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STORAGE EFFECT ON TEPA-TREATED SPERMS IN MALES AND FEMALES OF THE RED FLOUR BEETLE, TRIBOLIUM CASTANEUM HBST. (COl: TENEBRIONIDAE)

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Abstract. The effects of storage of tepa-treated sperms in untreated females and the effect of delayed matings of treated males on the resultant frequency of dominant lethal mutations were investigated. The genetic damage increased when sperm were stored in untreated females. Whereas in sperm from treated males the genetic damage varied with time. The fertility of celibate males varied with time after treatment in a manner similar to those mated immediately after treatment. The sperm from tepa-treated males negated the normal sperms and no residual effect in untreated females mated with tepa-treated males was observed. Some secondary effects of tepa in males have been suggested.

In several species of coleoptera[1-3] and diptera[4-7] chemosterilant-treated males recovered fertility at times after treatment which varied from species to species. This recovery of fertility also varied with the number of matings by treated males. In treated male *Musca domestica*, celibate males remained sterile, whereas males which mated repeatedly recovered their fertility in 7–8 days.[4] Similar results have been reported in *Aedes aegypti*[5,6] and in *Culex p. fatigans*.[7] This recovery of fertility has been attributed to a storage effect in males.

The effect of chemosterilants also varied when treated sperm were stored in untreated females. An increase in dominant lethal mutations was observed in treated sperms stored in untreated female *Drosophila*[8-11] and in *Callitroga*.[12] In contrast, North[13] reported a decrease of dominant lethality after sperm storage in untreated female *M. domestica* when males were treated with tretamine and hemel, while for tepa, metepa and hempa there was no increase in fertility. These contrasting observations have been found in short-lived insects and the present studies were undertaken to investigate the possible effect of storage of sperms in males and females of *T. castaneum*, a comparatively long-lived species, since in males, low doses of tepa had been found to induce complete sterility for a period of 6 days, after which a transitory recovery period occurred followed by permanent sterility.[14]

Methods

The culture of flour beetles was maintained on whole wheat flour with 5% yeast at controlled temperature (29±1°C) and humidity (55% R.H.). The sexes were separated at the pupal stage. Nine-day old adult males were exposed for 1 hr to a residual film of tepa applied to petridishes at 10 μg/cm². The beetles were paired by placing one male, either treated or untreated, and one untreated female in a 2×½ in glass vial with 0.5 g flour. The flour was changed on alternate days and assayed for fecundity by keeping any eggs found and carrying out a larvae census 10 days after the egg counts. The per cent sterility represented the percentage of dominant lethal mutations induced.

Experiments and Results

Treated Sperm Stored in Females. Normal females were mated with treated males for 4, 10 or 16 days, after which the males were removed and the females assessed for fecundity and fertility for 48 days. The females from which males were removed on the 10th day were repaired with the same males from the 26th day onwards.

The results from this experiment (Fig. 1) revealed that with the storage of sperm, no recovery of fertility occurred. The females mated for 4 day laid only infertile eggs. In the females which were mated for 16 days, the fertility decreased gradually for 12 days after the removal of the male, then became stable with no further change for 20 days. The females paired for 10 days were laying some fertile eggs at the time of removal of males, but 4 days later the eggs were infertile.

Treated Sperm Stored in Males. Treated males were not allowed to mate for 6 days, 14 days or 26 days after treatments. The males were then paired with females in 10 replicates at each level of celibacy and single-pair fecundity and fertility values determined for 40 days after pairing. In the control replicates, untreated males were paired with normal females, whereas in the treated control, the tepa-treated males were paired immediately after treatment. At the end of each experiment all females

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were dissected and observations made of the presence or absence of sperm in the spermathecae or bursa copulatrix.

The fertility of control pairs, of pairs made immediately after treatment and pairs with males kept celibate for various times after treatment are given in Fig. 2. In the untreated controls the fertility remained constant between 90–95% and in pairs made immediately after treatment a sterility period of 6 days was followed by a transitory recovery period of 26 days after which the eggs were again sterile. In celibate male treatments the fertility at pairing varied in a similar manner in relation to the time of treatment as it did with males not isolated after treatment.

The results of observations for the presence of sperms in mated females are summarized as follows:

(a) Controls
N ♂×N ♀

In all the females the spermathecae were full of motile sperms.

Treated controls
T ♂×N ♀ (No delay in mating)

No sperm. Thespermathecae were light yellowish and appeared to be filled with fluid.

(b) Treated males after 6, 14 and 26 days of celibacy had no sperm but filled with fluid as above.

(c) The treated males that had mated for 4 days and then taken off. The spermathecae were full of motile sperm.

The treated males that had mated for 14 days and then taken off, the spermathecae were full of sperms in 3 females, but some of them were immotile. In 2 females, the spermathecae were only half full and in 1 female spermathecae was empty and no sperms could be traced.

Alternate Matings with Sterile and Fertile Males. In one experiment, normal females were paired with either normal males or treated males. On the 4th-day treated males were replaced with normal males and normal males were replaced with other treated males. After four more days all the males were removed and the females assessed for fecundity and fertility for a further 10 days. Females which had last mated with treated males were transferred to 2×1 in glass vials with 2.5 g flour and were transferred to new fresh medium weekly for another 5 weeks. Each vial was kept for a further 5 weeks after which they were inspected for progeny.

The sperms from the last mating have precedence over those from previous inseminations (Fig. 3).

In another experiment the normal and treated males were paired with normal females. Each pair (1 ♀×1 ♂) was placed in 2 × 1 in glass vial containing 2.5 g flour. There were 10 replicates and the flour in each vial was replaced weekly. On the 34th day after treatment, each pair was transferred to ovipositional vials and egg counts of these pairs were made on the 36th and 38th day. On 38th day the treated males were replaced with normal males and the normal males interchanged with

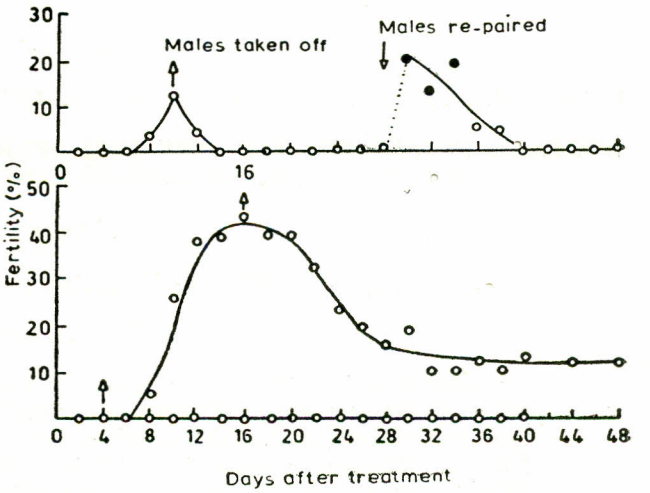

Fig. 1. The effect on egg fertility of sperm stored in females.

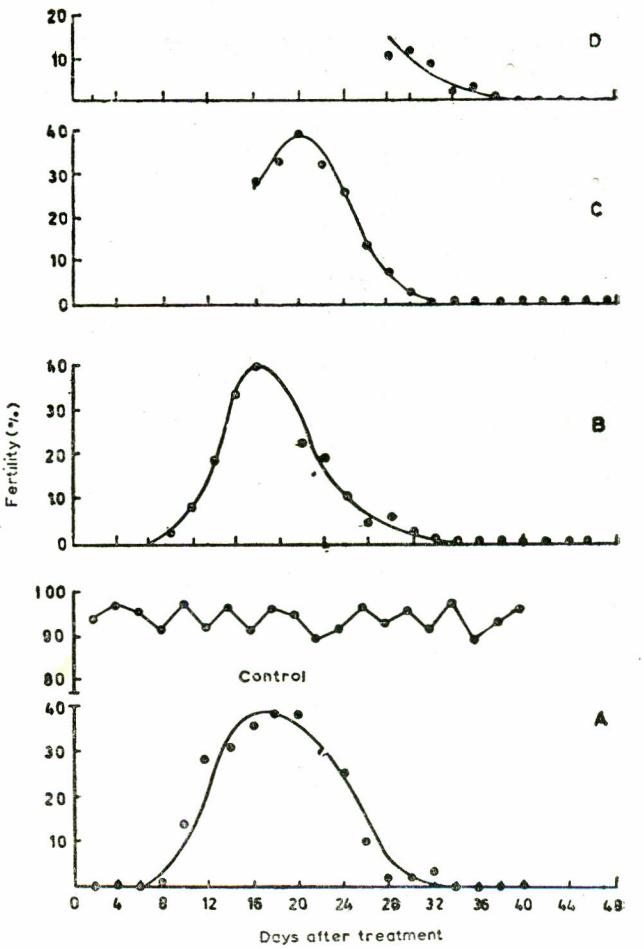

Fig. 2. The effect of tepa on the fertility of males paired at different times after treatment with untreated females: (a) Males mated immediately after treatment, (b) 6 days of celibacy, (c) 14 days of celibacy, and (d) 26 days of celibacy.

treated males. Mating was allowed for 2 days and then these males were removed and the fecundity

and fertility of the females were determined on the 40th, 42nd and 44th day.

In a similar experiment 5 pairs with treated males and 5 pairs with normal males were set up. On 34th day, the males were removed and each male paired with 15 nine-day old virgin females for 2 days; later the females were scored for the presence of sperm.

The females which were paired with treated males for 34 days laid infertile eggs for the next four days and after repairing with normal males, fertile eggs were laid (Fig.3). Thus the treated males had not transferred sufficient tepa or other active metabolites to affect subsequent inseminations. In the reverse combination, the fertile egg laying females when repaired with mated treated males, did not lay sterile eggs but continued to lay eggs with the same fertility found before re-pairing. Again the treated males appear not to transfer any active compounds with the spermathecae which could affect earlier inseminations and inspection of the females indicated that after 34 days of pairing, treated males either failed to mate or if mated, did not transfer sperms able to displace normal sperms from previous inseminations.

An insemination rate test revealed that normal males inseminated 11.38 ± 1.34 females each in 48 hr, whereas no sperm were observed in females which were paired with the treated males, however demonstrating that treated males are aspermic at this time after treatment.

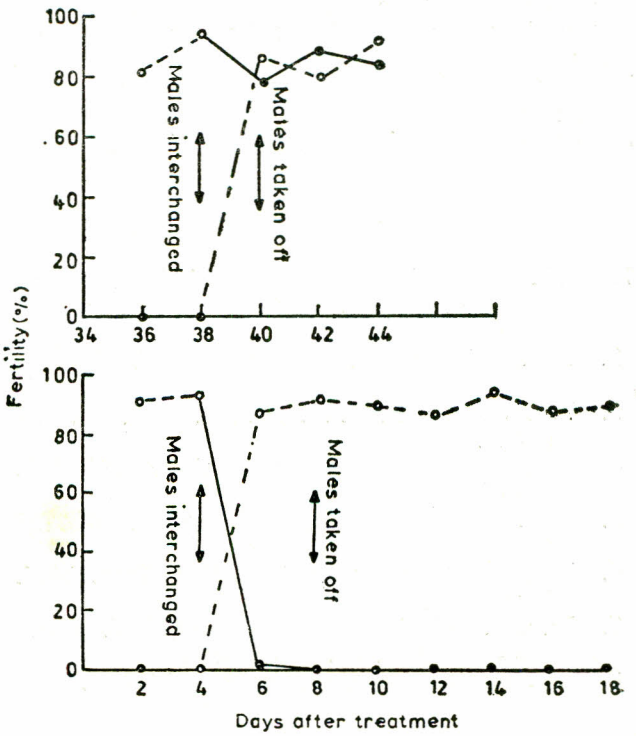

Fig. 3. The effect of mating untreated females with, alternately, normal and tepa-treated males. (closed circles, paired with treated males; (open circles, paired with normal males).

Discussion

Sperm Stored in Untreated Females. The present studies support earlier observations in *Drosophila*[8–10] and in *Callitroga*[12] that with sperm stored in untreated females, the frequency of dominant lethal mutations increased. The reverse occurrence has been reported in *M.domestica*[13] and no effect of storage has been reported by Kaplan.[15] These contrasting results suggest possible fundamental physiological differences in the storage effect between different species of insects tested and in the action of different chemicals being used. The increase in dominant lethal mutations due to storage of sperms in untreated females, could result from the possible residual transfer of small quantities of tepa to the female as found in *M. domestica*[16] and *Anastrepha ludens.*[17] The present studies did not support this hypothesis, however, for females first mated with treated males, commenced laying fertile eggs when re-paired with normal males.

Sperm Stored in Treated Males. These results do not parallel the results obtained by other workers. In *Drosophila*[15] treated with mustard gas and in *Musca*[9] treated with 1,3-propanediol dimethane-sulfonate, the genetic damage increased in celibate males. On the contrary in *Callitroga*[12] treated with *N,N*-tetramethylene bis(1-aziridinecarboxamide) and in *Musca*[18] treated with tepa, the genetic damage decreased in celibate males. In these experiments, the degree of genetic damage varied with time after treatment and in celibate males a similar change appeared to be operating. This pattern conflicts with the earlier observations on radiosterilized *Tribolium* males in which the rate of spermatogenesis slows down when the males are kept celibate and new sperm do not descend into the seminal vesicles until the older sperm have been used up.[19] Thus under these conditions the pattern found in the tepa-treated male pairs would be expected to show the same 6-day sterility period even when mated after a period of celibacy. Since this sterility period did not occur the inference is that some recovery mechanism may operate though this does not explain the subsequent decline in fertility which could be due to residual active chemical. Residual action is less likely due to the unstable nature of these chemicals.

The explanation which agrees most closely with the observations is that a differential susceptibility of existing sperm exists and that unused sperm in celibate males tends to be absorbed or expelled, unlike the condition in celibate radiosterilized males, at about the same rate as it is used up in normal mating. After 30 days the males become aspermic due to irrepairable germ cell damage occurring at the time of treatment.

Alternative hypothesis is that in *Tribolium*, there could be secondary and tertiary chemical reactions taking place sometime after treatment which influence the frequency of dominant lethal mutations and producing physiological damage after 30 days causing the males to be aspermic. This influence of residual tepa would be independent of the mating status of the male and affecting all stages of spermatogenesis with an intensity varying with time. Some tepa (5–20%) has been found to become polymerized and conjugated in insect tissue

in some form or the other,[20,21] although most is lost rapidly from the insect body within 48–72 hr.[22,23] The fate of the residual tepa is not known. Thus there remains a possibility of secondary reactions from this residual tepa which could show their effect at a later part in the life of the insect.

References

1. D.A. Lindquist, L.J. Goryzycki, M.S. Mayer, A.L. Scales and T.B. Davich, J. Econ. Entomol **57,** 745 (1964).
2. L.H. Reinecke, W. Klassen and J.F. Norland, Ann. Entomol Soc. Am., **62,** 511 (1969).
3. T.E. Skelton and P.E. Hunter, *ibid.*, **63,** 335 (1970).
4. L.E. LaChance, M. Degrugillier and A.P. Leverich, *ibid.*, **63,** 422 (1970).
5. D.A. Dame and H.R. Ford, Nature, **201,** 733 (1964).
6. D.S. Bertram, Trans. Roy. Soc. Trop. Med. Hyg., **58,** 296 (1964).
7. M.K.K. Pillai and K.K. Grover, Bull. World Health Organ., **41,** 915 (1969).
8. I.H. Herskowitz, Genetics, **40,** 574 (1955).
9. I.H. Herskowitz, *ibid.*, **41,** 605 (1956).
10. L.A. Snyder, Z. Verebunqs Lehre, **94,** 182 (1963).
11. W.E. Ratnayake, Mutat. Res., **5,** 271 (1968).
12. M.M. Crystal, Ann. Entomol. Soc. Am., **63,** 71 (1970).
13. D.T. North, Mutat. Res., **4,** 225 (1967).
14. M.M. Malik and D.J. Galley, Pakistan J. Sci. Ind. Res., **15,** 386 (1972).
15. W. Kaplan, *Proc. 9th Intern. Conf. Genet. Caryol.*, **6,** 693 (1954).
16. D.W. Meifert, P.B. Morgan and G.C. LaBrecque, J. Econ. Entomol., **60,** 1336 (1967).
17. M.W. McFadden, *ibid.*, **62,** 511 (1969).
18. C. Sacca and E. Stella, Riv. Parasitol., **25,** 279 (1964).
19. M.M. Malik, Ph.D. thesis submitted to the University of London (1970).
20. H.C. Cox, J.R. Young and M.C. Bowman, *ibid.*, **60,** 1111 (1967).
21. P.A. Hedin, G. Wiygul and N. Mitlin, *ibid.*, **60,** 215 (1967).
22. J.C. Maitlen and L.M. McDonough, J. Econ. Entomol., **60,** 1391 (1967).
23. S.C. Chang and A.B. Borkovec, J. Econ. Entomol., **59,** 102 (1966).