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THE EFFECT OF TEPA ON THE REPRODUCTIVE POTENTIAL OF THE RED FLOUR BEETLE, TRIBOLIUM CASTANEUM

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Abstract. Tepa when applied by a tarsal contact method, failed to produce complete sterility in *T. castaneum* without toxic effects. The females were more tolerant of the sterilizing and toxic effects of Tepa than the males. Males exposed to 20 μg/cm² treatment of Tepa were completely sterile but 79% of treated males died within 2 weeks after treatment. However, this dose did not produce complete sterility in females. The males exposed to 10 μg/cm² treatment of Tepa were completely sterile for 1 week after treatment, after which they recovered a transitory fertility. The Tepa-treated males failed to elicit the full oviposition potential of the females.

In the recent years the use of chemosterilants for insect pest control has become a possible alternative to radiation. Some of the insect species in which radiation treatment impaired the competitiveness of sterilized males, have been successfully sterilized by chemicals.[1–5] Malik[6] reported that the radiosterilized males of the red flour beetle, *Tribolium castaneum* (Herbst) failed to compete with normal males. Therefore, the present studies were undertaken to determine the doses of chemosterilant required for sterilization in males and females and their effect on reproductive potential and to compare these findings with those from radiation studies.

In the present studies tris-(1-aziridinyl) phosphine oxide, commonly known as Tepa, was used on a variety of insects with varying degree of success in the induction of sterility. Complete sterility with Tepa has been reported in *Musca domestica* L.,[7] *Ostrinia nubilalis* (Hubner),[8] *Prodenia litura* F.,[9] *Trichoplusia ni* (Hubner),[10] *Spodoptera frugiperda* (J.E. Smith),[11] *Carpocapsa pomonella* (L.),[12] *Heliothis virescens* (F.),[13] *Diparopsis castanea* Hmps,[14] *Papillia japonica* Newman,[15,16] and in *Anthonomus grandis* Boheman.[17] In *Hypera postica* (Gyllenhal) the males were temporarily sterilized with Tepa and recovery of fertility occurred subsequently.[18]

Materials and Methods

The culture of *T. castaneum* was maintained on whole wheat flour with 5% yeast at controlled temperature (29±1°C), humidity (55% R.H.) and 8 hr light per day under favourable conditions of low crowding and fresh medium. The sexes were separated at the pupal stage. In all the experiments 9 day old adults were tested.

The beetles were exposed to a residual film of Tepa in glass petri plates (35 beetles per petri plate) for 1 hr at 29°C. Fluorescent light was used to keep the insects actively moving during treatment. After treatment, the insects were transferred back to the C.T. room and offered fresh flour. The pairing was made 4 hr after treatment.

Experiments and Results

Effect of Tepa on Males

Experiment 1. To determine the relationship between dosages of Tepa and sterility induced in males, the males were exposed to 10, 20, 25, 50 or 100 μg/cm² Tepa for 1 hr. Five treated males were paired with 5 normal females in 2 × 1 in glass vials, in three replicates, at each dose level. The progeny produced by these females was assessed for 1 week after pairing. The females paired with treated males laid infertile eggs, hence 100% sterility was observed. High mortality occurred at all the dose levels of Tepa except at 10 μg/cm² dose level (Fig. 1). Thus all these doses were too high to establish any relationship between dosage and sterility. In another test, lower dosages ranging from 0.5 to 10 μg/cm² were used similarly. A probit analysis, based on the maximum likelihood method of Finney,[19] was carried out on the dosage response data (Fig. 2). No mortality was observed at any of these doses. From these results it was decided to use a 10 μg/cm² dose of Tepa for further studies. At this dose, complete sterility was expected but no mortality.

Experiment 2. The males were treated with a 10 μg/cm² dose of Tepa and paired with normal females at 1:1 ratio, in 10 replicates. The fecundity of the females was determined on alternate days for 40 days after treatment. Egg hatch was observed 8–10 days after eggs were incubated and per cent

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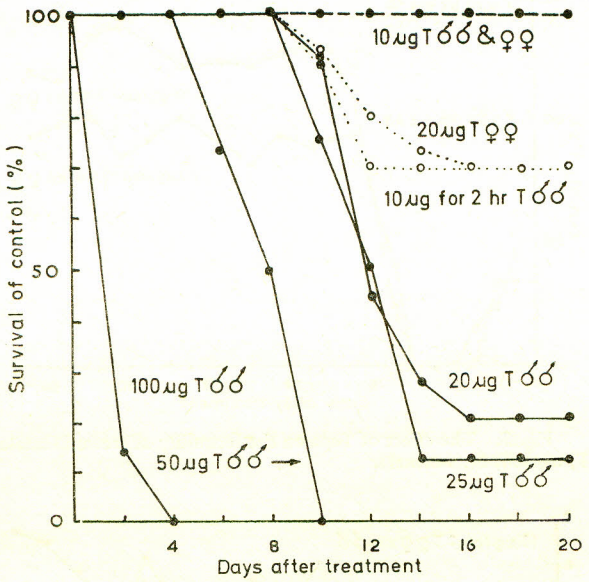

Fig. 1. The effect of Tepa Treatment on survival of males and females of *T. castaneum*.

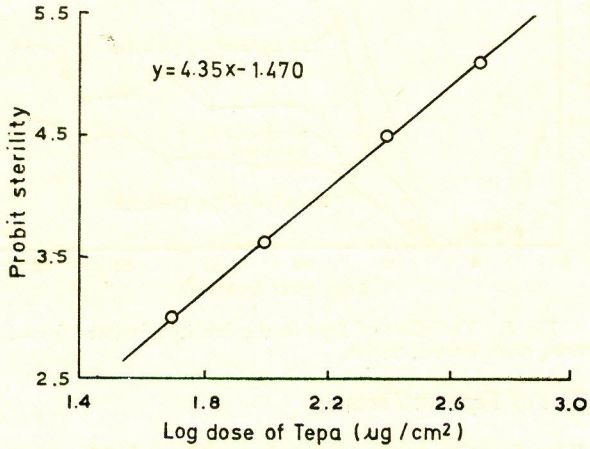

Fig. 2. Relationship between probit sterility and log dose of Tepa applied to males.

TABLE 1. EGG PRODUCTION, HATCH AND PER CENT FERTILITY OF NORMAL FEMALES PAIRED WITH NORMAL AND TEPA-TREATED MALES.

Females paired with	No. of eggs laid	No. of eggs hatched	Fertility (%)
Normal males (control)	491·1±13·4*	455·2±10·6	92·8±0·9
10 µg/cm² for 1 hr	357·8±19·1	56·9±10·4	15·7±2·7
10 µg/cm² for 2 hr	380·9±25·8	30·1±9·1	7·9±2·2
20 µg/cm² for 1 hr	435·0±21·2	0·0	0·0

*±S.E.

fertility computed (Table 1). The daily records of per cent fertility are shown in Fig. 3. Contrary to expectation females paired with treated males laid some fertile eggs. Partial recovery of fertility occurred from 6 to 20 days, then sterility increased and

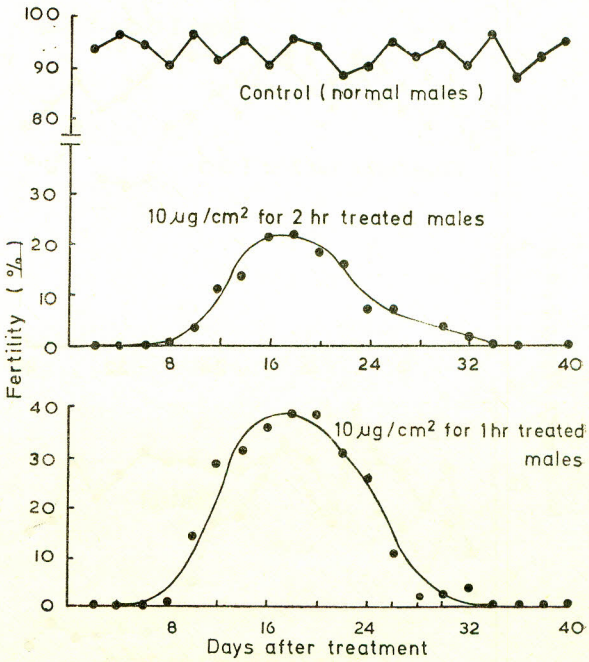

Fig. 3. The fertility of eggs from normal females paired with Tepa-treated males.

by the 32nd day females laid only infertile eggs. This suggests that the genetic cells at different stages of development show differential sensitivity to Tepa.

Experiment 3. Since at 10 µg/cm² of Tepa treatment, partial recovery of fertility took place, a further test was conducted with increased doses of Tepa. One batch of males was exposed to the same treatment but exposure time was increased to 2 hr and another batch of males was exposed to 20 µg/cm² for 1 hr. The control and treated males were paired with normal females in the same combinations and records of fecundity and fertility were made as before. At both exposures, survival was affected. Acute mortality occurred between 6–14 days, when 79% of the 20 µg/cm² and 30% of the 10 µg/cm² treated males died (Fig. 1). Complete sterility was observed in 20 µg/cm² treated males, whereas the males treated with 10 µg/cm² for 2 hr were only partially sterilized (Fig. 3). In the 2 hour-treatment the sterility and recovery of fertility was similar to that of males exposed to 10 µg cm² for 1 hr only, except that the maximum fertility on 16–18th day was 21%, compared with 40% in males treated for 1 hr. It is evident, as might be expected, that the effect of Tepa was increased with increased time of exposure and more dominant lethal mutations were induced.

Elicitation of Oviposition of Untreated Females by Tepa-Treated Males. From Table 1 it can be seen that the females paired with Tepa-treated males, laid fewer eggs than control females. Student's 't' test revealed that the differences between control females and females paired with treated males were significant. The differences between the fecundity of different females are further elucidated by Fig. 4 showing daily oviposition rates. It is evident that in control females, once the full oviposition was attained, it remained at that same level but with day to day

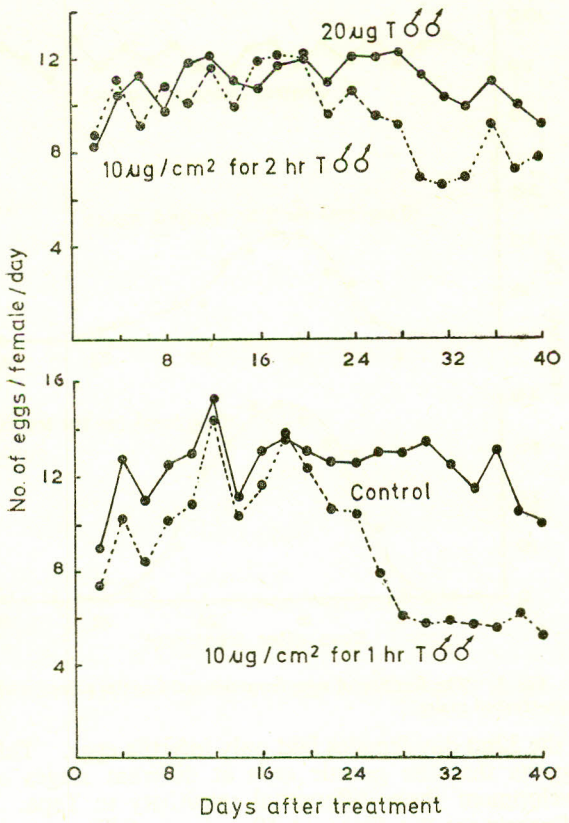

Fig. 4. The daily oviposition rate of normal females paired with normal or treated males.

TABLE 2. EGG PRODUCTION AND FERTILITY OVER A 50-DAY PERIOD OF TEPA-TREATED FEMALES MATED WITH NORMAL MALES.

Treatment	No. of eggs	No. of eggs hatched	Fertility (%)
10 µg/cm²	266·75±42·51*	193·25±30·75	72·58±4·14
20 µg/cm²	227·62±28·48	129·13±22·22	54·34±3·43
Control — normal females ×normal males, (for 40 days)	491·11±13·55	455·22±10·63	92·81±0·92
Unmated females			
10 µg/cm²	131·12±24·68	—	—
20 µg/cm²	84·87±19·09	—	—

*±S.E.

variation. In females mated with males which had been treated with 10 µg/cm² for 1 hr, the oviposition rate was not much different from the controls for 18 days; it later declined gradually for the next 10 days until it reached the lowest level. A similar reduction in females mated with 20 µg/cm² treated males and with males treated with 10 µg/cm² for 2 hr was not so marked. However oviposition rates in both these treatments were lower.

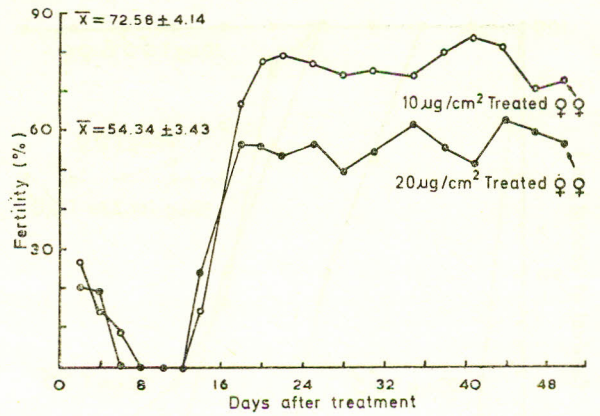

Fig. 5. The effect of Tepa on the fecundity of treated females (T, treated; N, normal).

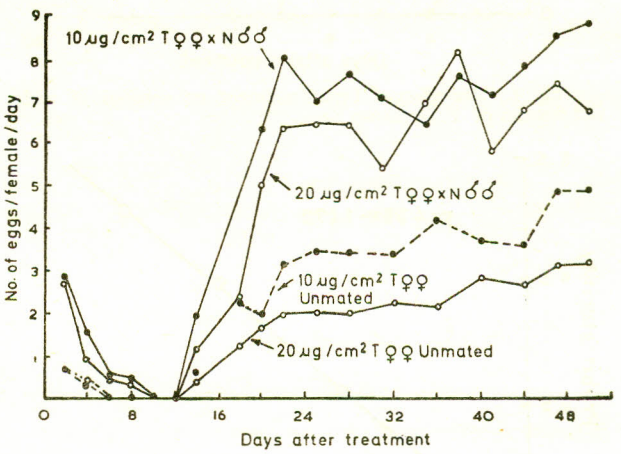

Fig. 6. The effect of Tepa on the fertility of treated femaels mated with normal males.

Effect of Tepa on Females

The females were exposed to 10 and 20 µg/cm² dosages of Tepa for 1 hr and after treatment they were paired with normal males and fecundity and fertility were assessed for 50 days (Table 2).

Toxicity. The survival of females treated with 10 µg/cm² Tepa was not affected. At 20 µg/cm² treatment, 30% of the females died between 10–16 days; afterward no mortality occurred. The females are more resistant than the males to the toxic effect of Tepa (Fig. 1).

Effect on Fecundity. The mean fecundity for 50 days of treated, untreated females and females mated with normal males is given in Table 2. It can be concluded that the Tepa adversely affects the fecundity of the females. The 20 µg/cm² treated females laid fewer eggs than 10 µg/cm² treated-females but the differences were not significant; this may be due to large variations in the samples. The daily oviposition rates (Fig. 5) revealed that at both levels of treatment, oviposition decreased immediately after treatment. The unmated treated females ceased to lay eggs by the 6th day and the mated females by the 8th day. On the 14th day both the mated and unmated females

resumed oviposition which subsequently increased until a maximum rate was attained by the 22nd day. After that the oviposition rates remained at about the same level with slight day to day variations.

Effect on Fertility. Complete sterility was not induced in females treated at both levels of Tepa. In 10 and 20 $\mu g/cm^2$ treated females respectively 72.58 ± 4.14 and 54.34 ± 0.43 per cent of the eggs hatched. The percentage of fertile eggs was not constant during the experimental period (Fig. 6). It was low for the first 6 days and was followed by a period of complete infertility. On the 14th day, the females started laying some fertile eggs and the maximum level of fertility was attained 18–20 days later, after which it remained constant at that level.

Discussion

Sexual Differences in Susceptibility. In these studies the females were less susceptible than the males to the toxic and sterilizing effect of Tepa. A similar conclusion has been reported in *Tetranychus urticae* koch,[22] *Spodoptera frugiperda* (J.E. Smith),[11] *Trichoplusia ni* (Hubner)[20] and in *Heliothis* virescens (F).[13] Various reasons have been suggested for the greater tolerance of females to the effects of chemosterilants. The greater susceptibility of males may be, in part, due to the differences in weights of males and females.[2] Jalil and Morrison suggested that differences in susceptibility can be due to differences in sclerotization as well as to differences in volume to surface area ratio of males and females.[22] However these reasons are not valid where males and females are of equal size and weight. Crystal[23] suggested that it is probably caused by a more rapid penetration of chemosterilant to the chromosomes of the small, relatively unprotected spermatozoa compared to the penetration of the large ova protected by nurse cells and yolk. This could be one of the possible reasons for the greater sensitivity of *Tribolium* males to Tepa though in these studies, it was observed in general that *Tribolium* males were more active than females. Thus, with the method of application used in these experiments where pick-up of the material is dependant on tarsal contact, there is a possibility that because of their greater activity, the males might have picked up more Tepa than females, resulting in higher sterility and mortality of males.

Effect on Fecundity. The Tepa-treatment inhibited the normal oviposition in females. Although it is well established that chemosterilants affect the fecundity, little is known about the causes of infecundity. Reduced size of ovariole contributes to the reduced fecundity and reductions in ovariole size have been observed in housefly and in tobacco bud-worm and boll-worm due to the effect of chemosterilants.[24,25]

The reduction in fecundity can also occur if the nurse cells are damaged, for the germ cells will then be unable to complete development. The nurse cells are most likely to be affected because they are undergoing endomitotic activity. Tepa is likely to disrupt the process and prevent the nuclei of the nurse cells from attaining the proper degree of ploidy and size required for normal vitellogenesis. This may be the cause of infecundity for the first 2 weeks in Tepa, treated *Tribolium* females. The nurse cells, however may recover to repopulate the vitellarium and then oviposition may occur as observed in these studies.

Female Fertility. The Tepa produced dominant lethal mutations in oocytes, however, complete sterility was not induced and Tepa-treated females recovered their fertility after times dependant upon the dosages of Tepa applied. In studies on mammalian and bacterial cells the uptake of proteins has been correlated with the rate of repair mechanism.[26] It may be that when females are not laying eggs the surplus proteins in female may be used in the repair of chromosomal damage induced by Tepa.

Male Fertility. In males, the degree of sterility depended upon the dose of Tepa and the exposure time. The males exposed to 20 $\mu g/cm^2$ dose were completely sterile for the test period. From fecundity data it can be suggested that these males were probably aspermic 8–10 days after treatment. Thus the sterility induced in these males for the first 8–10 days is due to dominant lethal mutations in mature and nearly mature sperms, thereafter sterility is due to aspermia in these males.

The males exposed to 10 $\mu g/cm^2$ dose of Tepa for 1 and 2 hr, showed a transitional recovery of fertility. A higher percentage of dominant lethal mutations being induced by the longer exposures, otherwise the recovery of fertility and permanency of sterility patterns were identical in both cases.

In insects, permanent sterility can be induced if all the genetic cells are affected by treatment, and dominant lethal mutations are induced in mature and immature stages of spermatogenesis as shown by LaChance and Leopold[27] in the adult housefly. The recovery of fertility is theoretically possible and could be due to a differential sensitivity of germ cells. If a chemical treatment produces a high level of dominant lethal mutations in mature and nearly mature sperms, but does not succeed in killing all the gonial cells, the surviving gonial cells will continue to divide and repopulate the germarium. Sperms produced from these cells would probably not contain dominant lethal mutations. When this happens, the male may recover fertility in later matings. This does not explain the return of sterility found here in *Tribolium*. Therefore, it can be suggested that different genetic cells at different stages of development show differential sensitivity to Tepa. In the mature and nearly mature sperms, dominant lethals are induced, while the spermatids and spermatocytes are resistant to the effect of Tepa and transitional recovery is observed. The primary germ cells were the most affected and failed to produce the sperm which can be correlated with the return of the second sterility period. This hypothesis is also supported by the evidence provided by corresponding radiation studies on *Tribolium*.[6,28]

References

1. G.C. LaBrecque, D.W. Meifert and C.N. Smith, Science, **136,** 388 (1962).
2. D.E. Weidhaas and C.H. Schmidt, Mosquito News, **23,** 32 (1963).
3. R.L. Fye and G.C. LaBrecque, J. Econ. Entomol., **59,** 538 (1966).
4. G.B. White, Nature, **210,** 1372 (1966).

5. D.A. Dame and H.R. Ford, Bull. Entomol. Res., **56,** 649 (1966).
6. M.M. Malik, Ph.D thesis, London University, (1970).
7. R.L. Fye, J. Econ. Entomol., **60,** 605 (1967).
8. J.A. Harding, *ibid.*, **60,** 1631 (1967).
9. A. Toppozada, S. Abdallah and M.E. Eldefrawi, *ibid.*, **59,** 1125 (1966).
10. A.N. Kishaba, T.S. Henneberry, P.J. Hancock and H.T. Toba, *ibid.*, **60,** 359 (1967).
11. J.R. Young and H.C. Cox, *ibid.*, **58,** 883 (1965).
12. D.O. Hathaway, L.V. Lydin and B.A. Butt, *ibid.*, **59,** 851 (1966).
13. H.M. Flint, W. Klassen, J.E. Norland and E.L. Kressin, *ibid.*, **61,** 1726 (1968).
14. D.G. Campion, Nature, **214,** 1031 (1967).
15. T.L. Ladd, J. Econ. Entomol., **59,** 422 (1966).
16. T.L. Ladd, C.W. Collier and E.L. Plasket, *ibid.*, **61,** 942 (1968).
17. D.A. Hedin, G. Wiygul, D.A. Vickers, A.C. Bartlett and N. Mitlin, *ibid.*, **60,** 209 (1967).
18. J.R. McLaughlin and R.G. Simpson, *ibid.*, **61,** 1730 (1968).
19. D.J. Finney, *Probit Analysis* (Cambridge University Press, Cambridge, 1952), second edition.
20. T.J. Henneberry, A.N. Kishaba, M.Z. Iqbal and B.B. Klingler, J. Econ. Entomol., **61,** 1536 (1968).
21. H. Kido and E.M. Stafford, *ibid.*, **59,** 1064 (1966)
22. M. Jalil and D.E. Morrison, *ibid.*, **62,** 393 (1969).
23. M.M. Crystal, *ibid.*, **59,** 580 (1966).
24. J.A. Wilson and S.B. Hays, Ann. Entomol. Soc. Am., **62,** 690 (1969).
25. D.E. Soto and J.B. Graves, J. Econ. Entomol., **60,** 550 (1967).
26. ϕ. Strmnaes, *Isotopes and Radiation in Entomology* (I.A.E.A., Vienna, 1968), pp. 341–354.
27. L.E. LaChance and R.A. Leopold, Can. J. Genet. Cytol., **11,** 648 (1969).
28. D.J. McDonald, Genetics, **46,** 1511 (1961).