MULTIFACTOR ECOLOGICAL ASPECTS OF POPULATION PERFORMANCE OF FLOUR BEETLES, *TRIBOLIUM CONFUSUM* AND *T. CASTANEUM*

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Interactions among biological, chemical and physical phenomena determine the distribution and abundance of the biota. Man manipulates these factors for his convenience, benefit and enjoyment. Knowledge of multifactor ecological data is prerequisite to a successful manipulative program, such as, pest control by radiation and/or the release of sterilized individuals.

Papers dealing with experiments on the effects of and control by radiation of flour beetles are reviewed, with special emphasis on radiation sensitivities of various life cycle stages; population performance of different species and strains in single and cohabitation regimes; population dynamics of adults exposed to acute and chronic radiations; and population maintenance after radiation and/or culture on various insecticides.

Flour beetles have many disadvantages which negate their control by radiations or modifications of the sterile-insect release technique. These include:

1) the adults as well as immatures damage grain, flour and other dried foods
2) females and males are promiscuous
3) sperms from multi copulations mix in the spermatheca. Since sperm from any copulation might fertilize eggs, the effects from sperm containing lethals might be reduced or eliminated.
4) their mobility and the lack of insect-proof storage facilities eliminates the effective control of insects whether incoming grain is radiated or whether radiation sources are implanted in the storage facilities.
5) large numbers of sterile adults cannot be added to populations in stored grain because of public health considerations.

INTRODUCTION: Man attempts to manipulate the physical, chemical and biological factors in the environment for his convenience, enjoyment and benefit. For example, inhospitable regions are made liveable due to heating and air conditioning; chemicals are used to eliminate pests, control reproduction and increase longevity; and biologically, selection results in earlier maturity, greater productivity and disease resistance.

Today more than ever, we appreciate the need to know the ecology of a target organism, whether the program is for control or eradication.

Multifactor ecological data are needed to describe the abiotic and biotic intra- and interactions and to predict the fate of the ecosystem. The successful application of the sterile-insect-release-technique emphasizes the need for detailed studies of the candidate organism not only in ecology but also, in radiation biology and in mass rearing technology. Recognition of the importance of biological control of pest populations is due to the effective application of the sterile-insect release technique for the screwworm and various other insect pests [1] and to the potential adverse ecological consequences of pesticides [2],[3],[4],[5].

This paper reviews some aspects of fitness of flour beetles and modifications due to various multifactor ecological results, particularly responses to radiations. Several experiments with flour beetles are reviewed from the standpoint of application for control by the sterile-insect-release-technique. Limitations and pitfalls of this technique to control effectively and economically stored foods insects are discussed.

MATERIALS AND METHODS: The experimental organisms were flour beetles (Coleoptera: Tenebrionidae), *Tribolium confusum* Jacquelin du Val wild-type strain "Chicago Standard" and *T. castaneum* (Herbst) wild-type strain "Brazil cI" and a black body mutant "sooty". Dr. T. Park (University of Chicago) supplied the wild-type stocks and Dr. A. Sokoloff (California State College at San Bernardino) supplied the "sooty" stock.

Methods are briefly reviewed in each subsequent section.

RESULTS AND DISCUSSION: Ontogenesis and Species Differential Radiation Sensitivity - X-rays were delivered at 1 kR/min from a General Electric Maxitron generator set at 30 ma and 250 kvp with 0.25 mm copper +1.0 mm aluminum filters and a 2.5 inch anode - subject distance. Five groups of 50-100 individuals per life cycle stage were exposed and kept at 29 ± 2 C and 70 ± 10 percent relative humidity. A dose was sterilizing if no offspring were produced; lethal if death occurred within 3-4 weeks post eclosion.

Although these two species are ecologically and morphologically similar, *T. castaneum* was markedly more x-radiation resistant than *T. confusum* throughout the life cycle except at the 1-3 hour old egg stage (Fig. 1). At this stage the same radiation dose was lethal to both species. The radiobiological studies during the life cycle which showed significant developmental changes indicated that the egg (1-3 hours post oviposition) was the weakest link in the life cycle of both flour beetle species and was killed by the 2 kR of x-ray. One could not radiate this stage and obtain a sterile adult.

Figure 1 also shows two other important radiobiological phenomena, namely, 1) the radioresistance for either induced sterility or lethality increased with increasing differentiation and development; and, 2) during the larval stage the sterilizing and lethal x-ray doses began to diverge. The exposures inducing

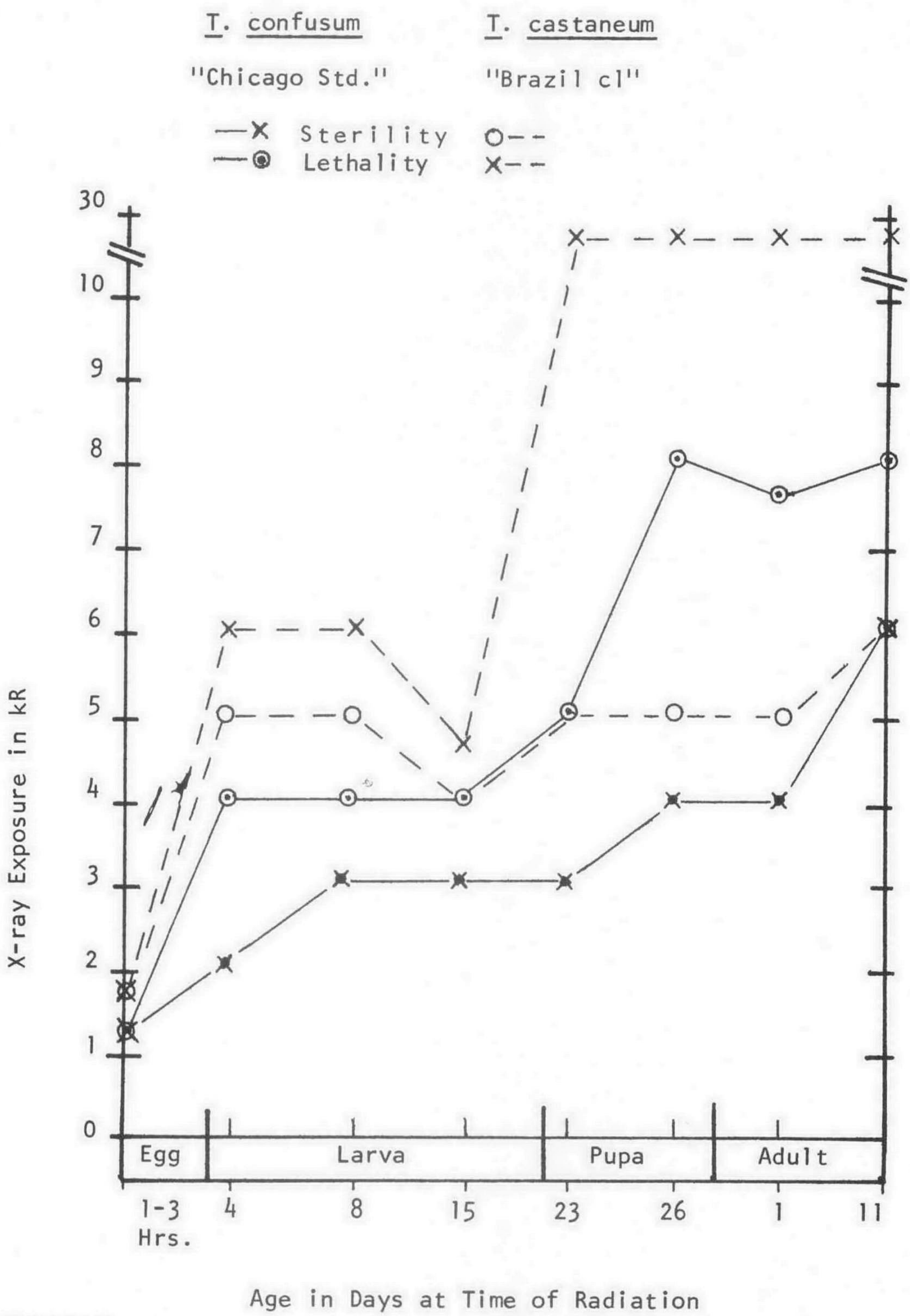

FIGURE 1. Comparative X-radiation effects in various life cycle stages of flour beetles, *Tribolium*.

lethality persisted higher than the sterilizing doses for the remainder of the life cycle. The 6 kR of x-rays induced sterility in adults of both species. Therefore, population control could be effected for these flour beetles by these x-ray exposures.

One must realize that radiobiological results are dependent on various factors, including:

a) species and strain [6], [7], [8], [9], [10].
b) age at exposure [6], [7], [11], [12], [13].
c) kind of radiation [14], [15].
d) dose rate [16], [11].

One cannot as yet very accurately predict the radiosensitivity of a species or strain. Sparrow and his colleagues [17], [18], [19] found that the numbers of mutant cells produced after exposure to radiation were correlated with nuclear volumes, interphase chromosome volumes, DNA per cell, and DNA per chromosome. The mutation frequency increased with increasing size of nuclear or interphase chromosome volumes and with increasing DNA content per cell or chromosome [18]. Their work was extended to a few amphibians [20]. Perhaps someday radiosensitivity predictability curves will exist for insects.

The age at exposure and the radiation effect, in general, agreed with Bergonie and Tribondeau's Law of Radiobiology which is the basis for radiation therapy of cancer, namely, the radiosensitivity of cells is in direct proportion to their reproductive activity and inversely proportional to their degree of differentiation [21]. Although some exceptions exist, this Law has been acceptable during the 70 years of radiation research.

The kinds of radiations having a progressively greater relative biological effect (RBE) are those with progressively increased distributions of energy along individual tracts (LET). From low to high RBE or LET these radiation types are gamma rays, x-rays, beta particles, neutrons, protons, and alpha particles [22]. High LET radiations would be poor disinfectors in stored-food product areas because of their low penetrability. On the other hand, low LET radiation sources implanted in a grain storage facility might be more advantageous except for the economics of shielding cost.

The rate at which radiation is given largely determines the biological effect. Usually, as the dose rate decreases, less adverse effects are produced. The rationale is that time allows biological systems to recover to various degrees. The longer the time interval between radiation induced events, the more recovery is possible; consequently, fewer mutations, less life shortening and fewer broken chromosomes are observed at lower than at higher dose rates [23]. A higher dose rate would be more effective in insect control than a low one.

Productivity Modification -

A. DDT + X-radiation [24], [25]. Sexually mature, virgin flour beetles were x-rayed and immediately 10 pairs were placed on 5 replicates each of which contained 20 g of standard food + various concentrations of DDT. These parental adults were transferred three times at 2-week intervals to comparable food. Old food containing the 2 weeks reproductive products were reincubated at 32 C and 70-75% relative humidity and adult F_1 progeny were counted as a measure of the productivity after the radiation assault and development on food containing DDT.

Figure 2 shows the inter- and intra-species sensitivites to combinations of x-ray and/or DDT. Reproduction after 1 kR x-ray was no different from that of the 0 kR group; however, 2 and 4 kR progressively reduced productivity. "Chicago Standard" (*T. confusum)* and "sooty" (*T. castaneum)* tolerated 20 ppm DDT in their food without significantly reducing their reproductive fitness from that

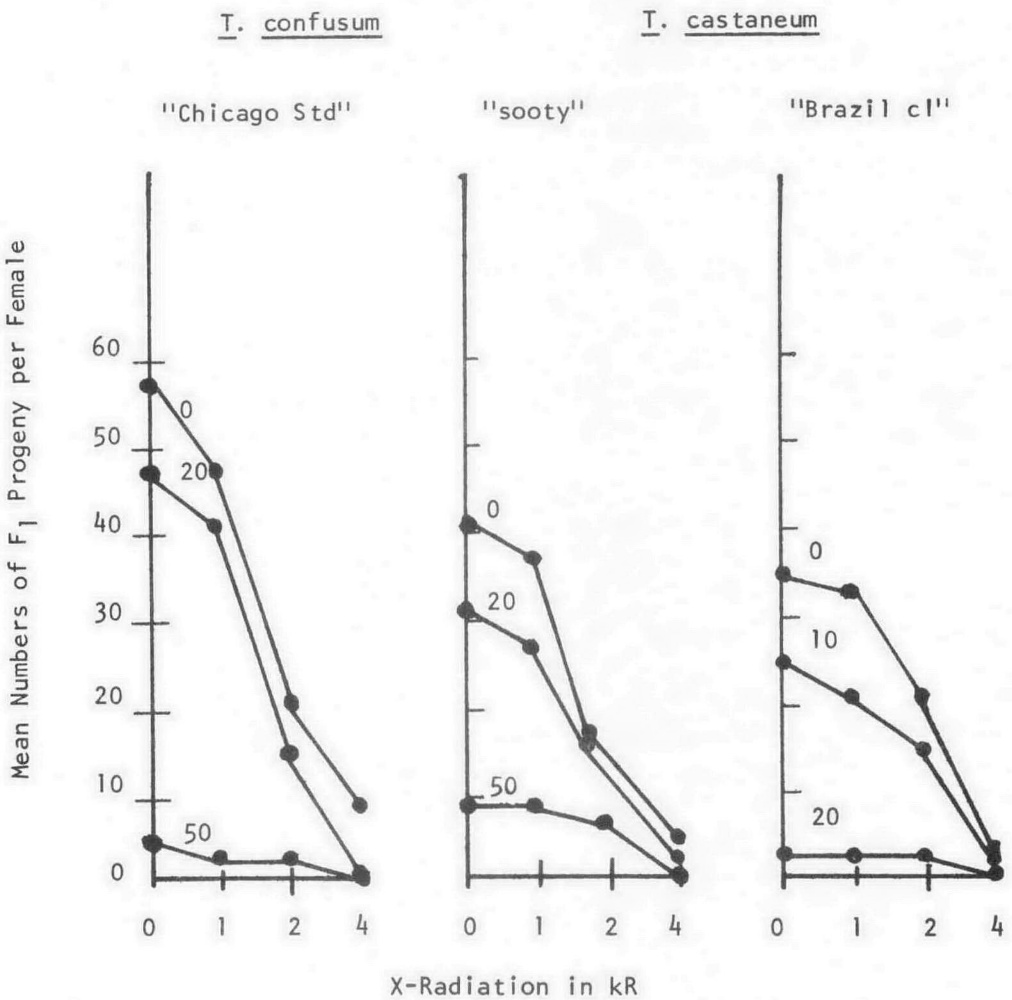

FIGURE 2. Mean numbers of F_1 progeny per female flour beetle for 6 weeks following a single x-ray exposure to parents and subsequent growth of F_1 on flour spiked with various concentrations of DDT. Numbers on the curves indicate ppm DDT. Species and strains of flour beetles, *Tribolium*, are given on the appropriate graph.

at 0 ppm DDT. The 50 ppm significantly decreased their reproductive capacity which was not eliminated except when the additional stress of 4 kR was applied.

Productivity of "Brazil cl" (*T. castaneum*) was most adversely affected by DDT; 20 ppm reduced productivity (measured as adult F_1) essentially to zero.

Although the exact danger level of DDT for humans is not known [26], data [27], [28] indicated that among those making DDT an intake 500 times greater than that of the average person was safely tolerated. Approximately 16 ppm was detected in human fat during the 1950's; whereas, by the late 1960's about 8 ppm was detected [26]. Reduction in use and exposure to DDT means a concommitant reduction in human exposure and storage of this insecticide.

The 50 ppm DDT will not eliminate all strains of *Tribolium;* however, in combination with 4 kR population maintenance was not possible. Radiation rather than DDT or in combination with DDT seemed a more economical control method for *Tribolium.*

B. DDT or Malathion with X-radiation - Experimental techniques with malathion [29] were similar to those with DDT (see above section) except that incubation was 29 C and 30-35 percent relative humidity. *T. confusum*, strain "Chicago Standard", was used. Differences in productivities of controls were attributed to temperature and relative humidity differences; since *T. confusum* performs better in temperate conditions compared to that in warmer, more moist climates than does *T. castaneum.*

The comparative effectiveness of DDT or malathion alone or in combination with x-radiation on the reproductive fitness of *T. confusum* is presented in figure 3. Four kR in combination with 20 ppm DDT or with 10 ppm malathion stopped the development and differentiation of progeny into adults. The LD_{100} for adult beetles was between 10 and 20 ppm malathion. If the end point were population extinction, malathion in combination with 4 kR x-ray was twice as effective as DDT. By itself, malathion was almost 5 times more effective than DDT in reducing the number of F_1.

Economics and environmental contamination must be considered in the application of pesticide and/or radiation control of pests. How many individuals can a population lose yet maintain its integrity and attain maximum size? How few in number can a pest population be and still be tolerated? Would 20 ppm DDT + 4 kR radiation be as acceptable as 50 ppm DDT + 0 kR? Perhaps one should use 5 ppm malathion + 4 kR or 10 ppm malathion + 0 kR?

C. Malathion and Population Autocide - Stress from 1 ppm or greater of malathion plus densities of 75 or more of *T. confusum* adults on 3 g food caused death of all individuals within 2-3 weeks [30]. This response was not observed for DDT [24]. The malathion and population size stimulated the beetles to produce a toxic gas consisting mainly of ethylquinones. Presence of the gas was indicated by the pink color of the flour. Although all the beetles died, this technique for control is not useful because the flour would be unacceptable for human consumption.

D. Adult Radiation - In three independent experiments [6], [31], [7] I found the sterilizing dose of 250 kvp x-rays was 6 kR to both species of adult flour beetles. For sterility in adult flour beetles from gamma radiation (^{60}Co) *T. confusum* required 7100 to 8400 R and *T. castaneum* 8600 to 9900 R [10], [32].

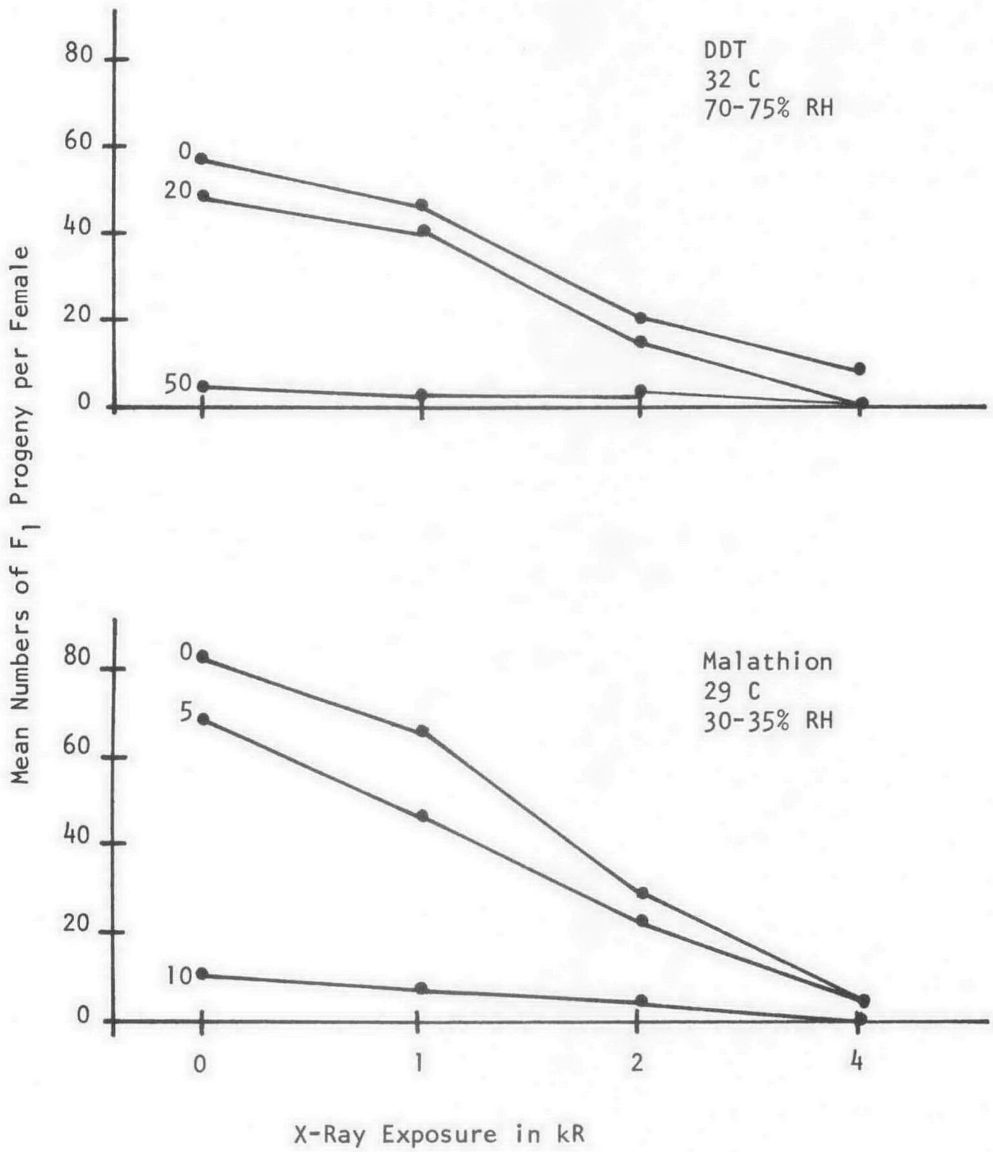

FIGURE 3. Comparative effects of insecticides, DDT and malathion, applied singly or in combination with x-rays on the reproductive fitness of the flour beetle, *Tribolium confusum* "Chicago Standard". Numbers on curves indicate insecticide in ppm.

Beetle strains and radiation techniques could account for the wide dose range; however, these doses are within the 16,000 R suggested [33] to induce sterility in 9 species of insects.

E. Addition of Radiated Males or Females to Populations- After exposure of sexually mature *T. castaneum* adults to 10.2 kR of ^{60}Co gamma radiation, the productivity measured as the mean numbers of F_1 adults per female per day for six weeks was 0.3 and 0.04 for exposed males and females, respectively. When similarly exposed individuals were added to control beetles in 20:1 ratios, the productivity was less but not significantly different from that of control populations. Comparable results were found for *T.*

confusum except that this exposure was sterilizing to females. Apparently radiated males of either species were not competitive and sterile females were not effective in eliminating unexposed sperm by acting as storage depots [34].

Population Performance With Chronic Radiation as an Environmental Factor - Twenty pairs of virgin, sexually mature flour beetles, *T. confusum* "Chicago Standard: and *T. castaneum* "sooty", were placed in test tubes on 5 g of standard medium. Three replicates of 5 populations (test tubes) of each species were kept at 25 C and 32 C with 70-75 percent relative humidity. Mixed-species populations were begun with 10 pair of each species and handling and analyses were similar to those for single species populations. Monthly, in each population, the numbers of larvae, pupae and adults were counted and placed on new food.

After 8 months, a ^{60}Co gamma radiation source was positioned such that the populations were subjected to one of three dose rates, namely 24, 49 and 170 R per day. Data collection continued as before radiation.

The reproductive performance and population maintenance, presented as mean proportions of larvae and adults, pre and post chronic radiation for single-species populations are presented in figures 4 and 5 for 25 and 32 C, respectively. During the first 8 months without radiation, these values represented the means of 15 populations; thereafter, the means were those for the 5 populations which received the 170 R/day.

Larval and adult proportions in populations given 24 or 49 R/day did not differ significantly and fluctuated within the shaded area indicated on the graphs. For radiation control, the dose rate must be greater than 49 R/day. Larvae were more radiosensitive than the adults regardless of temperature. As seen by their early, rapid decline in numbers and elimination at the 170 R/day dose rate. A species-temperature interaction was observed in the population exposed to 170 R/day [35].

T. confusum showed better survival of adults at 25 compared to that at 32 C, indicating its better adaptability in cooler climates. On the other hand, larvae and adults of *T. castaneum* were more radiation sensitive at 32 than at 25 C. This response was anticipated because progressively more adverse effects are correlated with increasing temperatures.

In mixed-species populations, the performance was comparable to that in single-species populations. (data not presented but available from the author). In the adult population segment, one consistently found *T. confusum* and no *T. castaneum;* thus, when radiation was an environmental factor, *T. confusum* had the selective advantage over *T. castaneum*. Effects due to co-habitation must be considered in radiobiological studies.

After 18 months of chronic radiation, the experiment was terminated. Total accumulated doses were 11780 R at 24 R/day and 82739 R at 170 R/day. At 25 C adult *T. castaneum* were eliminated by month 18 and larvae by month 8; whereas, *T. confusum* adults persisted but no larvae after month 6. At 32 C *T. confusum* adult

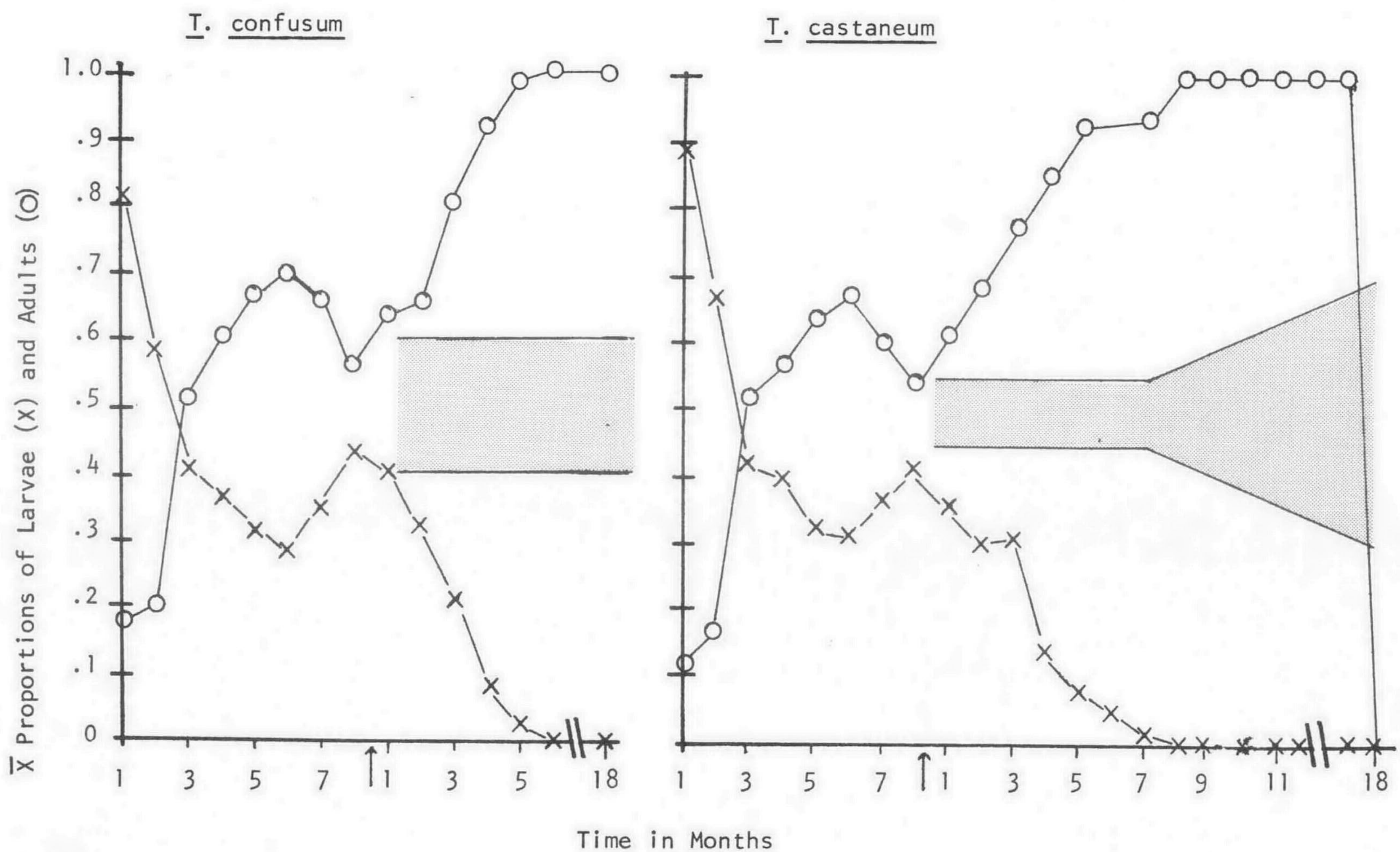

FIGURE 4. Population dynamics of flour beetles, *Tribolium*, given chronic ^{60}Co gamma radiation at 170/day beginning at arrow. See text for explanation of shaded areas.

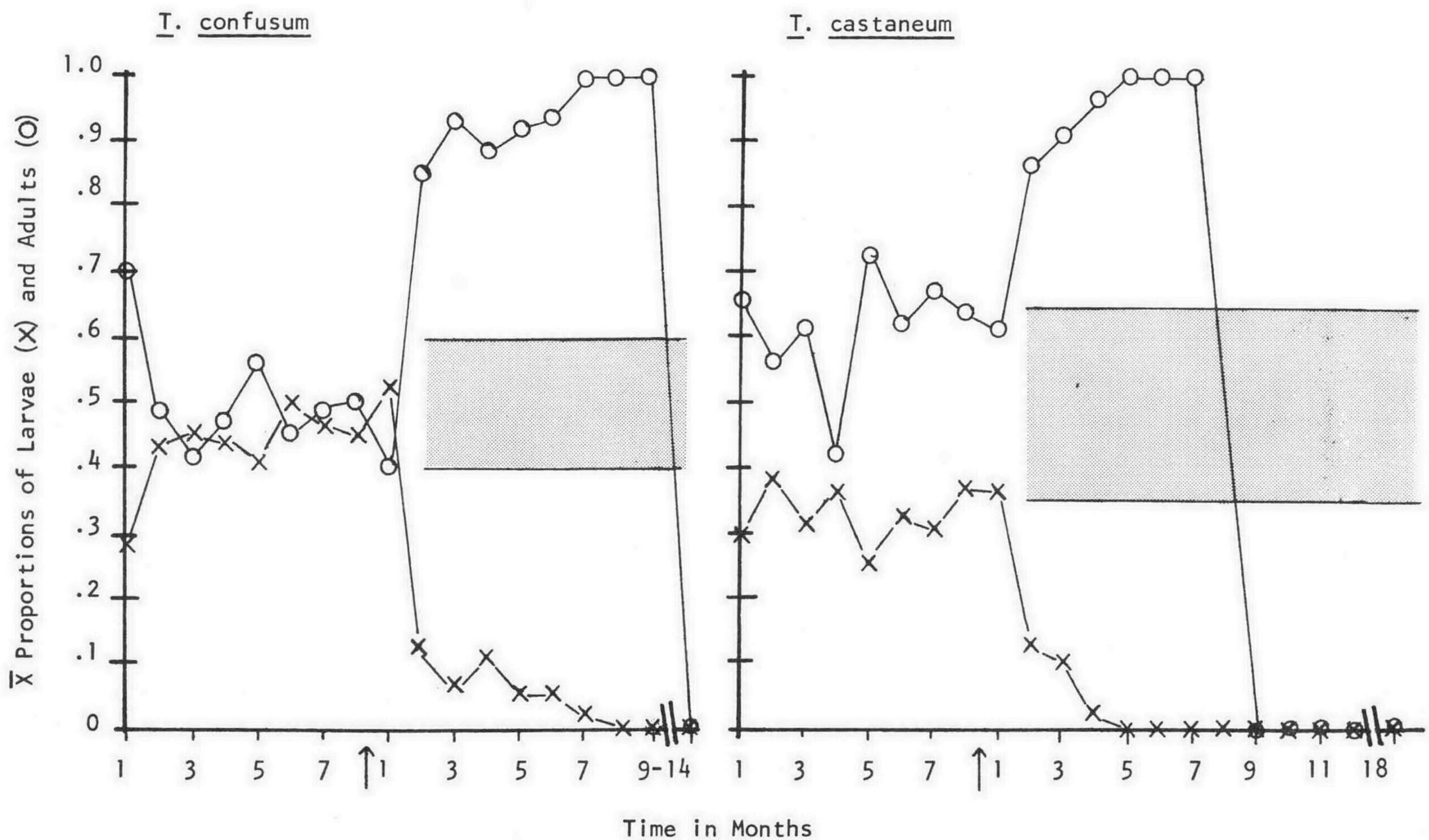

FIGURE 5. Population dynamics of flour beetles, *Tribolium*, given chronic ^{60}Co gamma radiation at 170/day beginning at arrow. Culture conditions were 32 C and 75% relative humidity. See test for explanation of shaded areas.

population segments were eliminated by month 14 and larvae by month 8; adult *T. castaneum* were eliminated by month 9 and larvae by month 4.

At the present time for the control of flour beetles neither radiation nor genetic manipulations are attractive methods. Integrated approaches seem feasible but these attempts for control depend on the insect. Perhaps the sex attractant method would be best for stored product insects since this method would flush adult insects from the stored products. Physical aspects such as sound, light and air currents need investigation.

CONCLUSION: The economics of shielding, the constant threat of re-infestation and the objections to adding even sterile insects to stored food products seem to preclude the use of radiation for control of stored product pests.

Radiation sources implanted in storage facilities must emit more than 170 R/day for quick, effective control of flour beetles. This control method does not seem feasible.

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