation are presented in Table II. These data show that 100% mortality was obtained at all dose levels of 10 krad and above on the 25th day after irradiation. At a dose level of 5 krad, about 14% underwent pupation, but adult emergence was completely inhibited. At all the other dose levels the larvae did not enter into the next instar and died in the first instar itself.

Table III gives the effects of radiation of the last instar and prepupal stages. It can be seen that the LD50 at the end of 48 h is 101 900 rad, with fiducial limits at 95% confidence level of 101 600 and 102 100 rad.

When adults which emerged from irradiated pupae were paired as indicated earlier it was observed that fecundity decreased with increasing dose (Fig. 2). When a mating combination was exposed to a dose of 6 krad all the eggs were found to be sterile. On the other hand when only the male in this combination was given a dose of 6 krad viability was observed to be 26.5%. A further reduction in the viability occurred when the dose was raised from 6 krad, and 100% sterility was obtained when the males received a dose of 16 krad (Fig. 1). These data indicate that the females can be sterilized by a dose of 6 krad while the corresponding sterilizing dose for the males is 16 krad.

The effects of temperature on the susceptibility of the larvae to gamma radiation are recorded in Table IV. It is evident from the data that the radiation susceptibility was more pronounced at 34 and 38 than at 30°C. The

TABLE II

RESPONSE OF FRESHLY HATCHED LARVAE OF TROGODERMA GRANARIUM EVERTS TO DIFFERENT DOSES OF GAMMA RADIATION

Dose	Mortality (%) after									Pupation
(krad)	9	15	25	36	47	53	66	70	73 d	(%)
0 (Control)	5.0	5.0	10.0	10.0	10.0	-	-	-	-	90.0
5	11.0	25.0	29.0	48.0	61.0	62.0	72.0	79.0	86.0	14.0
6	29.0	46.0	55.0	79.0	96.0	100.0				0.0
8	63.0	87.0	94.0	100.0						0.0
10	85.0	97.0	100.0							0.0
12	98.0	98.0	100.0							0.0
14	90.0	97.0	100.0							0.0
16	90.0	96.0	100.0							

TABLE III

RELATIVE SUSCEPTIBILITY OF THE LATE-INSTAR LARVAE AND PREPUPAL STAGE OF TROGODERMA GRANARIUM EVERTS TO GAMMA RADIATION

Heterogeneity	Regression equation	LD50 (rad)	Fiducial limits of LD50 with 95% confidence
χ^2 (6) = 5.76	Y = 4.771 x - 18.89	101 900	102 100 101 600

Y = probit kill; x = log dose; LD50 = Radiation dose calculated to give 50% kill. The data were not found to be heterogeneous at P = 0.05.

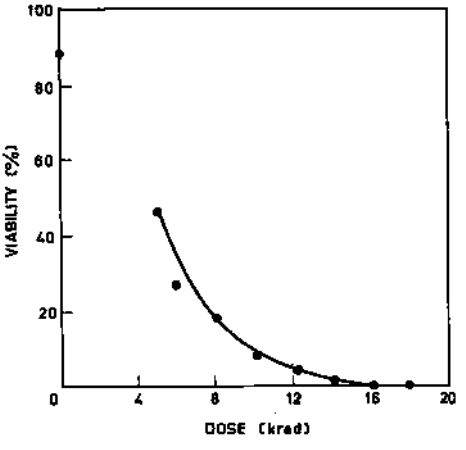

Fig. 1

Viability of eggs laid by normal female paired with male exposed to different doses of gamma radiation

LD50 values calculated from the probit analysis of mortality data were 59 980 rad at 30°C and 26 360 rad and 24 100 rad at 34 and 38°C respectively. The mortality response at doses of 14, 16 and 18 krad was independent of the post-irradiation temperature (Figs. 4, 6 and 8). However, at lower dose levels the influence of temperature on the mortality response was evident. It can also be noted that the pupation response was not only influenced by the radiation dose but also by the post-irradiation temperature treatment. At the three temperatures of 30°, 34° and 38°C pupation occurred at dose levels up to 8, 10 and 12 krad respectively. However, it was found that adults failed to eclose from the pupae formed at dose levels of 8 krad and above, irrespective of the post-irradiation temperature.

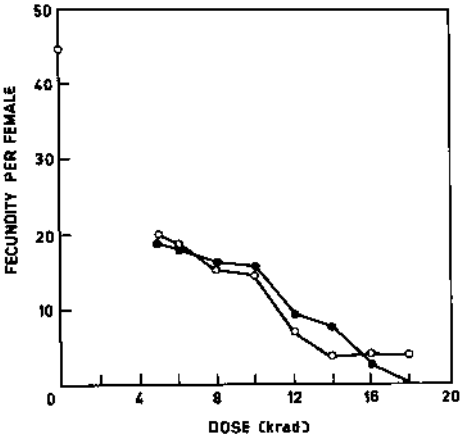

Fig. 2

Average number of eggs per irradiated female when paired with a normal or an irradiated male

● Irradiated ♂ × irradiated ♀
○ Normal ♂ × irradiated ♀

TABLE IV

RELATIVE SUSCEPTIBILITY OF THE LARVAE OF TROGODERMA GRANARIUM EVERTS TO GAMMA RADIATION IN RELATION TO POST-IRRADIATION TEMPERATURE

Post-irradiation temperature (°C)	Heterogeneity	Regression equation	LD50 (rad)	Fiducial limits of LD50 with 95% confidence
30	χ^2 (6) = 10.72	Y = 1.511 x - 2.224	59 980	63 360 56 780
34	χ^2 (6) = 7.3	Y = 1.953 x - 3.632	26 360	27 200 25 550
38	χ^2 (6) = 8.65	Y = 1.793 x - 2.875	24 100	25 060 23 120

Y = Probit kill; x = Log dose; LD50 = Radiation dose calculated to give 50% kill.
In none of the cases were the data found to be heterogeneous at P = 0.05.

DISCUSSION

It is evident from these studies that the females of the Khapra beetle are more susceptible to radiation than the males. A similar observation was made by CARNEY, G. C. [5] on the same species. Various factors

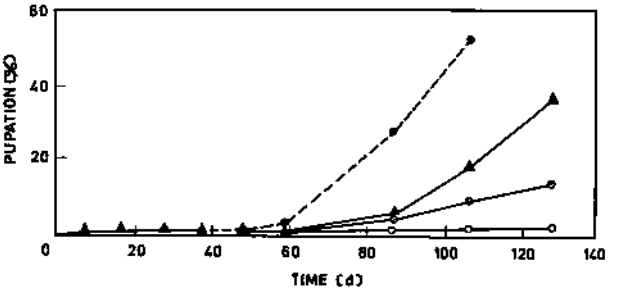

Fig. 3

Effect of post-irradiation temperature of 30°C on pupation

▲ 5 krad
⊙ 6 krad
○ 8 krad
● Control

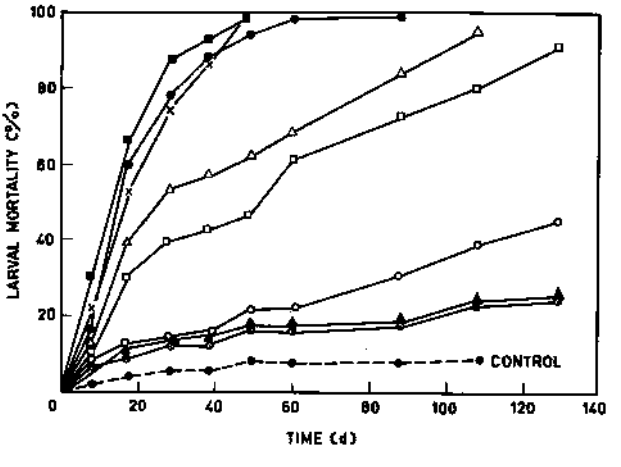

Fig. 4

Effect of post-irradiation temperature of 30°C on larval mortality

▲ 5 krad
⊙ 6 krad
○ 8 krad
□ 10 krad
△ 12 krad
● 14 krad
× 16 krad
■ 18 krad
—●—●— Control

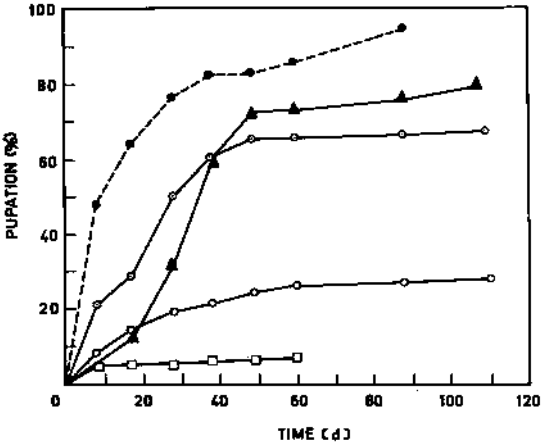

Fig. 5

Effect of post-irradiation temperature of 34°C on pupation

▲ 5 krad
⊙ 6 krad
○ 8 krad
□ 10 krad
—●—●— Control

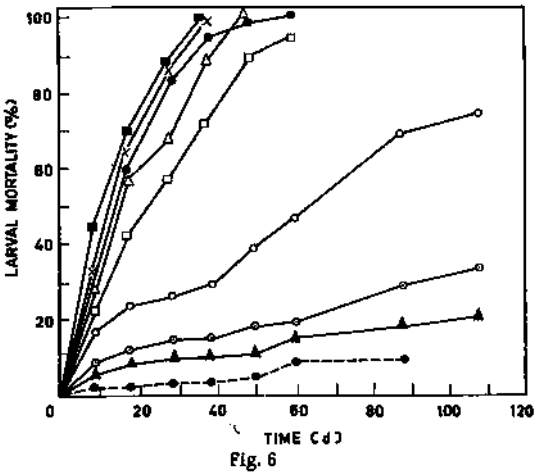

Fig. 6

Effect of post-irradiation temperature of 34°C on larval mortality

▲ 5 krad
⊙ 6 krad
○ 8 krad
□ 10 krad
△ 12 krad
● 14 krad
× 16 krad
■ 18 krad
—●—●— Control

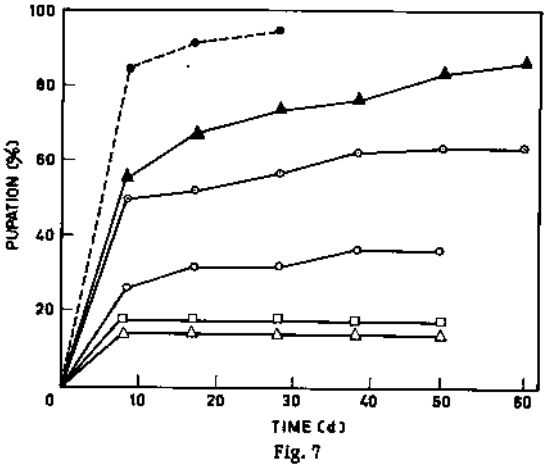

Fig. 7

Effects of post-irradiation temperature of 38°C on pupation

▲ 5 krad
⊙ 6 krad
○ 8 krad
□ 10 krad
△ 12 krad
—●—●— Control

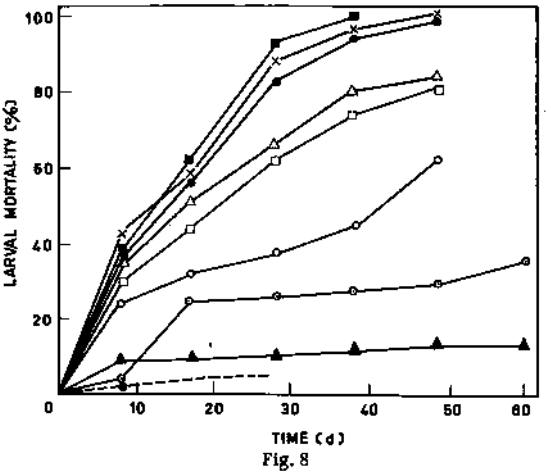

Fig. 8

Effects of post-irradiation temperature of 38°C on larval mortality

▲ 5 krad
⊙ 6 krad
○ 8 krad
□ 10 krad
△ 12 krad
● 14 krad
× 16 krad
■ 18 krad
—●—●— Control

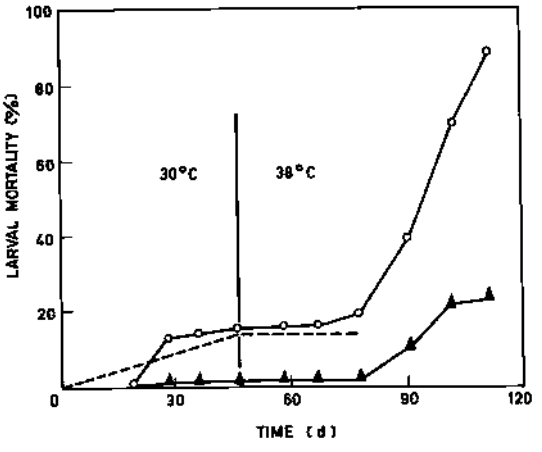

Fig. 9

Effect of higher temperature (38°C) on irradiated larvae maintained for long periods at low temperature

▲ 5 krad
○ 8 krad
--- Control

appear to contribute to this differential susceptibility between male and female. Among these the one that seems most plausible is the body volume. The average body volume of the female is approximately three times that of the male; hence it is presumed that the total energy deposited is correspondingly more in the female than in the male. COLE, M.M. et al. [7] determined the LD50 of insects of different body sizes. According to them the LD50 decreased with the increase in body size. It is therefore reasonable to expect a greater biological damage for the same dose in the female than in the male. Another factor which may contribute to the damage differential is probably the oxygen tension of the tissues. Preliminary studies on oxygen uptake by males and females have shown that the female consumes more oxygen than the male. If the oxygen tension of the tissue is also greater in the female than in the male, it is possible that the biological damage in the female is also more severe, since higher oxygen tension is known to enhance radiation damage [1, 10]. Only a detailed investigation would throw more light on these and other factors which are also involved in enhancing radiation damage in the females. Such studies are in progress.

Irradiation of the larvae at different instars has shown that there is a progressive increase in resistance to radiation as larval development proceeds. The first-instar larvae could not develop any further when the dose applied was 6 krad; 50% mortality occurred on the 20th day after irradiation. On the other hand, when the larvae above third instar were exposed to a dose of 8 krad, 50% mortality was observed on the 43rd day. Pupation was completely inhibited at 6 krad in the first instar, while the corresponding dose for complete pupal inhibition was 14 krad.

BALDWIN, W.E. [2] has shown that the lethal effects of X-rays can be drastically modified by post-irradiation temperature. This he observed in Dahlbominus fascipennis, which was first exposed to X-rays and im-

mediately thereafter transferred to higher temperature for a short time. He concluded that irradiation in some way or the other sensitized the insect to heat which was normally sublethal. When this process was reversed, i.e., X-ray treatment following heat, a negligible killing occurred. In contrast to this, PENDLEBURRY, J.B. and BANHAM, E.J. [12] found in Sitophilus granarius that heating before irradiation sensitized the insect at doses of 5100 and 7700 rad, though at doses above 7700 rad complete kill was obtained within fourteen days when the post-irradiation temperature was 30°C. From our studies it is apparent that post-irradiation temperature does modify radiation response, as is evidenced from the different LD50 obtained for each temperature treatment (Table IV). The arrest of growth noticed at 30°C is associated with a fall in metabolic rate. BURGES, H.D. [4] has shown that the level of respiration of the diapause larvae of T.granarium is considerably lower than that of the normal ones. Since it is known that high oxygen tension can enhance radiation damage, it is likely that the increase in LD50 observed in the "diapausing" larvae may be due to, among other factors, the low oxygen tension in its tissues.

It is evident from the data obtained that, within certain limits, the stage at which the radiation damage manifests itself is largely dependent on the ambient temperature. In the irradiated larvae maintained at 30°C pupation was arrested above a dose of 8 krad. In those maintained at 34°C and 38°C, doses above 10 and 12 krad respectively were required to bring about the same effect. It is well known that reduction in ambient temperature prolongs the larval stage. This is reflected in this study by the fact that at low temperature mortality occurred in the larval stage itself, whereas at higher temperature, as a result of accelerated development, it was seen in the pupal stage. Though pupation occurred at these dose levels, adult emergence was completely inhibited at all doses above 6 krad irrespective of the temperature treatment. In all cases where adult emergence occurred, the adults were found to be sterile.

Though most of the studies carried out by earlier workers have been on the influence of temperature on the radiation susceptibility, no attempt has previously been made to study the effects of radiation on "diapausing" insects. It has been shown that when hibernating mammals such as marmots [14] and squirrels [9] were irradiated in the hibernating stage, mortality was greatly reduced; but when the survivals were awakened the animals died after the same period of sickness as non-hibernating controls. In our studies too, when the larvae were irradiated and maintained in the "diapausing" state, the larval mortality was remarkably low; but when the "diapause" was broken by increasing the temperature, radiation damage began to manifest itself (Fig. 5). This shows that the development of radiation damage was postponed but by no means prevented by "diapause".

SUMMARY

Different stages in the development of Khapra beetle were exposed to various doses of gamma radiation and their effects recorded. It was observed that the females were more susceptible to gamma radiation than the males.

REFERENCES

[1] BAKER, W.K. and SGOURAKIS, E., Proc. Natl. Acad. Sci., 36 (1950) 176-84.
[2] BALDWIN, W.E., Rad. Res. 5 (1) (1956) 46-51.
[3] BURGES, H.D., Bull. entomol. Res. 50 (1959) 407-22; Nature, 184 (1959) 1741-2.
[4] BURGES, H.D., J. Insect Physiol. 5 (3-4) (1960) 317-34.
[5] CARNEY, G.C., Nature 183 (1959) 338-9.
[6] CORNWELL, P.B. and MORRIS, J.A., AERE R 3065 (1959).
[7] COLE, M.M., LABRECQUE, G.C. and BURDEN, G.S., J. econ. Entomol. 52 3 (1959) 448-50.
[8] DAVIES, D.R. and WALL, E., Int. J. Rad. Biol. 2 3 (1960) 261-7.
[9] DOULL, J. and DUBOIS, K.P., Proc. Soc. Exptl. Biol. Med. 84 (1953) 367.
[10] GILES, N.H., BEATTY, A.V. and RILEY, H.P., Genetics 36 (1951) 552-3.
[11] MARTIN, V.J., BURSON, D.M., BULL, J.O. and CORNWELL, P.B., AERE R 3893 (1962).
[12] PENDLEBURRY, J.B. and BANHAM, E.J., AERE R 3503 (1962).
[13] PENDLEBURRY, J.B., BANHAM, E.J., COOPER, B.E. and BLAND, C.M., AERE R 364 (1962).
[14] SMITH, F. and GRENAN, M.M., Science, 113 (1951) 686.

DISCUSSION

P. NARDON: You state that an increase in the lethal effect of irradiation was observed when the temperature was raised. Might this not be due to the fact that irradiation interferes with the metabolism of the insect? Since insects do not regulate their own temperature, if you raise the external temperature you increase their rate of metabolism. It is not surprising under such conditions that the primary effects of irradiation should also be accelerated. Could you comment on this, please?

K.K. NAIR: You are quite right. It is true that at low temperatures the metabolic rate is also low. When an insect is irradiated in this state, the radiation damage does not manifest itself very rapidly. On the other hand, if the external temperature is raised, it accelerates the metabolic rate, which results in the manifestation of radiation damage.

E.E. TURTLE: The theory that the lower susceptibility of T. granarium at lower temperatures is a consequence of the lower metabolic rate at lower temperatures is supported by the fact that the insect is also less susceptible to fumigants at these temperatures. Some very detailed work is being done to find out for one or two fumigants what is the (concentration × time) needed to kill the various stages of this insect. There again, as the temperature is lowered you need a much higher (concentration × time) to get the same kill, and I think this is explainable by assuming that the effect is due to the lower metabolic rate at lower temperatures.

RADIOSENSITIVITY OF VARIOUS STAGES OF CALLOSOBRUCHUS CHINENSIS L.

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Abstract — Résumé — Аннотация — Resumen

RADIOSENSITIVITY OF VARIOUS STAGES OF CALLOSOBRUCHUS CHINENSIS L. The effects of irradiation on the eggs, larvae, pupae and adults of Callosobruchus chinensis L., a destructive pest of leguminous seeds, have been studied. Since the entire life-cycle except the egg and adult stages of this insect is passed in the seed itself, control by conventional means is very difficult.

Insects were obtained from local grain shops and reared in an incubator in the laboratory on moong (Phaseolus mungo L.) seeds at a temperature of 29 ± 1°C and a humidity of 70-75%. Under these conditions the insect completed its life-cycle in 18-22 d.

A 1-c iridium-192 source was used for initial experiments in irradiation. Later this was increased to 4 c. Exposures were made at very close range and the dose-rates were calculated on the basis of a measurement made at 50-cm distance with a Victoreen condenser r-meter.

Eggs were irradiated at a distance of 0.5 cm from the source and larvae, pupae and adults at 1.0 cm from the source, the respective dose-rates being 80 kr/h at 0.5 cm and 20 kr/h at 1 cm for a 4-c source.

It was found that a dose of 15 krad gave 100% mortality in the case of eggs. Different doses below this level gave somewhat variable results and it is possible that there is a sensitive stage for a short period during the first 24 hours of development of the eggs.

In the case of 8-day-old larvae 100% mortality was obtained with a dose of 20 000 rad. The pupae seem to be less radiosensitive than the eggs or larvae and doses of 47 000 rad were needed to give 100% mortality.

Doses of 42 000 rad "sterilized" the males and females in the sense that, though mating took place after irradiation, the eggs produced when either of the parents had been irradiated with a dose of 42 000 rad did not hatch. Production of "sterile" eggs continued even when either or both parents had been exposed to doses of 67 000 rad - the highest dose tried.

RADIOSENSIBILITÉ DU CALLOSOBRUCHUS CHINENSIS L. AUX DIVERS STADES DE SON DÉVELOPPEMENT. On a étudié les effets des rayonnements sur les œufs, les larves, les pupes et les adultes du Callosobruchus chinensis L., insecte nuisible aux graines de légumineuses emmagasinées. Il est très difficile de lutter contre cet insecte par les moyens classiques, du fait qu'il passe toute la période larvaire et la pupation dans la graine elle-même.

Des insectes obtenus dans des graineteries locales ont été élevés en laboratoire, dans un incubateur, sur des graines de mungo (Phaseolus mungo L.), à une température de 29 ± 1°C et un degré d'humidité de 70 à 75%. Dans ces conditions, le cycle vital de l'insecte est de 18 à 22 j.

Pour les premières expériences d'irradiation, on a utilisé une source d'iridium-192 de 1 c. Son intensité a ensuite été portée à 4 c. L'exposition a été effectuée à une très faible distance; le débit de dose a été calculé d'après une mesure faite à 50 cm avec un dosimètre à condensateur de Victoreen.

Les œufs à irradier ont été placés à 0,5 cm de la source et les larves, pupes et adultes à 1 cm, l'intensité de dose étant de 80 kr/h et de 20 kr/h, respectivement, avec la source de 4 c.

Une dose de 15 000 krad a provoqué une mortalité de 100% dans le cas des œufs. Diverses doses de plus faible intensité ont donné des résultats quelque peu variables; il est possible qu'il existe un stade sensible pendant une brève période des premières 24 h du développement des œufs.

Une dose de 20 krad a provoqué une mortalité de 100% des larves âgées de 8 j. Il semble que les pupes soient moins radiosensibles que les œufs ou les larves; il a fallu des doses de 47 krad pour obtenir une mortalité de 100%.

Des doses de 42 krad ont provoqué la stérilisation des mâles et des femelles, en ce sens que les œufs produits après l'irradiation de l'un des parents ne sont pas parvenus à éclosion. La ponte d'œufs «stériles» a continué même après que l'un des parents ou les deux aient été exposés à des doses de 67 krad, maximum appliqué dans ces expériences.

ЧУВСТВИТЕЛЬНОСТЬ К ОБЛУЧЕНИЮ У РАЗЛИЧНЫХ СТАДИЙ CALLOSOBRUCHUS CHINENSIS L. Было изучено воздействие облучения на яйца, личинки, куколки и взрослые особи Callosobruchus chinensis L. - вредного насекомого, поражающего запасы бобовых культур. Так как весь жизненный цикл этого насекомого, за исключением времени пребывания в состоянии яйца и взрослой особи, протекает внутри самого боба, наблюдать за этими насекомыми обычными средствами чрезвычайно трудно.

Насекомые были взяты из местных зерновых складов и выращивались в лабораторном инкубаторе на семенах мунго (Phaseolus mungo L.) при температуре в 29°C ± 1°C и при влажности в 70 - 75%. В этих условиях насекомые завершали свой жизненный цикл за 18 - 22 суток.

Для первоначальных экспериментов с облучением был использован источник с иридием-192 мощностью 1 кюри. В дальнейшем мощность была увеличена до 4 кюри. Облучение производилось на очень близком расстоянии, и дозы подсчитывались на основании измерений, сделанных на расстоянии 50 см при помощи конденсаторного дозиметра типа Викторин.

Яйца облучались на расстоянии 0,5 см от источника, а личинки, куколки и взрослые особи - на расстоянии 1,0 см от источника, причем соответствующие дозы были 80 000 рад/час на расстоянии 0,5 см и 20 000 рад/час на расстоянии 1 см для источника мощностью 4 кюри.

Было обнаружено, что для яиц доза 15 000 рад давала 100-процентную смертность. Разные дозы ниже этого уровня давали довольно различные результаты, и представляется возможным, что во время первых 24 часов развития яиц имеется непродолжительная чувствительная стадия.

Для личинок в возрасте 8 суток 100-процентная смертность наступала при дозе 20 000 рад. По-видимому, куколки менее чувствительны к облучению, чем яйца или личинки, и для достижения 100-процентной смертности были необходимы дозы в 47 000 рад.

Дозы в 42 000 рад "стерилизовали" как самцов, так и самок в том смысле, что хотя после облучения и происходило спаривание, но из отложенных яиц потомство не развивалось, когда один из родителей подвергался облучению дозой в 42 000 радов. Откладывание "стерильных" яиц продолжалось даже в тех случаях, когда один из родителей или они оба подвергались облучению дозой в 67 000 рад, которая была наивысшей опробованной дозой.

RADIOSENSIBILIDAD DE LAS DIVERSAS FASES DE LA METAMORFOSIS DEL CALLOSOBRUCHUS CHINENSIS L. Los autores han estudiado los efectos de la irradiación sobre huevos, larvas, ninfas e imagos del Callosobruchus chinensis L., insecto que ataca a las semillas de leguminosa almacenadas. Como todo el ciclo de desarrollo del insecto - exceptuando el huevecillo y las fases adultas - tiene lugar en el interior de la semilla, es muy difícil combatirlo por medios convencionales.

Los insectos se recogieron en almacenes de granos y se cultivaron en una incubadora del laboratorio, con semillas de Phaseolus mungo L., a una temperatura de 29°C ± 1° y una humedad de 70 a 75%. El ciclo de desarrollo del insecto duró de 18 a 22 d.

Para los experimentos iniciales de irradiación, se empleó una fuente de iridio-192 de 1 c cuya intensidad se aumentó más adelante a 4 c. Las irradiaciones se efectuaron a muy corta distancia y la intensidad de las dosis se calculó a base de una medición efectuada a 50 cm de distancia con un contador Victoreen, de condensador, calibrado en roentgens.

Los huevecillos se irradiaron a medio centímetro de la fuente, y las larvas, ninfas e imagos a un centímetro; la intensidad de la dosis con la fuente de 4 c fue de 80 kr/h en el primer caso y de 20 kr/h en el segundo.

Se pudo observar que con una dosis de 15 000 rad se obtenía una mortalidad del 100% entre los huevecillos. Con dosis inferiores se obtuvieron resultados variables, y es posible que durante un corto período de las primeras 24 h de desarrollo de los huevecillos haya una fase de mayor sensibilidad.

Con larvas de ocho días, la mortalidad total se obtuvo tras una dosis de 20 000 rad. Las ninfas parecen ser menos sensibles que los huevos y las larvas, y se precisaron dosis de 47 000 rad para alcanzar el mismo resultado.

Una dosis de 42 000 rad «esterilizaba» a los machos y a las hembras en el sentido de que, aunque copularan después de la irradiación, los huevos producidos cuando uno cualquiera de los padres había sido irradiado con esta dosis resultaban hueros. La producción de huevos «estériles» continuó incluso cuando se expuso a uno o a ambos padres a dosis de 67 000 rad, que fue la más intensa que emplearon los autores.

INTRODUCTION

Beetles belonging to the family Bruchidae (order Coleoptera) are very destructive to stored lentils in Turkey, Iran, Pakistan and the Middle and

Far East [1,2,3]. The genera Bruchus and Callosobruchus are particularly serious, and moderate to heavy infestations have been reported in the region. The insects lay their eggs on the outside of the seed and the larvae bore directly into the grains, where they feed on the inside, pupate and emerge as adults by making a hole in the seed coat. Since the life-cycle is a short one – under ideal conditions about 18 - 22 d for Callosobruchus chinensis – there are several generations in a year and the damage is consequently heavy.

Because the insects pass a major portion of their life-cycles inside the seed, conventional means of control are not very successful.

Irradiation offers a promise of control and in view of the paucity of literature on the Bruchidae family, we thought it useful to work out the radiosensitivity of different stages of Callosobruchus chinensis.

IRRADIATION SOURCE AND DOSE RATE

A source of Ir^{192} was used for irradiation. To start with we had a source of about 1 c, but later this was replaced by one of 4.4 c. The source used is by no means ideal for this type of work because, firstly, it gives a whole spectrum of γ-rays and some β-rays [4]; secondly, it has a short half-life; and thirdly, because of its low strength, it is necessary to give exposures at a very close range and consequently the dose-rate cannot be accurately determined.

The dose-rate was obtained in two ways: firstly it was calculated from the 4π flux at 5 cm from the source, assuming a 2×2-mm source to be a point source; secondly it was measured by a Victoreen r-meter at a distance of 50 cm from the source and then calculated at 5 cm distance by the inverse-square law. The two figures were within 10% of each other.

The calculated dose-rate for a 4.4-c source is 820 rad/h at 5 cm. By applying the inverse-square law, which will hold true approximately, the dose at 1 cm is about 20 000 r/h and at 0.5 cm 80 000 r/h.

The measured dose rate at 50 cm distance for the same source is 8.8 r/h and therefore at 1 cm 22 000 r/h.

The dose-rate due to β-radiation has been neglected in view of the self-absorption in the source, β-absorption in the specimen container and β-absorption by the seeds themselves.

MATERIALS AND METHOD

Callosobruchus chinensis L. [5] adults were obtained from local grain shops in the town and were reared in an incubator in the laboratory on moong (Phaseolus mungo Linn.) seeds at a constant temperature of 29 ± 1°C and a humidity of 75 - 80%. While mentioning the incubator temperature, it is necessary to point out that the temperature of the infested grain is not always the same as the temperature of the incubator. Seed temperatures up to 35°C have been recorded while the incubator remained at a constant temperature of 29 ± 1°C.

To obtain eggs, clean Phaseolus mungo seeds were placed with adult Callosobruchus at 4 p.m. in the incubator and the eggs laid overnight were

irradiated at 9 a. m. the next morning. The eggs thus irradiated were up to 17 h old.

Eight-days-old larvae, the age being counted from the day the egg was laid, were irradiated in the intact seed, and so were the pupae.

RESULTS AND DISCUSSION

Table I gives the results of irradiation of eggs at different doses within 30 d. Irradiation at doses of 2 000 r gave variable results and mortalities varying from 18 to 26% were obtained. After making corrections for the

TABLE I

RADIOSENSITIVITY OF EGGS OF CALLOSOBRUCHUS CHINENSIS L.

Radiation dose (rad)	Mortality (%)
0 (control)	3.8
2000	18 - 26
5000	38
10000	58
15000	100

control, we still find this variation statistically significant and feel that this is due to the variation in age of the eggs. Possibly the radiosensitivity differs at different levels of development of the egg.

Table II shows the effect of γ - irradiation on 8 - d - old larvae of Callosobruchus. In the case of larvae, 100% mortality has been obtained by a dose of 20 000 rad.

Pupae seem to be less sensitive to γ - irradiation than either eggs or larvae, and Table III shows the results of irradiation of the pupae. To obtain 100% mortality in this case, a dose of 47 000 rad is required.

Unmated males and females emerging overnight were irradiated separately and after irradiation were paired as follows:

1. Irradiated female and normal male.
2. Irradiated male and normal female.
3. Irradiated male and irradiated female.
4. Normal male and normal female (control).

Doses of 42 000 rad were needed to "sterilize" both the males and females. When either sex exposed to this dose was paired with a normal adult of the opposite sex, eggs were laid on the seeds but the eggs failed to hatch.

Exposures at higher doses were tried and the highest dose-rate given was 67 000 r. Even at this dose the females did not stop oviposition, though the eggs, as with the lower doses mentioned above, were all sterile.

TABLE II

MORTALITY OF LARVAE OF CALLOSOBRUCHUS CHINENSIS L. AT DIFFERENT DOSES OF γ - IRRADIATION

Radiation dose (rad)	Mortality (%)
0 (control)	3.8
10 000	70
15 000	98
20 000	100

TABLE III

MORTALITY OF PUPAE OF CALLOSOBRUCHUS CHINENSIS L. AT DIFFERENT DOSES OF γ - IRRADIATION

Radiation dose (rad)	Mortality (%)
0 (control)	0.8
8 000	50
12 000	54
47 000	100

This is only a preliminary study and the present results have been presented to show that simple irradiation studies in the case of stored-grain pests can be carried out with small sources.

We are now in the process of installing a 100-c Co^{60} source which will enable us to continue this work on a larger scale.

ACKNOWLEDGEMENTS

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REFERENCES

[1] FREEMAN, J. A., Stored Products Infestation and its control in Turkey, Ministry of Agriculture, Fisheries and Food, London (1957).
[2] FREEMAN, J. A., Infestation of Stores Products in Iran, Ministry of Agriculture, Fisheries and Food, London (1958).
[3] FREEMAN, J. A., Infestation of Stored Products in Pakistan, Ministry of Agriculture, Fisheries and Food, London (1958).
[4] ALLEN, R. A., MILLETT, R. J. and SMITH, D. B., Radioisotope data, Wantage Radiation Laboratory, Wantage, Berks. (1959).
[5] Encyclopédie Entomologique, Série A XXII, Paul Lechevalier, Paris (1944).

DISCUSSION

D. A. CROSSLEY: Did you observe any difference in duration of the larval or pupal stages following irradiation?

M. S. QURAISHI: No, this could not be done because either the insects emerged as adults during the period of observation or the seed was broken open to expose the insect.

K. K. NAIR: Could you tell me the technique of your irradiation experiments?

M. S. QURAISHI: The insects and seeds were put into open tubes with cellophane on one side and pressed by means of a small cord against the cellophane.

K. K. NAIR: Do you feel that by immobilizing the insects on cellophane tape the radiation response will be different from what you would get if they were irradiated in an active state?

M. S. QURAISHI: The small source we were using was not a very good source to use for this purpose, I agree, and we shall continue this work with the bigger source that we have now.

DISINFESTATION OF DRIED FIGS BY GAMMA RADIATION

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Abstract — Résumé — Аннотация — Resumen

DISINFESTATION OF DRIED FIGS BY GAMMA RADIATION. Research has been carried on for many years on the disinfestation of stored agricultural products. Recently gamma radiation has been successfully applied in such investigations. Dried figs are among the main export products of Greece. Serious damage is caused by insect attack each year.

The research work aimed at: (1) Determining the doses of radiation required to destroy certain species of insects attacking dried figs at their various stages of development, or to destroy their eggs and thus prevent reproduction; and (2) Determining the physical and chemical changes of the texture of dried figs caused by radiation.

Studies have been made on the following species: Plodia interpunctella, Ephestia cautella, Carpophilus hemipterous, Oryzaephilus surinamensis and Lasioderma serricorne.

The optimum doses required are discussed. At these doses, irradiation damage does not become apparent in the texture of the figs.

DÉSINSECTATION DES FIGUES SÈCHES PAR LES RAYONS GAMMA. Des recherches sur la désinsectation des produits agricoles en magasin sont en cours depuis de nombreuses années. On a récemment utilisé avec succès les rayons gamma dans ces études. Les insectes font chaque année de grands ravages dans les figues sèches, qui sont l'un des principaux produits d'exportation de la Grèce.

L'objet des recherches était le suivant: 1. Déterminer les doses de rayonnements nécessaires pour détruire certaines espèces d'insectes qui, à différents stades de leur développement, attaquent les figues, ou détruire leurs œufs et les empêcher ainsi de se reproduire; 2. Déterminer les modifications physiques et chimiques que les rayonnements produisent dans la texture de la figue sèche.

Les travaux ont porté sur les espèces suivantes: Plodia interpunctella, Ephestia cautella, Carpophilus hemipterous, Oryzaephilus surinamensis et Lasioderma serricorne.

Le mémoire étudie les dises optimum d'irradiation, qui ne provoquent aucun dommage visible dans la texture de la figue.

ДЕЗИНФЕСТАЦИЯ СУШЕНОГО ИНЖИРА С ПОМОЩЬЮ ГАММА-ИЗЛУЧЕНИЯ. В течение многих лет проводятся исследования по вопросам дезинфестации сельскохозяйственных продуктов в хранилищах. Недавно гамма-излучение было успешно применено при проведении таких исследований. Сушеный инжир составляет один из основных продуктов экспорта Греции. Ежегодно насекомые наносят серьезный ущерб этому продукту.

Цель исследования: 1) определение доз излучения, необходимых для уничтожения некоторых видов насекомых, поражающих сушеный инжир, на различных стадиях их развития, и для уничтожения их яиц и предотвращения таким образом их размножения; и 2) определение физико-химических изменений текстуры сушеного инжира под действием излучения.

Исследования проводились со следующими видами: Plodia interpunctella, Ephestia cautella, Carpophilus hemipterous, Oryzaephilus surinamensis и Lasioderma serricorne.

Обсуждаются необходимые оптимальные дозы. При этих дозах не наблюдается видимого нарушения текстуры инжира под действием облучения.

DESINFESTACIÓN DE HIGOS SECOS MEDIANTE RAYOS GAMMA. Des hace muchos años se están efectuando investigaciones sobre la desinfestación de los productos agrícolas almacenados. En esas investigaciones se han utilizado recientemente con éxito los rayos gamma. Los higos secos constituyen uno de los principales productos de exportación de Grecia y los insectos ocasionan pérdidas importantes.

Las investigaciones tenían las siguientes finalidades: a) determinar las dosis de radiación necesarias para destruir ciertas especies de insectos que atacan los higos en diversas fases de su desarrollo, o destruir sus huevos impidiendo así que se reproduzcan; b) determinar las modificaciones físicas y químicas que las radiaciones producen en la contextura de los higos secos.

Se han estudiado las especies siguientes: Plodia interpunctella, Ephestia cautella, Carpophilus hemipterous, Oryzaephilus surinamensis y Lasioderma serricorne.

En la memoria se estudian las dosis óptimas de radiación que no producen daños aparentes en la contextura de los higos.

1. INTRODUCTION

Since 1930, the disinfestation of dried figs has been compulsory in Greece. Fumigation is the main method used for the destruction of insects infesting the figs. Until 1952, CS_2 was applied at a dose of 80 g/m^3 of space for a duration of 8 h; later, however, this chemical was replaced by methyl bromide given in special fumigation chambers at a dose of 25 g/m^3 of space for 12 h.

The main disadvantages of fumigation with methyl bromide are:

(a) Methyl bromide is an odourless and very toxic gas, so that special precautions need to be taken during its use and the figs must be well aerated after treatment;

(b) To ensure good penetration by the fumigant, the figs must be treated before packing;

(c) Fumigation kills adults and larvae but has a limited effect on the viability of the eggs, so that it is necessary to repeat the treatment after a certain time of storage, in order to kill the larvae which have emerged in the meantime from the unaffected eggs.

In the search for a more effective method of disinfesting dried figs, we decided to use the radiation method, which had proved satisfactory in the treatment of stored agricultural products. To satisfy our needs, the method of treating figs with gamma radiation should meet the following requirements:

(a) It should be possible to treat the figs after packing;

(b) The method must be more efficient than the previous ones in that not only adults and larvae but also all the eggs must be killed;

(c) There must be no side-effects on the texture, appearance and nutritional value of the treated products; and

(d) The large-scale application of the method must be physically and economically feasible.

2. MATERIALS AND METHODS

In view of the very large number of published papers dealing with various aspects of insect disinfestation of stored agricultural products by means of ionizing radiation no attempt will be made here to review the pertinent literature (a short Bibliography is appended). In this preliminary paper we describe briefly the methods and the results so far obtained in our own experiments.

The research work was divided into two parts. In the first part of our experiments, we examined the effects of gamma radiation on the insects infesting Greek figs. To obtain large numbers of these insects, very heavily infested figs were collected from Calamata, the main export centre for this

product, and placed in suitable insectaria. They served also as experimental media for rearing the insects.

The species found in the examined figs were identified as:

(a) Plodia interpunctella (Lepidoptera)
(b) Ephestia cautella (Lepidoptera)
(c) Carpophilus hemipterous (Coleoptera)
(d) Oryzaephilus surinamensis (Coleoptera)

All radiation exposures were made in the gamma irradiation facility of the Democritus Nuclear Research Centre, where a 500-c Co^{60} source was avail-
All radiation exposures were made in the gamma irradiation facility of the Democritus Nuclear Research Centre, where a 500-c Co^{60} source was available. The dose-rate of the source was 30-40 krad/h. Calibrations were made by the Fricke chemical dosimetry method, using ferrous sulphate solution.

Eggs, larvae and adults were irradiated separately in groups of 50 for the eggs and of 20 for the other metamorphic forms at various doses ranging from 25 to 400 krad. Controls were placed under the same conditions except for irradiation. Counts of dead and living insects were made every day, together with studies on developmental and reproductional changes.

All insect cultures were maintained in the incubator under a constant temperature of 28°C and at a relative humidity of 40%.

3. EXPERIMENTAL RESULTS

(a) Eggs

In general, doses below 25 krad do not seriously affect the hatching of the eggs of the different species. The dose of 25 krad reduces hatching of

TABLE I

MORTALITY AND RELATED RESPONSES TO GAMMA RADIATION OF INSECTS INFESTING FIGS

SPECIES	EGGS		LARVAE		PUPAE	ADULTS	
	Dose (krad)	Hatch (%)	Dose (krad)	T_{50} (d)	No-eclosion dose (krad)	Dose (krad)	T_{50} (d)
Plodia interpunctella	25	15	100	2	100	100	4
	50	7	250	1		400	1
Ephestia cautella	25	10	100	3	100	150	4.5
	50	2	300	1		400	1
Carpophilus hemipterus	25	12	100	3	100	150	3
	50	5	300	1		400	1
Oryzaephilus surinamensis	25	30	100	2	100	150	3.5
	50	10	250	1		400	1.5

the eggs to 10% for Ephestia, 12% for Carpophilus, 15% for Plodia and 30% for Oryzaephilus. At a dose of 50 krad, hatching is reduced to 2% for Ephestia, 5% for Carpophilus, 7% for Plodia and 10% for Oryzaephilus, but the enclosed larvae in general were not viable. At a dose of 100 krad, hatching was practically nil (Table I).

(b) Larvae

The effect of the radiation on the larval stage is expressed here as "T_{50}", which is the time required for 50% of the irradiated population to die after exposure to a given dose of radiation. For a dose of 50 krad, T_{50} varied between 5 and 6 d, depending upon the age of the larvae and the species. At a dose of 100 krad the value of T_{50} was 2 - 3 d, and a dose of 250 krad reduced this value to 1 d (Table I).

(c) Pupae

In general, there was no eclosion of the pupae after doses of 50 krad and above (Table I).

(d) Adults

At a dose of 50 krad, the T_{50} was between 6 and 8 d, depending upon the species, Oryzaephilus being the most resistant. At a dose of 150 krad the T_{50} was between 3 and 4 d. A dose of 400 krad killed all insects in less than 48 h (Table I).

4. EFFECTS OF IRRADIATION ON THE PRODUCTS

In the second part of our experiments, we examined the effects of various doses of gamma radiation upon the colour and the sugar content of the dried figs, as a preliminary check on possible side-effects of irradiation on the texture and appearance of the treated figs.

Colour

For the colour estimation, we used the following standard method. 5 g of figs were extracted with a mixture of equal volumes of alcohol and 10% trichloroacetic acid at a constant temperature of 45°C. The extract was mixed with 0.5 g Supercell and filtered. The colour of the filtrate was then determined in a Lumetron colorimeter in 420 nm, using as blank a mixture of alcohol and trichloroacetic acid. The results are expressed as per-cent transmission in Table II.

As shown in the Table, no significant changes of colour result after irradiation with doses ranging between 25 and 400 krad.

Sugars

For sugar estimation we applied the Lane and Eynon method, which permits determination of total and reducing sugars. Table III gives a picture

TABLE II

PHOTOMETRIC ESTIMATION OF THE COLOUR OF DRIED FIGS

(% transmission)

Dose (krad)	Irradiated	Not irradiated
25	33	40
50	33	40
100	33	40
150	34	41
200	35	39
250	32	38
300	36	43
350	35	40
400	38	43
500	33	40
600	22	35

TABLE III

SUGAR ESTIMATION OF IRRADIATED FIGS

Dose (krad)	Total (%)	Reducing (%)	Non-reducing (%)
Blank (average)	52	46.2	5.8
25	52	46.5	5.5
50	49.25	45.44	3.81
100	49.25	45.44	3.81
150	49.25	45.44	3.81
200	49.10	45.40	3.70
250	49.10	45.40	3.70
300	48.90	45.20	3.70
350	49.00	46.28	3.72
400	48.80	45.40	3.40

of the doses employed and the changes produced in sugar content after irradiation of the figs.

Table III shows that the total sugar content is not significantly affected by radiation; the slight difference between the total sugar contents of the

blank and of the irradiated figs is probably due to some decomposition of carbohydrates caused by radiation. There is also a slight diminution of non-reducing sugars, which is due to hydrolytic reactions caused by radiation.

5. CONCLUSIONS

Preliminary experiments have shown that the disinfestation of figs by gamma radiation seems to be a very promising treatment, since at the moderate doses of 100 - 200 krad needed for the destruction of the different stages of the infesting insects, no significant changes result in the texture, appearance and nutritive value (related to carbohydrates) of the figs.

Of course many problems are still to be solved and much work has to be done to overcome the difficulties associated with the large-scale application of this method. Some of these problems are the economical feasibility of the method for commercial use, the development of new packaging materials, and better conditions of storage in order to prevent re-infestation of irradiated figs.

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I wish to express my deepest appreciation to Professor G. Pantazis and F. Hangate for their useful suggestions and encouragement during the performance of this research.

BIBLIOGRAPHY

PROCTOR, B.E. et al., Food Technol. 8 (1954) 536.
BROWNELL, L.E. et al., Eng. Res. Inst., Project M 943, April 1955.
BAKER, V.H. et al., Agric. Eng. 34 (1953) 755.
CARNEY, G.C., Nature 183 (1959) 338-339.
PENDLEBURRY, J.B. et al., AERE-R 3503 (1962).
RUCK, J.A., Chemical methods for analysis of fruit and vegetable products, Con. N° 350, Canadian Department of Agriculture.

DISCUSSION

P. PELEGRIN: Is there any legislation in Greece to control the irradiation of foodstuffs?

A. PAPADOPOULOS (on behalf of the author): No, there is no such legislation. I think we shall have to carry out tests for several years to be sure the method is safe. I have heard something about the use of irradiated potatoes in the USSR and to a limited extent in Canada.

P. PELEGRIN: Yes. Potatoes may be irradiated in Canada and the USSR, I believe, and in the United States the irradiation of bacon with 4500 Mrad has been authorized since 15 February 1963. That is quite a high dose which probably represents a new direction in the conservation of food products by irradiation.

M. FRIED: I might add that disinfestation of grain by irradiation is now under consideration in the United States and a decision is expected shortly.

B. DARIS: Have you experimented with dried figs of varying water content?

A. PAPADOPOULOS: No.

B. DARIS: Did you have any trouble through the appearance of sugar crystals at high humidities? I have made certain experiments on raisins and I found that with high water contents of about 20-22% sugar crystals soon appear and this modifies the behaviour of the dried fruit.

A. PAPADOPOULOS: We had no such trouble. The fact that you tested without a plastic package might explain your difficulties.

SYMPOSIUM ON THE USE AND APPLICATION OF RADIOISOTOPES AND RADIATION IN THE CONTROL OF PLANT AND ANIMAL INSECT PESTS

Athens, 22-26 April 1963

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RADIATION AND RADIOISOTOPES APPLIED TO INSECTS OF AGRICULTURAL IMPORTANCE

Corrigenda

E. HORBER: Eradication of white grub (Melolontha vulgaris F.) by the sterile-male technique

p. 316: Opening words of first complete paragraph were omitted; it should begin: "It should be noted that males heavily outnumber females. . . ".

p. 321, sixth line up: for "and the are" read "and they are".

p. 322, last two lines of first paragraph in 214: for "vermiculate" read "vermiculite".

p. 330, 15th line down: for "isotopic dolution" read "isotopic dilution".

BACCIO BACCETTI and RAFFAELLA de DOMINICIS: The effects of gamma radiation on the ovaries of Dacus oleae Gmel.

p. 389, fourth complete paragraph: delete last sentence, beginning "Since research of this kind. . . " and substitute: "This research was performed under IAEA Research Contract No. 169/RB."

pp. 395-406, Figs. 9-20: In each caption the magnification factor should read "6677 diam."

Chairmen of Sessions, Secretariat of the Symposium and list of Participants

p. 493: For "G. PANKRAZIS" read "G. PANTAZIS"
For "G. B. SCHMIDT" read "C. H. SCHMIDT"

p. 498: For "Pankrazis, G." read "Pantazis, G."

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