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 NEW MUTANTS

REPORT OF P.S. DAWSON

Tribolium confusum

1. Depressed (dep) – Dawson, 1968. Sex-linked recessive, similar to thu in expression. Good viability through pupal stage, but reduced in adults. Located about 3 units from red.
2. Red (r u) -- Dawson, 1968. Allelic to red. Eye color remains light in adults, thus making this allele much more useful for mapping sex-linked genes. Good viability.

REPORT OF G. JOHNSON and SOKOLOFF

Tribolium castaneum

A spontaneous mutation appeared in a wild type strain of T. castaneum. Its expression is variable: in inbread strains beetles may have one or both eye reduced or missing altogether. The cranium is reduced behind the gena. Tests of allelism between this mutant and microcephalic (mc) indicate the two are allelic. While the effect on the eye is not uniform, the effect on the cranium is rather uniform suggesting complete penetrance for this gene. We are naming this allele of microcephalic mc-1, Johnson and Sokoloff.

REPORT OF A. SOKOLOFF

Tribolium confusum

Miss Maria Korunic Zlatko, of the Agricultural Faculty, Institute for Plant Protection, Zagreb, Yugoslavia, sent us a black mutant found in a local strain of T.confusum. Preliminary crosses with wild type suggested it was an sutosomal, semi-dominant gene, the heterozygote being identifiable from either homozygote. Tests of allelism with McGill black indicated the two mutants were allelic. We are designating the new mutant black z – Korunic Zlatko and Sokoloff.

REPORT OF M. P. TAGARRO and F. OROZCO

Tribolium castaneum

1. Eye mutant. Autosomal recessive found in “Consejo” strain affecting the pigment of the eye, it appears dark red surrounded by a black circle, identifiable in pupa and adult stage. Good viability and expressivity, complete penetrance.

 Test for alleliam with pink, chestnut, ruby, ivory and maroon was negative.

 Linkage tests proved negative for II, III, V, VI and VII groups. Test in the VIII group shows a possible linkage. At present, doing a three points test-cross for IV, V and VIII groups.

1. Elytra mutant. Found in “Consejo” strain. Different tests show an irregular dominance, variable expression and good viability. It shows a similar phenotype to abbreviated appendages (aa) or split (sp). Test for allelism shows variable results owing to the changeable dominance.
2. Melanotic stink gland-like. Spontaneous in the eye mutant stock, it shows a dark spot in both sides of anterior prothorax, the abdomen appears also affected. Similar to melanotic stink gland. The mutation expression appears after 21 days of adult stage, with variable expressivity and penetrance.

 NOTES - RESEARCH

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Epigenetic studies of the egg in Tribolium castaneum.

Flour beetle of the genus Tribolium constitute important primary and secondary pests in all kinds of cereal products. For this reason they have attracted the attention of investigators with a broad spectrum of interests. Since Chapman (1924) used and stressed the usefulness of Tribolium in population studies, Tribolium castaneum has received considerable attention from ecologists and geneticists. It has been used extensively in testing quantitative genetic theory; as a result the knowledge of its reproductive fitness has assumed considerable importance, this being one of the very important development of the egg is closely tied up with the components of fitness and it also is a prerequisite for studying the developmental genetics of cogenesis, it is necessary to fully understand the development of the egg before meaningful conclusions can be drawn with respect to fitness components.

To date several distinct approaches to the problem of yolk synthesis have been used. The development of sectioning and staining methods resulted in a period of extensive histological investigation of the ovary. This modern period of research on the insect ovary seems to have been initiated by Stein (1847) with his histological study of the female reproductive organs of beetles. The second major approach to insect vitellogenesis was stimulated by the appearance of special cytological methods (osmic acid, silver method; Janus green staining, etc.,) insect vitellogenesis has been studied by various authors.

There is no report on the vitellogenesis in Tribolium castaneum. The study was taken up in two phases, in the first phase emerging females were dissected out and ovaries were teased. The ovariole number was recorded. The stages of maturation of egg were studied by staining of whole mounts with Feulgen stain. In the second phase the stages in development of the egg were studied by dissection of adult mature females and then a study of the stages of development from microtome sections after using appropriate stains was undertaken.

The ovarioles are described was panoistic type which lack nurse cells. Average ovariole number per ovary is 5.76 and per individual 11.51. Each ovariole anteriorly has a germarium which continues into a germarial filament. These filaments are quite long. The germarium is followed by various egg chambers. The entire ovariole is surrounded by a thin membranous epithelial sheath. The germarium consists of germarial cells. The oocytes are formed posterioly and on sides of follicle cells in the construction region of the ovariole.

Early follicle cells are small and spindle shaped. They are seen in a group in the posterior part of the germarium. These follicle cells encircle oocytes. The follicle cells increase in size with time. The oocyte nucleus increases in size and is of variable position. Diring the process when oocytes are formed oooplasm is laid and goes on increasing in successive stage of development. The composition of ooplasm changes from dense mass in which vacuoles are laid which finally develop into yolk spheres. The whole cognesis has been divided into seven stages according to our criteria. The time taken for the maturation of ovary has been studied. It has been seen that the ovary becomes mature to lay eggs 96 hours after emergence.

The time taken for the maturation of egg was studied in ovarioles from 0 hours to 168 hours of age. When the ovary was 48 hours old 54% of the follicles were formed. The oocytes were formed between 24 to 48 hours of age. If we suppose that oocytes were formed at 36 hours of age then it would take approximately 60 hours for the maturation of egg. Cytochemical studies for RNA, DNA Polysaccharides and alkaline phosphatase activity were undertaken. The presence and absence of these at various stages of the development of egg has been studied in detail.

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Chapman, R. N. 1924. Nutritional studies on the confused flour beetle Tribolium confusum Duval. J. Gen. Physiol. 11:565-585.

1. Stein, F. 1847. Vergleichende Anatomie und Physiologie der insekten, I, Die weiblechen Geschlectsorgane der Kafer. Dunckeru, Humblot. Berlin, 139 pp.

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\*Activity in T. castaneum.

Using the technique described by Amos (1967), the activity of T. castaneum adults is being examined to determine the possible existence of rhythmicity. Both sexes are being studied at two humidities (75 and 42% RH) under stable conditions (in darkness) and under diurnally fluctuating conditions where both temperature and light are varied. Analysis of data so far obtained suggests the absence of an activity rhythm under stable conditions whereas a distinct rhythm is present under fluctuating conditions. Partial correlation analysis indicates that activity is positively correlated with temperature and negatively correlated with light. Further work is underway using other stable (in light ) and fluctuating conditions to determine more precisely the nature of adult activity.

 Literature Cited

Amos, T.G. 1967. Apparatus for studying locomotor activity of small animals with the aid of time-lapse photography. J. Econ. Ent. 60:886-887.

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\*The biology and behavior of a stored products moth Ephestia (Cadra) cautella (Walker) (Lepidoptera, Phycitidae), as studied in controlled environments.

Laboratory studies using E. cautella tested the versatility of a gradient environment apparatus developed for use with Tribolium spp. (Onyearu, A. K. 1967) and also gave life history biology data to supplement that previously based on the uniformly controlled conditions of the constant temperature room.

Three different populations of E. cautella were employed, a laboratory one and two field populations (ex Nigeria, and ex Kenya). In the gradient environment the populations were able to select conditions most suitable for particular stages in their life history.

Adults of all three populations responded similarly in that they favoured the cool regions in the gradient environment. All laid their eggs in generally warmer conditions although the actual distributions of their oviposition sites differed in a number of respects. This suggests that adults move from the cooler to the warmer regions in order to oviposit. Further evidence for this view comes from the work of Amos et al1968 who studied isolated E. cautella adults. They found that a female adult spent periods in the cool region of a gradient environment (17 0 to 32 0 C) interspersed with periods of a much shorter duration in the warmer regions, where oviposition occurred. The distribution of the pupal sites were generally in warmer conditions than those of the oviposition sites.

In a number of ways the field populations were more strongly favoured over a much wilder range of conditions than the laboratory one, although they too differed between themselves.

Details of this study are being prepared for publication elsewhere.

This work was supported by grants R 1964 and R 1939 from the Ministry of Overseas Development.

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Amos, T. G., F. L. Waterhouse and N. A. Chetham 1968. Temporal distribution of Tribolium castaneum Herbst and Cadra cautella (Walker) on temperature gradients. Experientia 24:86.

Graham, W. M., a. k. Onyearu and F. L. Waterhouse 1965. Temperature and moisture gradient equipment. Can. Ent. 97 (8):880-886.

Onyearu, A. K. 1967. The behavior and biology of flour beetles, genus Tribolium as studied in laboratory gradients of temperature and humidity. Tribolium Inform.

 Bull. 10:121-122.

FLORENTINE, G. J.

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Food-seeking behavior of stored product insects.

A study is in progress to determine whether a particular constituent of white flour can serve as the primary stimulus for Tribolium castaneum in its food-seeking behavior. In conjunction with this work phase, electrophysiological techniques are being developed which will provide a basis for correlating behavioral responses with neural responses.

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Fecundity of T. castaneum females.

In an experiment to evaluate the reproductive performance of males from a line selected for large 21 day pupa weight, selection line males were mated, each to two BC1 females. BC1 was a highly fertile line, whereas the selection line was in reproductive difficulty. Egg collections were taken for 24 hours from individual females which had previously been held with the male for 6 days. The number of eggs were counted at the end of the collection period and fertility of larva on the 7the day. Of a total of 86 matings, 31 had no larva indicating either that mating did not take place or the male was sterile. The mean egg number for these 62 females was only 8.5 per 24 hours. The comparable figure for the remaining 150 females was 21.7. These data suggest the existence of a male stimulating effect on egg laying.

GOUVEIA, M. E. S.

Laboratorio da Defesa Fitossanitaria dos Produtos Armazenados

Lisbon, Portugal

Methyl bromide and phosphine residues in stored beans.

Studies on the degradation of methyl bromide and phosphine residues in beans treated with one or various applications of the fumigants.

GOUVEIA, M. E. S.

Laboratorio da Defesa Fitossanitaria dos Produtos Armazenados

Lisbon, Portugal

Pesticides residues in cacao beans.

The effect of residues of fumigants such as methyl bromide and phosphine on cacao beans has been under study in order to determine the influence of moisture content of the seeds on the absorption and fixation of the fumigants.

An identical study has been undertaken with contact insecticides based on DDT, Lindane, malathion and pyrethrins.

On both cases, a wide range of doses have been used, the residues analysed several times after the treatment.

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Hissar, Harayana

Preliminary studies on PAU-I strain of Tribolium castaneum.

I Life Cycle

It was considered essential to study various stages of the life cycle and growth pattern in PAU-I strain of |Tribolium castaneum before any quantitative genetics experiment could be started. The stok was maintained in the Population genetics laboratory of the Department of Genetics at Punjab Agricultural University, Hissar. The stock has been developed from samples collected from various part of Hissar town. The samples were mixed and the beetles allowed to breed at random under the laboratory conditions in a population cage for three generations. The random mating wild population was gradually adapted to standard laboratory conditions. The beetles were fed on medium containing 94.5% whole wheat flour, 5% dried yeast and 0.5% of Rovimix (Vitamin mixture containing A, B2 and D3). The whole wheat flour was sifted through a filter sieve 60 (0.250 m.m.).

The beetles are maintained in the air conditioned laboratory inside the temperature and humidity controlled cabinets maintained at 32 0 C and 70% relative humidity (32 + 1 0 C; 70 + 6% RH). All experimenta populations are maintained on medium containing 94.5% whole wheat flour, 5% dried yeast and 0.5% vitamin mixture containing vitamins A, B2 and D3. This will refer to standard laboratory conditions if otherwise not mentioned.

The experiment was run in two replicates. In each replicate, five males were mated to five virgin females each. After 48 hours the females were removed from these vials and number of eggs laid were counted. Two progeny per dam were secured at random from among the larvae hatched on the third day for studying the larval period, pupal period and other aspects of life cycle. The following observations have been made.

Nos. Character Replicate I Replicate II Overall Avg.

 1 Number of eggs laid

 Per female 18.2 19.8 19.0

 2 Percent hatchability 92.31% 95.96% 94.21%

 3 Percent eggs hatched

 On Day 1 1.20% 1.05% 1.12%

 On Day 2 54.76% 40.00% 47.00%

 On Day 3 41.67% 58.84% 49.72%

 On Day 4 0.00% 1.05% 0.56%

 On Day 5 2.38% 1.05% 1/68%

 4 Average larval period

 (days) 28.33 28.22 28.25

 5 Average pupal period

 (days) 7.50 7.10 7.30

 6 Average developmental

 Cycle (egg to adult) in days 35.33 35.18 35.25

 7 Percent Mortality 5.00% 10.00% 7.51%

The same strain after being adapted to the laboratory conditions of 32 0 C temperature and 70% relative humidity for a period of about eight months showed that the average larval period was 17.00 days in PAU-I as against 17.16 days in a strain from Brazil and 18.05 days in Chicago wild. The pupal period was 5.81 days in PAU-I and 5.76 days in Brazil and 5.50 days in Chicago wild.

The figures from the second experiment are quite comparable to those of Brazil and Chicago stocks which have long been kept under laboratory conditions. It might, therefore, be due to the constant suitable environmental and feeding conditions provided to the beetles of PAU-I that their life cycle was stabilized after eight months in this laboratory.

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Hissar, Harayaa

Preliminary studies on PAU-I strain of Tribolium castaneum.

II. Comparison of growth curve of PAU-I, Brazil wild and Chicago wild strains of Tribolium castaneum.

The synthesis and the life cycle of PAU-I strain of T. castaneum has been described in a communication in this bulletin elsewhere. The other two stocks Brazil and Chicago wild were obtained from Dr. Sokoloff than at |Department of Genetics, University of California, Berkeley in 1967 and maintained by Dr. Bhat, Assoc. Prof., Department of Genetics, Punjab Agricultural University, Hissar. The three stocks were kept under standard laboratory conditions, bred at random for a number of generations before the start of the experiment. The beetles were kept on medium containing 94.5$ whole wheat flour, 5% dried yeast and 0.5% vitamin mixture containing vitamin A, B2 dna D3.

60 pair matings were kept from each strain. The adults were removed after 24 hours of mating. Four days after the eggs were hatched, one larva each was secured at random from each mating. The larvae were weighed from the fifth day of hatching and daily weights recorded up to 26 days after hatching.

Two growth curves have been drawin based on the data of body weight records.

Figure 1 shows the growth curve of the three strains drawn from the averages of the individuals irrespective of their stage of life. The growth pattern for the three strains does not seem to differ very much. Highest body weight was attained on 12th day after hatching by PAU-I beetles as against the 14th day by other two strains. There was regular loss of body weight weight from there onwards. A drop in weight between the 14th and 16th days is one of the characteristic features which may be compared to pubertal flextion in large animals. The decrease in body weight continued upto 22nd day in PAU-I, 23rd day in Chicago wild and 24th day in Brazil wild stocks. Further increase in body weights was not significant.

Figure 1 does not give a lucid idea about the growth pattern with regard to the stages of life cycle. Figure 2 is a modification of the first one in that the averages of body weights have been plotted strictly according to the stages of life cycle. The body weights of the larvae have been plotted from 5th day to 17th day in PAU-I and Brazil stocks and upto 18th day in Chicago strain of T. castaneum. This is based on the fact that average larval period in these strains were 17.00, 17.16 and 18.05 days respectively. From day 18 in PAU-I and Brazil and day 19 in Chicago the body weights of the puape have been plotted. The day of pupation in all the cases was taken as a zero day and the growth curve of the three strains was drawn. The discontinuity of the curves marks the end of larval growth period and zero day of the pupal growth period.



The curves are therefore divided into two larval and pupal growth curves. The larval curves are almost similar to that in Figure 1. The highest body weight is attained on day 13 in PAU-I (2.13 mg.), day 14 in Brazil (2.47 mg.) and day 14 in Chicago (2.10 mg.). A gradual decline in larval weight thereafter is indicative of the fact that the larvae were preparing for the next stage – the pupation (the larvae seem to eat at a lesser rate but the rate of moulting is maintained). This may be a possible reason for the lose in body weight of the larvae.

The pupal growth curve is characterized by a gradual and regular decline in the body weight. Continuous feeding at its own stored energy may be a cause for this regular decline in the body weight of pupae. Average time taken for a pupa to become an imago was 5.81 days in PAU-I, 5.76 days in Brazil wild and 5.50 days in Chicago wild strains of T. castaneum.

LOSCHIAVO, S. R., A.J. MCGINNIS and D. R. METCALFE

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\*An insect bioassay for feeding quality of cereals.

The confused flour beetle was used to detect differences in nutritive value among cereal varieties (Loschiavo, McGinnis and Metcalfe, 1969). Cereals to be assayed were finely ground in a ball mill. Since the length of larval period varies with the fineness of the grind and since the effect of particle size on larval development is not consistent among varieties, all material was pelleted prior to assay. Newly hatched larvae were placed in the medium, and the length of larval period (in days) used as the criterion of nutritive value. Feeding trials have been conducted with chicks to determine whether the beetles’ assessment of nutritive value was meaningful for farm animals. Results of these comparative studies using five varieties of barley and three varieties of wheat were in general agreement.

 Literature Cited

Loschiavo, S. R.., A. J. McGinnis, and D. R. Metcalfe, 1969. Nutritive value of barley varieties assessed with the confused flour beetle. Nature 224:288.

McGINNIS, A. J., AND S. R. LOSCHIAVO

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\*Repellency to Tribolium confusum of diethyl ether extracts of wheat germ.

Soxhlet extracts of raw wheat germ with either diethyl either or n-hexane caused adults of Tribolium confusum to aggregate. An extract prepared by stirring wheat germ with diethyl ether for 18 hours repelled the beetles. Extracts similarly prepared with n-hexane stimulated aggregation. The level of repellency in the extracts varied widely depending upon the source and age of the diethyl ether. We have concluded that the repellent is an artifact of extraction and is the result of oxidative processes.

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\*Sub-zero mortality responses of flour beetles.

Further studies of mortality at several sub-zero temperatures show that there is a delayed mortality effect up to one week after exposure. These studies also show that some species of Tribolium, e.g. T. madens, are very considerably more resistant to cold than species investigated so far (Nowosielski-Slepowron, et. Al. 1968).

 Literature Cited

Nowosielski-Slepowron, B.J.A., F.L. Waterhouse, and D.E.A. Strevens 1968.

 Sub-zero mortality responses of Tribolium confusum Duval

 (two stocks) and T. castaneum (Herbst) (two stocks) analyzed by weighted regression lines based on individual temperature

 L. D. 50’s. Physiol. Zool 41:440-446.

PRESCOTT, R. A.

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Some effects of auto-conditioned flour on various aspects of the biology of

Tribolium castaneum.

The defensive quinoid secretion produced by the so-called “stink glands” of Tribolium species is rapidly taken up by a flour medium, which in time assumes a pinkish colour and has a characteristic odour. (Ladisch et al 1967). The effects of such auto-conditioned flour on the behavior of T. castaneum adults, oviposition with respect to female orientation, and the developmental period are being investigated. Using various degrees of conditioned flour, a repulsive effect on adult beetles was demonstrated, the adult distribution declining rapidly with increasing levels of conditioning, and a marked preference being shown for fresh flour. The definite avoidance reaction of the beetles would seem to indicate that the adult response is olfactory in nature. In a gradient environment the normal distribution of adults is altered by inserting a region of conditioned flour, there being a decrease in adult numbers in that region. This effect, however, is less marked at the higher, preferential temperatures.

The effect on oviposition was found to be correlated with adult behavior, the egg distributions obtained being similar to those of the adults. However a higher egg/female ratio was sometimes obtained in the more heavily conditioned flour than expected, this possibly being associated with the long-term survival of the population in unrenewed flour.

The rate of oviposition was found to decrease with increasingly conditioned flour, the adults being retained in the same flour medium over a period of time, and the egg output measured at intervals.

The developmental periods were found to be proportionately lengthened in increasing levels of conditioned flour and the mortality of eggs and small larvae increased.

These effects are being further investigated and the responses compared, in T. confusum, and other Tribolium species, such as T. brevicornis, and T. destructor, which in general produce larger amounts of the quinones.

Such factors as the inhibition of oviposition, and the lengthening of the developmental period, may be thought to have a contributory effect on the self-limiting mechanisms postulated to enable populations to survive for long periods in unrenewed flour.

 Literature Cited

Ladisch, R. K., S. Ladisch and P. Howe 1967. Quinoid secretions in grain and

 Flour beetles. Nat. 215, No. 5104.

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The effects of exposure to low temperatures on survival and fecundity in the flour

Beetles, Tribolium confusum and T. castaneum.

The survival and fecundity responses of flour beetles following exposures of the adults to subzero temperatures were investigated. Four populations were tested: an outbreeding stock and the inbred CFI-B of Tribolium confusum Duval, and an outbreeding stock and the inbred CS-5 of T. castaneum (Herbst).

The general aspects of the postexposure survival and fecundity responses were examined with the outbreeding T. confusum population exposed to -5 0, -10 0 and –

15 0 C for various durations. The survival response was influenced by latent lethal effects the expression of which became more immediate as the exposures became more harsh. The survivors showed 3 types of fecundity responses as compared to the unexposed beetles: sustained fecundity increase, temporary fecundity increase, and fecundity decrease The increased postexposure fecundity was stimulated by exposures having sublethal and low lethal effects on survival.

Interspecies and intraspecies differences of postexposure survival and fecundity responses to – 5 0 C were shown. T. confusum was more tolerant than T.castaneum, especially between the outbreeding stocks. Within species, especially confusum, the outbreeding stocks were more tolerant that the inbred stocks. Postexposure fecundity was stimulated in all the populations tested by certain exposure durations, but the inbreds had lower capabilities to interact favorably with the effects of the exposure than the outbreds.

The postexposure fecundity of the outbreeding T. confusum population following sub-lethal exposures to – 5 0 was studied in greater detail. The results show: (1) The increased fecundity was stimulated by as short an exposure as 2.5 minutes.

(2) Sublethal exposures stimulated the subsequent egg production of very young. Adults, less than 1 day old, and of adults 32 days of age. Physiological ageing effects both the postexposure survival and fecundity responses, however. (3) Exposure of the male sex did not contribute to the increased fecundity of exposed females, but differential exposure of the female and male sexes augmented the effects of delayed mating (lower fecundity level) when mating was experimentally curtailed until after the exposure. This augmentation was attributed to the changes in the adult’s behavior and physiology. (4) Exposed beetles burrowed more quickly in the medium, and re-surfaced less frequently, in contrast with unexposed beetles which were surface-active.

(5) Exposed females had higher oxygen consumption reflecting an increased metabolic rate. (6) Fecundity was disrupted by male absence less drastically with exposed than with unexposed females. However, egg fertility dropped more immediately in the former. (7) Low temperature exposure rendered the beetles insensitive to the fecundity-depressing factors found in adult-conditioned medium. (8) Low temperature exposures moderated the negative effect of high adult density on fecundity. There was a reduced egg cannibalism rate and an increased real fecundity among the exposed beetles. A relaxation of the density-dependent, self-regulating mechanism following sublethal exposures to low temperatures was implied by these findings.

(NOTE: The above abstract is of a paper which will appear in Ecological Monographs sometime in 1970. Ed.)

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Influence of antennae clipping on egg laying rate of Tribolium castaneum females.

A good system to differentiate individuals of Tribolium in the adult stage is to clip antennae (the right or the left one, both or none). It was of interest to know how that removal of antennae could possibly affect egg laying since a great part of the genetical research carried out in this laboratory is on that quantitative character. It seemed logical to assume that such a stress would probably reduce egg laying rate, and
a series of experiments were run to check the validity of this assumption.

Four consecutive experiments were run, each one designed according to the results of the preceding one. The strain “Consejo” was used in all four experiments. Egg laying rate was evaluated from the 7th to the 11th days after adult emergence for both virgin or fecundated females. Since the presence of the male is definitive to increase the egg laying rate (fecundated females lay highly significantly more than virgin ones), the influence of clipping was studied both in males and females.

Experiment 1. The following single pair matings were made:

 20 females with NO antennae with TWO antennae males
 20 females with ONE antenna with TWO antennae males
 20 females with TWO antennae with TWO antennae males
 20 females with TWO antennae with ONE antenna males
 20 females with TWO antennae with NO antennae males

Average values of egg laying rate and statistical analysis are shown in Table 1. From that analysis no significant differences are detected. So a second experiment with two replications was run.

Table 1 - Average egg laying rate (four days) and analysis of variance of the Experiment 1.
No. of No. of antennae Analysis of Variance
antennae in females
in males 2 1 0 S. of V. d.f. M. S. F

 2 70.95 60.35 69.85 Treatments 4 422.97 1.95

 1 71.40 - - Error 94 217.00

 0 70.00 - - Total 98

Experiment 2. Same type of matings as in Experiment 1, but with two replications. Table 2 gives the average figures for the egg laying rate and the statistical analysis. From that analysis it is shown that the treatments were highly significant, so it could be tentatively concluded that treatments have probably some effect on egg laying. For that reason a more complete experiment was run afterwards.

Table 2 - Average egg laying rate and analysis of variance of Experiment 2.

No. of Repli- No. of antennae Analysis of Variance
antennae cation in females
in males 2 1 0 S. of V. d.f. M.S. F

 2 69.97 66.33 76.43 Treatments 4 6584.47 9.62++
 1 I 64.53 - - Replications 1 38.34
 0 68.67 - - Trat. X Rep. 4 1887.38 2.76 +

 2 60.87 67.83 90.03 Error 189 684.74
 1 I/II 56.37 - -
 0 67.93 - - Total 198

 2 65.42 67.08 83.23
 1 I/II 60.45 - - ++ Significance at 0.01 level
 0 68.29 - - + Significance at 0.05 level

Expertiment 3. In order to see the possible difference between the effect of clipping males and females on the egg laying rate of these last ones, a complete set of single pair matings was prepared combining the three possibilities (2, 1 or 0 antennae) in males with the three in females, completing a factorial design with the possibility of testing the effect of treatments both in the males as in the females. In each of those nine combinations were tested 22 matings. Table 3 has the average figures obtained for the egg laying rate and the corresponding statistical analysis. It is shown that both males and females give statistical significance, but while the males only present that significance at the 0.05 level, the females present on extremely high “F” value. The most important point is that it seems that in females the less antennae they have the more egg laying rate they give. The effect of the males is discussed later on.

Table 3 - Average egg laying rate and analysis of variance of Experiment 3.

No. of No. of antennae Analysis of Variance
antennae in females
in males 2 1 0 mean S. of V. d.f. M. S. F

 2 64.96 72.99 54.23 74.06 Males 2 383.10 4.32+
 Females 2 9458.33 106.66+++
 1 58.23 72.73 85.41 72.12 M x F 4 151.78 1.71
 Error 387 88.68
 0 65,96 73.73 91.05 76.91
 Total 395

 Mean 63.05 73.15 86.90 74.37 + ) Significance at 0.05 level
 ++ ) Significance at 0.001 level

Experiment 4. This last experiment was centered only in females since the effect of clipping antennae seems to be very important only in them. It was divided in two parts: with fecundated and with virgin females. A new treatment was introduced, crossed with number of antennae as a factorial design, in order to understand better the clipping effect; it was the amount of medium in the individual female vial. When fecundated females were used, the corresponding males were normal, i.e. with both antennae. 48 females were evaluated for each of the nine combinations of “number of antennae” and “amount of medium”. Finally, the three treatments of this last factor were:

 (P) A little bit (about 0.25 grs.)
 (N) Normal amount for egg laying evaluation (2 to 3 grs.)
 (T) Three times the normal amount

Tables 4 and 5 present the averages of egg laying rates and statistical analysis for the virgin and fecundated females respectively. It can be seen that both factor analyzed result with effects highly significant and the lay of eggs increases when more medium is available and when less antennae are present, both in virgin as in fecundated females.

Table 4 – Average egg laying rate and analysis of variance of the Experiment 4.
 Virgin females.
Amount No. of antennae in Analysis of Variance
 of females
medium 2 1 0 mean S. of V. d.f. M.S. F

 P 17.17 18.77 39.11 25.02 Antennae 2 30,417.68 115.65+++

 N 25.79 34.38 51.17 37.11 Medium 2 11,737.32 44.57+++
 A x M 4 1,080.63 4.10++
 T 26.29 38.52 63.91 42.91 Error 419 263.35

Mean 23.08 30.56 51.40 35.01 Total 427

 ++ ) Significance at 0.01 level
 +++) Significance at 0.001 level

Table 5 – Average egg laying rate and analysis of variance of the Experiment 4.
 Fecundated females.

Amount No. of antennae in Analysis of Variance
 of females
medium 2 1 0 mean S. of V. d.f. M.S. F

 P 52.15 56.02 61.68 56.62 Antennae 2 6,201.65 16.93+++

 N 75.15 79.65 93.63 82.81 Medium 2 59,177.41 161.52+++
 A x M 4 445.38 1.22
 T b 90.69 97.77 102.13 96.86 Error 418 366.38

Mean 72.66 77.81 85.81 78.75 Total 426 +++) Significance at 0.001

Considering the total of the four experiments, and by giving more importance to the two last ones due to the fact that they included a greater number of individuals, it seems reasonable to draw the following conclusions:

Most important conclusion is that, surprisingly enough, removal of antennae in Tribolium castaneum instead of reducing, enhances egg laying, either on virgin or on fecundated females. It seems also that females with both antennae clipped lay more than those with only one removed. What could be the cause for that significant effect, it is difficult to determine without doing more precise and diverse research, but two tentative explanations are suggested in a very simplified way; explanations related with two biological facts more or less verified in general: (a) Mechanism of reducing the reproductiveness when the environment is far away from the optimum. (b) Mechanism of increasing the reproductiveness when the individual does not feel itself well. In the case of (a) it is probably that they detect the density of eggs per certain amount of medium and they slow down the laying if that density is high, and so with one or both antennae removed they do not detect well the number of eggs and so the oviposition continues. In the (b) case, perhaps the lack of an important organ in the female decreases its vitality and mobility and so the organism reacts against that by activating its reproductive capacity.

 As a consequence of the first conclusion it is clear that for experiments on egg production, clipping antennae as a system to mark individuals (e.g. three females mated to the same male) is not practical at all.

The possible effect of clipping antennae in males must be considered in a very different way. This could be an indirect effect through the facility or difficulty of female fecundation. When considering different males, a possible individual effect on the egg laying rate of fecundated females was shown to exist 9TIB-9), March 1966), independently of the fecundation “per se”. Since in the Experiment 3 no female gave as low figure as to consider it non-fecundated, some quantitative effect of the males could be considered to exist. However, given that the means for males with 0, 1 or 2 antennae do not follow a regular pattern as in females and that the significance is very far away from that in those females (F = 4.32 v. F = 106.66), it is difficult to conclude something definitive from these results. The replication of Experiment 3 would have probably drawn different rank orders for the three types of males and so by pooling replicates could cancel out those significant differences. In summary, clipping antennae in males does not seem to be as important as in females but caution must be taken with this fact before starting an egg laying experiment.

The conclusion of the positive influence of the amount of medium in the vial on the egg laying rate of individual females had been shown in experiments run before and it relates closely with point (a) in the conclusion 1) on the ability of the female to control the density of eggs on a certain amount of medium in order to prevent a stressful situation for the offspring. But given the results in Experiment 4 where the females without antennae were also influenced by the amount of medium, the interpretation of having more lay in the clipped females with the suggestion (a) seems less reasonable than with the (b) one.

The highly significant effect found for the interaction between antennae and medium with virgin females is only quantitative and not qualitative (same rank order inside of each factor) and it could be interpreted through the high level of significance of both main factors (as residual effect) or by some more essential facts related with the problems under (a) and (b); interpretation which deserves more investigation (more replications of the last experiment).

Finally, the problem of the cannibalism of eggs was not considered because little is known of it in this laboratory. The influence of cannibalism in reduction or increase in egg laying found could be studied by adequate methods.

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Microclimatic studies in cacao beans warehouses.

Climatic and microclimatic studies are in progress in some areas of S. Tome Island where cacao beans warehouses are built.

The different temperature and relative humidity values in the warehouses have been determined in order to study the relation of these values with the presence of insect pests.

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A survey of the interest of inert dusts against pests of stored foodstuffs.

The most commonly used inert dusts and their efficacy as insecticides either in laboratory, experiments or large-scale trails are described in the present work. Most of the treatments, according to the literature of the subject, are made by mixing the inert dust with the product but its insecticidal efficacy depends upon biological and ecological factors, dust characteristics and method of application. The efficacy changes with age, sex and “instar”; immature stages of insects are usually more susceptible to inert dusts; for a given stage it seems that efficacy is greater for more advanced “instars”. The resistance of adult insects seems to reach a maximum sometime after emergence and then a gradual fall takes place.

It has been observed that in some way the insecticidal power increases with increasing fineness and this is to a certain degree related to dust adhesiveness. Apart from exceptions, efficacy increases also with increasing hardness.

Generally, insecticidal efficacy increases with increasing temperature and decreasing both relative humidity and product moisture content.

As a rule they are the more effective the more their concentration is high and the more homogeneous has been the mixing of product and dust.

Although it has been assumed that inert dusts were harmless to man and domestic animals, some diseases may be due to them, v. g. silicosis, asbestosis and dermatitis. Moreover the dust dirties the product, the appearance of which becomes often bad, and a suitable cleaning processes must follow.

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\*Feeding of Tribolium confusum on world species of Fusarium and Gibberella zeae.

Stored product insects are frequently associated with fungi infecting stored grain and other foodstuffs. Many of these insects have also been demonstrated to feed on fungi (Sikorowski 1964; Sinha 1965, 1966, 1968;Lenz 1968a, 1968b). As a part of general ecological study on interrelations among insects, mites and fungi in granary ecosystems (Sinha et al. 1969) a project was undertaken in 1961 to study the feeding specificity of adult Tribolium confusum on world species of Fusarium and Gibberella. Dr. W.L. Gordon, who was the foremost authority on the taxonomy of the fungus Fusarium, collaborated with me in this study by providing 116 strains of wild and mutant forms belonging to 11 cosmopolitan species of Fusarium (Gordon 1960) and 2 species of Gibberella. Unfortunately, the study had to be discontinued due to the sudden death of Dr. Gordon in 1963. This paper reports some results of the feeding experiments with T.confusum and each of 111 forms of Fusarium and 5 forms (2 species) of Gibberella isolated from a large variety of foodstuffs, such as cereals, coffee, potato, and ground nuts. The experimental procedures described earlier by Sinha (1966) were used and are briefly summarized here. Each strain of fungus was grown axenically on potato-sucrose agar (PSA) at pH 6 in 200 x 20 mm test tubes plugged with cotton. Test insects were taken from laboratory stock cultures maintained at 27+ 1 0 C and 70 + 2% RH on a mixture of wheat flour and brewer’s yeast powder (20:1). Five surface sterilized adults were introduced into each tube; adults placed in PSA tubes without fungus served as controls. The insects were surface sterilized by immersing them in 1% sodium hypochlorite for 4 minutes and then rinsing in sterile distilled water 3 or 4 times; no detectable damage was apparent. Each tube was replicated 3 times within an experiment. The tubes were examined 3 and 7 days after the insects were introduced to determine amount of feeding. Production of excreta of the same color as the mycelium and spores in the culture indicated feeding.

No evidence of egg laying or breeding was observed on any fungal cultures. The fungal cultures tested are divided into 4 broad categories on the basis of their suitability as foods for T. confusum; figures in parenthesis indicate numbers of strains or species in each group. I. Generally favorable, rarely poor (12); II. Moderately favourable, occasionally poor (43): III. Generally poor, occasionally favorable (43); and
IV. Unfavorable, rarely acceptable (12).

Because of the shortage of space, only the fungi of the first and the last category are listed with their original hosts and geographical origins.

I Generally favorable fungal diet, rarely poor

Gibberella zeae Type VII (Schw.) Petch. (Stat. conid. Fusarium graminearum Schwabe); barley; Balmoral, Man., Canada. G. zeae Type X (Schw. ) Petch.; barley; Balmoral, Man., Canada. G. zene Type VI (Schw.) Petch.; wheat; Normandin, Quebec, Canada. G. zene Type XIII (Schw.) Petch.; wheat ear blight; New Zealand. F. graminearum Schwabe; wheat; Normandin, Quebec, Canada. Gibberella zeae (Schw.); mutant 120; barley; Balmoral, Man., Canada. F. avenaceum (Fr.) Sacc.; Ontario, Canada. F. avenaceum, mutant J, salmon colored mutant; wheat ear blight; New Zealand. Gibberella zeae, mutant a; wheat ear blight; New Zealand. F. sambucinum Fckl. Var. coeruleum Wr.; pine seedling (Pinus contorta Dugl.); British Columbia, Canada. F. oxysporum Schlecht f. dianthi (Prill. & Del.) S. & H.; carnations; Guernsey, Channel Is. F. sporotrichioides Sherb., wild type; winter wheat; British Columbia, Canada. F. culmorum (W. G. Sm.) Sacc.; scab, wheat; Winnipeg, Man., Canada.

IV. Unfavorable fungal diet, rarely acceptable

F. xylarioides Steyaert, Gibberella (Carbuncularia) xylarioides Heim et Saccas; coffee; Africa. F. merismoides Cda.; perar; Washington, U.S.A. F.sambucinum Fck1. Var. coeruleum Wr., mutant, pine seedlings (Pinus contorta); British Columbia, Canada. F. moniliforme Sheldon; maize; India. F. coccidicola P. Henn.; citrus species; Trinidad. F. oxysporum Schlecht f. cepae (Hanz) Snyd. & Hansen; onion bulb root; Winnipeg, Man.,Canada. F. stilboides Wr., mutant; coffee; Tanganyika. F. sambucinum (Fekl.) & ff6, Wr., mating type a; potato; Alberta, Canada. F. solani (Mart.) App. & Wr., dwarf mutant; roots of Citrus; West Bengal, India. F. semitectum Berk & Rav.; Manila hemp; North Borneo. F. tumidum Sherb.

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Effect of insect infestations on the baking and taste qualities of farinaceous foods.

Insect-infested flour is often rejected for aesthetic and probable health reasons without consideration of certain undesirable effects which may be related to changes in population density level and infestation period. In order to understand certain undesirable consequences of insects in flour, experiments are being conducted to ascertain the effects of insect infestations on the baking and taste qualities of bread prepared from insect infested flour.

Bread prepared from flour previously infested with Oryzaephilus surinamensis (L.), Tenebrio molitor L., Tribolium castaneum (Herbst), and Tribolium confusum (Duval) ebony strain, disclosed a variety of baking and taste quality changes after 1, 2, and 3 month infestation periods.

Although bread made from flour infested with O. surinamensis and T. molitor revealed only minor canges in physical attributes, a “chemophenolic” taste and odor was detected. Bread prepared from flour infested with T. castaneum and T. confusum showed many changes including a progressive darkening of the crumb, reduction in slice size, and a distinct, offensive taste and odor which intensified after each infestation period.

Although the results are inconclusive, our data lend support to the hypothesis that quinine secretions of Tribolium spp. adversely affect the taste qualities of bread made from insect-infested flour, and may be an important factor in lowering its baking qualities.

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X-ray analysis of infested cacao beans.

Different films and exposure times are being used in order to obtain the best conditions for the X-ray analysis of cacao beans containing insect larvae.

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Hygroscopic equilibria of cacao beans.

Little bags containing cacao beans from S. Tome Island were stored in eight different relative humidities in the range 60% to 95%. Daily weighing of the bags gave the rate of change in moisture content. For the highest humidities equilibrium was not obtained as mold growth was observed.

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\*Aggregation response by adult Tribolium confusum to stimuli from components of the fungus Nigrospora sphaerica.

Nigrospora sphaerica is a fungus that is sometimes associated with grain in storage. We have found that flour beetles aggregate on pith discs treated with extracts of this fungus. More detailed extensive studies have shown that triglycerides present in the mycelia, and free fatty acids were important factors eliciting aggretation. A monounsaturated triglyceride fraction, obtained by separation according to the degree of unsaturation, was the most active. Bioassays of other components separated from the extracts, and of some synthetic triglycerides have contributed to an understanding of structure-activity relationships. The question of whether part of activity is due to synergism or to a potent minor component is under investigation.

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Characterization of substances in wheat germ eliciting aggregation response by Tribolium conusum.

Strong aggregation response of Tribolium confusum has been demonstrated on cereal products by an elaborate bioassay method (Loschiavo, 1965a, b). n-Hexane extract of wheat germ, which elicited a strong aggregation response, was used as a starting material. The aggregation factors were isolated and purified by employing column, thin layer, and gas-liquid chromatography. The aggregation factors have been shown to be triglycerides having 2 to 3 double bonds in one molecule and at least three compounds are responsible for the aggregation phenomenon. Candidate compounds are 1-palmito-2, 3-diolcin, 2-linoleo-1, 3-dipalmitin, and 1-palmito-2-linoleo-3-olein.

Synthesis of these and other triglycerides are under way to elucidate the relationship between chemical structure and biological activity.

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\*Larval weight and pupation in the absence of food in T. castaneum strain (a preliminary report).

Introduction. Robertson (1965) mentions the divisibility of the larval growth of a holometabolous insect into two phases: 1) a more or less exponential growth to
a critical stage, in which a hormonal change occurs; 2) the period following this stage, when an individual can pupate and eclose even without food. When food is limited, the ability of individuals to reach the critical stage may determine their chance of leaving progeny.

In the laboratory, with a fixed amount of food and space and the absence of emigration (Zyromska-Rudzka, 1966), food becomes progressively limited with the increase in population density. In their discussion of the types of population response to increased density, Sokal and Karten (1964) note that in Tribolium, both survival and individual weight decrease with an increase in density. If the food supply were the only limiting factor and the population responded instantaneously to it, there should not be
a decrease in both parameters.

The experiment reported here was designed to find out whether an individual Tribolium may attain a critical weight beyond which it can pupate and eclose in the absence of food. An obvious second step would be to find if, in a dense culture, individuals can reach this critical weight, thus indicating whether or not the diminishing food supply is the direct cause of the decrease in survival. This and other steps are planned, and the results should be considered preliminary.

Materials and methods. Of each of two T. castaneum strains (wild type (++) and black (bb) – see stock list), 120 larvae were recovered and weighed individually on a microanalytical balance to the nearest 10 micrograms. Similarly 40 hybrid (+b) larvae were weighed. Each larva was then transferred to a numbered empty vial. The vials were inspected daily and death, moulting, pupation or eclosion were recorded. Exuviae were removed from the vials.

Pupae were weighed and returned to the vials to eclose. Adults were weighed alive and then dried overnight at 100 0 C and weighed again.

The data were originally grouped into larval-weight classes ranging from 0 to 3.80 mg with a class interval of 0.2 mg. For parts of the final analysis they were regrouped into three categories – 0-1.60, 1.61-2.40, and more than 2.40 mg.

Results and discussion. The frequency distribution of the recovered larvae is shown in Table 1, together with that of the pupating and eclosing individuals. Larvae smaller than 1.60 mg never pupated without food (there were 42 ++ larvae, 38 bb larvae and 12 +b larvae in this category).

In the second category, 15 of 26 ++, 19 of 29 bb, and all 10 +b larvae is shown in Table 1, together with that of the pupating and eclosing individuals. Larvae smaller than 1.60 mg never pupated without food (there were 42++ larvae, 38 bb larvae and 12 +b larvae in this category).

In the second category, 15 of 26 ++, 19 of 29 bb, and all 10 +b larvae pupated. The difference between the two pure strains was not significant, but +b was significantly different from them (P 0.05, by a test of proportions given in Snedecor, 1956, 9.9.1).

Almost all the larvae in the third category pupated (50 of 52 ++, and all 53 bb and 18 +b larvae). Pupal mortality was very low and only 3 of the 165 pupae did not eclose.

Larvae remained alive for long periods without food. Larvae less than 1.4 mg when recovered survived upto 12 days without food, and larvae 1.4-1.6 mg in weight lived up to 22 days in the empty vials. However, all but 2 of the 165 larvae which pupated did so no later than 9 days after removal from food.

The results seem to indicate a critical weight beyond which there is a good chance that food abundance will not be directly important for survival. The cannibalism by larvae (Park et al., 1965; Wool, 1969) and the harmful effects of flour conditioning (Karten, 1965) may, however, be increased if the food supply is diminished, thus resulting in low survival.

It is interesting to investigate the ability of starved larvae to recover and develop to pupation when food becomes available. If they do to a considerable extent, Tribolium could serve as an experimental model of a natural population well adapted to the uncertainty of environmental conditions.

The assistance of Mrs. Meral Kence is gratefully acknowledged.

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Table1. Frequency distribution of larvae, when removed from food, and of pupae and adults.

Larval ++ bb +b
weight
classes larvae pupae adults larvae pupae adults larvae pupae adults

1. -0.20

0.21-0.40

0.41-0.60 2 - - 2 - - 1 - -

0.61-0.80 3 - - 6 - -

0.81-1.00 3 - - 6 - - 4 - -

1.01-1.20 12 - - 10 - - 2 - -

1.21-1.40 14 - - 8 - - 3 - -

1.41-1.60 8 - - 12 - - 2 - -

1.61-1.80 9 3 3 12 5 5 2 2 2

1.81-2.00 6 5 5 5 4 4 3 3 3

2.01-2.20 6 3 3 3 2 2 2 2 2

2.21-2.40 5 4 4 9 8 6 3 3 3

2.41-2.60 7 6 6 9 9 9 3 3 3

2.61-2.80 11 11 11 14 14 14 11 11 11

2.81-3.00 14 14 14 11 11 11 3 3 3

3.01-3.20 9 9 8 6 6 6

3.21-3.40 6 6 6 10 10 10 1 1 1

3.41-3.60 2 2 2 2 2 2

3.61-3.80 1 1 1 1 1 1

3.81-4.00 1 1 1

Total 120 65 64 120 72 70 40 28 28

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\*Development studies of X-irradiated Tribolium confusum eggs.

The effects of environmental factors, including temperature, magnetic field, ionizing radiation, etc., on the development of T. confusum have been investigated for many years in our laboratory (1, 2, 3). Recently we studied the dynamics of embryonic development and the effects of x-irradiation, delivered to the early egg stage, on the development at all stages. About 750 adults, less that 2-months-old, were placed in a 1-liter jar containing about 500 ml medium (4% flour-yeast) for 4 hours at 30 0 C and R.H. 30%. Eggs were then separated from adults and medium by the usual sifting methods, and x-irradiated at 24 0 C with a therapeutic x-ray unit (Philips) at 15 mA and 180 kV with a 1.0 mm A1 filter. A dose rate of 300 R/min was used. The hatchability, number of eclosions, and the malformations of adults from irradiated eggs were carefully checked, and results are shown in Tables 1, and 2, and Figure 1.

When the batchability in percentage is plotted against dose on a semi-log paper, the lethal dose of 50% was found to be about 400 R, and the extra-polated number about 2. Results suggest that a simultaneous two hits will cause hatching failure and that the egg could be at single cell stage. Using histological methods, we found that the 6-hr old egg is a multi-nucleated single cell. It seems that two simultaneous chromosome breaks will prevent a successful embryonic development of the 6-hr old egg.

The percentage of eclosion showed also a decrease with increase of dose delivered at an early egg stage. The effect of x-irradiation on eclosion, however, was found to be less severe than on hatchability. With a dose of

Table 1. Hatchability and Eclosion of X-irradiated T. confusum Eggs.

Dose ( R ) No. of Total % Hatchability Total % Eclosion
 eggs larvae Adults

 0 200 175 87.5 (100) 166 95.0
 100 “ 151 75.5 ( 86) 137 90.8
 200 “ 133 66.5 ( 76) 111 83.5
 300 “ 95 47.5 ( 54) 84 88.2
 400 “ 75 37.5 ( 43) 65 86.6
 500 “ 73 36.5 ( 42) 62 85.0
 600 “ 84 42.0 ( 48) 78 92.8
 700 “ 45 22.5 ( 26) 37 82.2
 800 “ 32 16.0 ( 18) 25 78.3
 900 “ 18 9.0 ( 10) 12 66.8
 1000 “ 18 9.0 ( 10) 13 72.1

Table 2. Frequency and Type of Malformation of T. confusum Adults.

Dose (R) No. of Total % Malfor- Type of Deformity Observed
 Eggs Adults mation

 0 200 166 0.00 -------------------------------
 100 “ 137 0.73 abnormal elytron
 200 “ 111 1.80 twisted abdomen
 300 “ 84 1.20 curved elytron
 400 “ 65 3.17 “pocket” abdomen
 500 “ 62 3.28 twisted abdomen
 600 “ 78 1.35 incomplete median projection
 700 “ 37 27.03 twisted abdomen,
 abnormal abdominal sclerite,
 fused ntnnal segments.
 800 “ 25 4.00 abnormal elytron
 900 “ 12 8.33 fused antennal segments
1000 “ 13 7.69 abnormal elytron

900 R, for example, hatchability was decreased to 9%, but eclosion only to 66.8%. Possibly ome radiation infuries were recovered at the larvaland pupal stages, since the ability of recovery has been demonstrated in larvae and pupae (4, 5).

Deformity of the abdomen, of the antenna, and of the elytra were all observed at adult stage, as shown in Table 2 and Figure 1. An antennal deformity seems to be induced at relatively higher doses, and abnormal abdomen was found most frequently in this study. The frequency of the deformity generally showed an increase with dose. Since the number of adults that survived in this experiment is small at higher doses, a precise



quantitative relationship between dose and amount of deformity can not be obtained at present, and more data are needed. Our preliminary data indicate that the type of deformity at adult stage is age dependent. Irradiated 2- and 3-day old eggs at 30 0 C,for instance, only showed abnormal antennae and elytra.

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\*Kinetics of recovery of X-irradiated Tribolium confusum pupae.

Recovery from injuries induced by ionizing radiation has been demonstrated recently in Tribolium larvae and adults (1, 2). In this laboratory young pupae have been extensively used to study the effects of radiation on differentiation and morphogenesis of organisms (3). A wing abnormality consisting of median elytral split, elytral blistering, and protrusion of the underlying membranous wings generally will develop to the adult stage when a dose of X-ray over 1200 R is given to 20- to 30-hr. old pupae. In this study, we chose wing abnormalities as the endpoint to determine the kinetics of recovery in pupae.

The methods used for collecting defined aged pupae have been reported elsewhere
(4). In brief, prepapae, collected from stock culture, were placed in a 30 0 C incubator. At the end of 6 hours, pupae were harvested and kept at 30 0 C until they reached 18- to 24-hr. old. Pupae were irradiated at 24 0 C with a Philips x-ray unit at 180 keV and 15 mA with a 1.0 mm A1 filter. The distance from the target to the specimen was 30 cm with a dose rate of 300 R/min. At the end of the second dose, pupae were transferred from 24 0 C back to 30 0 C incubator and scored after they eclosed as adults.

A quite rapid recovery was found in pupae at 24 0 C, as shown in Table 1. Wing abnormalities dropped from 75.0% to 18.4% within 7 hours, slightly rose to 28.6% at 8 hours, and dropped again to 17.4% at 9 hours. Since the radiosensitivity of pupae aged from 18- to 30 hr. old (at 30 0 C) is the same, as shown be Buckhold and Slater (4), the change of number of abnormalities at different intervals observed in this experiment represents recovery, not change of radiosensitivity. This rapid and complicated pattern of recovery is very similar to that found in larvae. The reason for this similarity is probably due to the fact that in both larvae and pupae radiation injuries are occurred in some tissues containing embryonic cells. It has been suggested, from radiosensitivity studies (4), that the wing formation in T. confusum is not completed until 39 hours postpupation (at 30 0 C). In the present study, the maximum interval used was 10 hours at 24 0 C, which, as the Q 10 for pupal period is about 3.8, would approximately be equivalent to 5 hours at 30 0 C; the age of the pupae at the end of the second irradiation was, therefore, less than 30 hours old, and the observed changes in response to radiation with intervals represents the recovery of embryonic cells in wings.

Table 1. Radiation Response and Recovery in T. confusum Pupae.

Conditioning Second Interval No. of % Wing

Dose ( R ) Dose ( R ) (hr) pupae Abnormalities

 0 -- -- 100 0.0

1350 -- -- 100 5.0

1500 -- -- 100 26.0

1650 -- -- 100 48.0

1800 -- -- 100 76.0

2000 -- -- 100 94.0

 0 -- -- 100 0.0

1800 -- -- 100 75.0

 900 900 0.5 50 75.0

 900 900 1.0 50 75.0

 900 900 2.0 50 66.7

 900 900 3.0 50 64.0

 900 900 4.0 50 58.3

 900 900 5.0 50 31.3

 900 900 6.0 50 27.1

 900 900 7.0 50 18.4

 900 900 8.0 50 28.6

 900 900 9.0 50 17.4

 900 900 10.0 50 16.3

By comparing the per cent of wing abnormalities following split-doses with that of
a dose-response curve produced by single exposures, we found that the residual injuries at 7 hours, the first peak, is about 450 R. This gives only about 39% recovery. The fact that no complete recovery in pupae is reached at first peak shows agreement with results obtained in larvae.

This work was supported by NASA and U.S. AEC.

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 Radiation Res. 37:567-576.

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Detection of lindane and DDT in beans using thin-layer chromatography process.

The technic described is mainly based on a method developed and improved by Martin F. Kovaks.

The insecticides are extracted by hexane and spotted on pre-washed plates of silica-gel after extract concentration.

The dilution has been done with an hexane mixture, acetone 98: 2 and it was used with the Mitchell chromogenous reagent with a base of silver nitrate.

Under U. V. lamps the insecticides are seen as brownish stains.

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Pest control of stored cacao beans.

The principal insect pests found in stored cacao beans of S. Tome Island are the species Oryzaephilus Mercator Fauv. And Cadra cautella Wlk.

Some studies are being carried on fumigation methyl bromide either in atmospheric chamber or under sheet, in order to control those insect pests.

Some contact insecticides are also under study.

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Application of the thin-layer chromatography technic to the detection of stored beans malathion.

Detection of malathion residues in silica gel G layers using as first solvent a mixture of hexane-acetone and as second a solution of 0.5% palladium chloride.

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Detection of organochlorinated insecticides in cacao beans.

Thin-layer chromatography technic is experimentally under study in the detection of organochlorinates in bagged cacao beans. The insectivides were used as dusts and sprays applied externally in the bags.

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Application of insecticide smokes to disinfest warehouses used for storage of bagged cacao beans.

In S. Tome Island the species Cadra cautella (Walk.) has been detected very often
as the main pest of the warehouses and also of the cacao beans.

Local experiments are under study to determine the efficacy of lindane, pyrethrins and piperonly butoxide against that pest, using the Swingfog, model SN 8.

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An alternate feeding medium for the Natidulid Carpophilus hemipterus.

The following is a highly suitable medium for rearing Carpophilus hemipterus:

 5 cups Chicken feed
 3 cups Raisins
 cup Honey
 5 tbls. Glycerin
 ½ cup Water

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Evaluation of hygroscopic equilibria of grain – a new technique.

The grain is enclosed in little nylon tulle bags and stored within large dessicators where different constant relative humidities are maintained until equilibrium is reached. Daily weight increments are converted into moisture content.

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Hygroscopic equilibria of cereal grains and pulses

The technique we referred above is being used for the establishment of hygroscopic equilibria curves for wheat, maize and beans, either by drying damp grains or moistening dried ones.

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A comparative study of different oven methods for the determination of cacao beans water content.

Three oven methods (98-100 0 C x 2.5 h; 100-101 0 C x 4 h; 101-105 0 C x 16 h) are under test. The water content of cacao beans must be determined before they are introduced in different desicators where constant relative humidities from 70% to 95% have been established. The moisture increase of cacao beans added to the initial water content determination must be equal to the final evolution of water content.

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Legislation and regulations on pesticides used in foodstorage.

A survey of legislation and regulation from various countries concerning those pesticides used in stores and stored foodstuffs is being carried out, especially relating to residue tolerances, dose of applications and protection of operators.