

behavioral experiments with the tench the nervous excitation elicited by the alarm substance<sup>6-9</sup> was explored. The goal was to discover by which bundle this excitation was conducted from the bulbus olfactorius to the telencephalon. **Material and methods.** The tench, *Tinca tinca* L., studied were approximately 30 cm in total length. Each individual lived in a 60-l tank. The water was changed twice a week. In total we operated on 29 fish under urethane narcosis. A dentist's drill and scalpel were used while the fish were artificially respired. The wounds were closed with a tissue adhesive, Histoacryl®. We always performed the corresponding operation on the right and left tractus olfactorius. To prevent regeneration, which would otherwise be fast, we not only cut through each nervous bundle but in every case cut out a 5-mm piece. The following bundles were operated upon bilaterally: a) the lateral bundle alone (8 fish), b) both the lateral and central bundles (6 fish), c) the central bundle alone (4 fish), d) both the central and medial bundles (4 fish), e) the medial bundle alone (7 fish). In addition, 8 fish with intact tractus olfactorii served as controls. All operated fish fed normally a few days after the operation.

The behavioral experiments were performed approximately 3 weeks after the operation. In these experiments skin extract containing the alarm substance from a conspecific was poured into the tank. The behavior of the tench was then observed. The technically difficult micro-operations were first carried out by U. Mangold-Wernado in 1980 and they were continued by P. Neustetter. All behavioral results or reactions by the fish to the alarm substance were evaluated by the senior author without prior knowledge of the kind of operation performed.

**Results.** Out of the 8 control fish with intact tractus olfactorii, 7 responded to the alarm substance with a fright reaction. Of the 8 tench in which only the lateral bundle was separated, none reacted. The same was true for the 6 fish in which both the lateral and central bundles were operated upon. Of the 4 specimens in which only the central bundle was impaired, 2 responded. Of the 4 individuals in which both the central and medial bundles were cut, 1 responded. Of the 7 fish in which only the medial bundle was cut, 5 demonstrated a fright reaction. To summarize; out of the 14 fish, in which either the lateral or the lateral and central bundles were cut, we found that none responded

to the alarm substance. However, 8 out of 15 individuals with intact lateral bundles showed a fright reaction, although either the medial or the central or both bundles had been operated upon. The difference between the operated individuals with intact lateral bundles and those with impaired lateral bundles is significant. The fright reaction of the fish may indicate that the nervous excitation elicited by the alarm substance is conducted exclusively by the lateral nervous bundles of the tractus olfactorii from the bulbi olfactorii to the telencephalon.

**Discussion.** Since the tractus olfactorius in other species of Cyprinidae has not been studied histologically and electrophysiologically as thoroughly as that in the tench, it cannot be decided at this time whether our observation is specific for *Tinca tinca* or also valid for the other Ostariophysi. The operation on single nerve bundles in specimens of certain small species such as the European minnow *Phoxinus phoxinus* (L.) would be technically very difficult if not impossible. However, since the alarm substance is identical in the Ostariophysi studied it seems also probable that the organs of perception are accordingly similar, though minor differences might occur. Further work must be performed with a number of other species. The fishes should be large enough to ensure successful operations upon single nerve bundles, and their tractus olfactorii should be studied histologically and electrophysiologically in advance, as they have been in the tench.

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## Mating competitiveness and fertility of thiotepa-sterilized flies

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**Summary.** In the present investigation, an attempt has been made to study the mating competitiveness and fertility of males as well as females of *Dacus dorsalis* Hendel (Diptera: Tephritidae) treated topically with thiotepa. In the mixed population, treated flies of either sex were found to be sexually more vigorous than untreated flies. However when the mating competitiveness of either sex was determined separately using various methods, treated males were found not to differ significantly in sexual competitiveness from untreated flies. Receptivity of treated and untreated females to males was also studied by the single choice method. Thiotepa-treated and untreated females were found to be equally receptive to males.

One important consideration, while evaluating sterile male release technique, is to make sure that there are no adverse effects on sexual behaviour of insects treated with chemicals. The chemosterilized insects should not lose their ability to find their mates and the competence to mate successfully with untreated insects in the field. LaChance and Leverich<sup>3</sup>

considered the induction of aspermia, inactivation of sperms or gametic aberrations in the sperm as sterilization effects. However, in *Dacus oleae* (Hendel) only aberrations in gametic material were considered as sterilization effects<sup>4</sup>. It was suggested by laBrecque et al.<sup>5</sup> that chemically sterilized males were more vigorous sexually than

normal ones. Studies on the sheep blow-fly, the Mexican fruit fly, the screwworm fly and a wasp (*Habrobracon*) also showed that chemosterilized males were more vigorous sexually than normal ones, a phenomenon known as "hypercompetitiveness"<sup>6</sup>. Hypercompetitive behavior of chemosterilized *Anopheles stephensi* was also observed by Sharma et al.<sup>7</sup> after thiotepa treatment. Tsiropoulos and Tzanakakis<sup>8</sup> reported no major differences in mating frequency in artificially reared *Dacus oleae* males sterilized at the late pupal stage with 8krad of  $\gamma$ -radiation. Fytizas<sup>4</sup> reported that competitiveness between tepa-sterilized and normal *Dacus oleae* males was not affected either at the level of individuals or of spermatozoa. But Economopoulos and Gordon<sup>9</sup> sterilized the males of *Onchopeltus fasciatus* with tretamine and noted that sterilized males were only half as active as normal ones. In the insect species with polygamous females, the presence and mating of sterile and fertile males with females may significantly affect the importance of the first sperm supplement. *Dacus dorsalis*, a serious pest of most of the fruits and vegetables throughout India, was taken as the experimental insect in the present study.

**Materials and methods.** Laboratory reared 0-24-h old adults of both sexes were immobilized by keeping them in the freezer for 3-5 min. The chilled flies were easy to handle and mortality was negligible. They were treated topically with 1  $\mu$ l solution of thiotepa in acetone of different concentrations with the help of a calibrated syringe controlled by a micrometer. The concentrations tested were 0.1, 0.5, 2, 3, and 5%, but flies treated with 0.5% thiotepa were used in the present study because it proved to be an optimal dose for complete sterility in either sex. The control flies were treated with an equal amount of acetone alone after chilling. Different methods were used for assessing mating competitiveness of males and females. The use of a single method only is not advised since in cases of multiple matings the results might be misleading. The methods used were as follows: a) Sterilized flies of either sex were caged in plastic jars with an untreated pair in different ratios to examine their sexual competitiveness. Ratios studied were 0:0:1:1, 1:1:0:0, 1:1:1:1, 2:2:1:1,

3:3:1:1 and 9:9:1:1 of T $\delta$ :T $\phi$ :U $\delta$ :U $\phi$ , where T and U represent treated and untreated flies. To avoid overcrowding, plastic containers of different capacities were used according to the density of flies. Egg laying and hatching were scored in each combination for a period of 60 days. 5 replicates of each combination were studied. The competitiveness of sterilized flies was determined by applying the procedure devised by Ahmed et al.<sup>10</sup>, which is as follows:

$$CV = \frac{EO}{EU \times PU + Ei \times Pi}$$

where CV = competitiveness value of treated flies,  
where EO = observed egg infertility in test,  
where EU = egg infertility in control (U $\delta$   $\times$  U $\phi$ ),  
where Ei = egg infertility of treated males (U $\phi$   $\times$  T $\delta$ ),  
where PU = the proportion of untreated males in test,  
where Pi = the proportion of treated males in test.

When CV is near one, it indicates the sterilized insects are fully competitive. The extent to which CV exceeds or falls short of one is a measure of increased and decreased competitiveness. The results are summarized in table 1.

b) Mating competitiveness of thiotepa-treated males was evaluated by caging 20 treated males with 20 untreated males of the same age group (10-13 days old). After 24 h, 20 virgin females (10-13 days old) were introduced in each cage. They were allowed to copulate and eggs were collected; the percent sterility in each cage was scored.

5 replicates were studied for this experiment. Nonhatchability of eggs was used as a criterion for calculating sterility. The competitiveness of treated males was determined by calculating the quotient of the percentage of expected egg hatch divided by the percentage of observed egg hatch to obtain the competitive index (CI). When CI is > 0.5, it indicates that treated males are as sexually competitive as untreated males. The results obtained are summarized in table 2. This method was used by Crystal<sup>11</sup> to determine the mating competitiveness of treated males of *Cochliomyia hominivorax* (Coquerel).

c) Mating competitiveness of males using this method was noted by visual observation when the insects were in copula rather than by egg hatching. This method was applied by Kumuda and Naidu<sup>12</sup> while studying the sexual behavior of tepa-treated *Dysdercus cingulatus*. In this method a mixed population of males was introduced into a cage containing virgin females (10-13 days old). The ratio of treated males, untreated males and untreated females was maintained at 10:10:10. 5 experiments were carried out and each experiment was triplicated. Nail polish was used as a marker, and treated and untreated flies were marked in alternate experiments to avoid any effect of the marker on the mating competitiveness. The significance of the results obtained was tested by analysis of variance and summarized in table 3.

Table 1. Mating competitiveness and fertility of thiotepa-treated (5  $\mu$ g/fly) flies of *Dacus dorsalis* mixed with a pair of untreated flies in different ratios

Ratio of population		Percent egg hatch	Percent egg sterility		Competitiveness value
Treated	Untreated		Expected	Observed	
$\delta$ $\phi$	$\delta$ $\phi$				
0:0	1:1	84.50	—	15.50	—
1:1	0:0	00.00	—	100.00	—
1:1	1:1	38.24	57.75	61.76	1.07
2:2	1:1	25.70	71.83	74.30	1.03
3:3	1:1	26.30	78.87	73.70	0.93
9:9	1:1	00.00	91.55	100.00	1.09

Table 2. Influence of thiotepa treatment on the sexual competitiveness of *Dacus dorsalis* males in a mixed population of 20 untreated males, 20 treated males and 20 untreated females

Replicate number	Percent egg hatch		Competitive index (CI)
	Expected*	Observed	
1	42.25	52.21	0.80
2	42.25	55.39	0.76
3	42.25	65.14	0.64
4	42.25	69.80	0.60
5	42.25	76.61	0.55

\* Expected percent egg hatch was calculated according to Fried<sup>23</sup>.

Table 3. Mating competitiveness of thiotepa-treated males (5  $\mu$ g/fly) of *Dacus dorsalis*

Test number	Number of copulations of 20 males	
	Treated	Untreated
1	2	11
2	8	7
3	9	7
4	8	6
5	12	9
Total	39	40

Analysis of variance at 5% level of significance showed that the difference between untreated and treated values is not significant.

d) Here a treated male has to compete with an untreated male for a female mate; this is known as the single choice method, and it was devised by Smart and Gilmour<sup>13</sup>. For the experiment a treated and an untreated male of the same age were confined with a single mature female in a plastic container. One male in each container was marked with a spot of nail polish on the thorax. 25 trials were made at a time, and we recorded the number of trials in which the male was successful in copulating with the untreated female. The experiment was replicated 5 times on different dates. The results were recorded in table 4 and an analysis of variance was calculated to test for the significance of the differences.

e) In this experiment the attractiveness of thiotepa-treated females to males was investigated by the single choice method devised by Smart and Gilmour<sup>13</sup>. For this experiment a treated and an untreated female of the same age were kept with a single mature, untreated male in a plastic container. One of the 2 females in each group was marked with nail polish on the dorsal side. Each time, 25 such groups or trials were studied in 25 different containers. Observations were made of which female was chosen by each male for mating. Results obtained were tested by analysis of variance and are recorded in table 5.

**Results.** The results of tests in which both sexes of *Dacus dorsalis* were treated with thiotepa (5 µg/fly) and mixed with paired untreated flies at various ratios, are presented in table 1. At these ratios, the percentage of eggs hatching decreased from 84.5 (0:0:1:1) to 0% (9:9:1:1). The decreasing trend in egg hatching showed that an increasing number of treated pairs in the population enhanced the sterility effect. Competitiveness values indicated that sterilized flies were more competitive as compared to untreated flies. At a 9:9:1:1 ratio treated flies were found to be highly competitive as evidenced by the fact that no eggs hatched. This showed that at this combination a complete suppression of the fly population is possible.

The results of tests with treated males are reported in tables 2-4. Table 2 shows the competitive index (CI) of treated males, while tables 3 and 4 show the number of copulations

of treated and untreated males with untreated females. The values of CI remained above 0.5, indicating that treated males are sexually as competitive as untreated males. No significant differences were observed in the number of copulations made by treated and untreated males with untreated females, which fact indicated equal mating competitiveness of treated and untreated males. The minor bias in favor of treated males (table 4) was not statistically significant.

The results of receptivity of females to males in which 1 male was confined with 2 females (one untreated and another treated) are shown in table 5. No significant differences were observed between values for untreated and treated females.

From this study it can be concluded that 1 µl of a 0.5% solution of thiotepa per fly sterilized flies of either sex. The sexual vigor of the treated flies was equal to or greater than that of untreated flies.

**Discussion.** The increased mating ability of thiotepa-sterilized males of *Dacus dorsalis* confirmed the results of various researchers. Hypercompetitiveness has already been reported in male insects of 4 dipteran species: *Musca domestica*<sup>5,14</sup>, *Phaenicia sericata* (Meigen)<sup>15</sup>, *Cochliomyia hominivorax* (Coquerel)<sup>16</sup> and *Aedes aegypti* (L.)<sup>17</sup>. Crystal<sup>18</sup> also observed a chemosterilant-induced increase in the mating ability of male screwworm flies. Our results showing the hypercompetitiveness of treated flies are in accordance with the results reported by Ahmed et al.<sup>10</sup>, who studied the competitiveness of irradiated Indian meal moths. LaBrecque et al.<sup>5</sup>, suggested that chemosterilized male houseflies might have supernormal sexual vigor or that second matings with treated males might tend to nullify the effects of prior matings by untreated males, which was substantiated by the findings of Crystal<sup>18</sup>. Ascher and Avdat<sup>14</sup> suggested that some of the matings with chemosterilized male houseflies might have happened with oligospermic or aspermic individuals. But the actual mode of action by which a chemosterilant causes an increase in mating ability is unknown, perhaps it might be due to hormonal mechanisms that enhance sexual attraction in chemosterilized insects.

Our present findings that thiotepa-treated males are as competitive as untreated ones, are in agreement with the findings of Mohammad et al.<sup>19</sup>, Smart and Gilmour<sup>13</sup> and Haniotakis<sup>20</sup>. In contrast, males sterilized with tretamine and uredepa failed to compete with untreated males<sup>21,22</sup>.

The results of the equal attractiveness to males of thiotepa-treated and untreated females in single choice experiments are in agreement with the results reported by Smart and Gilmour<sup>13</sup> in *Lucilia cuprina* treated topically with N, N-tetramethylene bis (1-aziridine carboxamide). To conclude, sexual competitiveness of *D. dorsalis*, whether measured by mating activity or egg hatching is not affected by thiotepa sterilization. From this experiment it was concluded that both sexes of sterilized flies can be released in the field. Released females cannot produce any hazardous effects as they are already sterile, and they will dilute the effectiveness of normal males present in the natural population, which would mate otherwise with normal females.

Table 4. Sexual competitiveness of thiotepa-treated males (5 µg/fly) of *Dacus dorsalis*. In each trial one female was confined with a treated and an untreated male, 25 trials/test

Test number	Number of copulations/test Treated ♂	Untreated ♂
1	4	0
2	2	1
3	7	6
4	9	7
5	5	2
Total	27	16

According to analysis of variance at 5% level of significance the differences among treated and untreated values are not significant.

Table 5. Attractiveness of thiotepa-treated (5 µg/fly) and untreated females to males, 25 trials/test

Test number	Number of copulations/test Treated ♀	Untreated ♀
1	4	11
2	11	3
3	9	2
4	5	7
Total	29	23

Analysis of variance at 5% level of significance showed that differences among values for treated and untreated flies are not significant.

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## Pineal - related changes in cyclic AMP levels of rat medial basal hypothalamus<sup>1</sup>

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**Summary.** Pinealectomy (Px) in adult male rats resulted in increased cyclic AMP accumulation by medial basal hypothalamic (MBH) explants 3 and 7 days after surgery. 24 h after superior cervical ganglionectomy (Gx) an augmented MBH cyclic AMP accumulation was observed. The effects of Px and Gx were additive, as revealed in animals subjected to Gx 3 days after Px.

It is now accepted that the mammalian pineal gland is an endocrine organ that produces hormones with activity on the neuroendocrine system<sup>3</sup>. One of these hormones, melatonin, is secreted as a function of time of day in all vertebrates studied as yet, and acts on the brain to influence adenohypophyseal function. Receptors for melatonin are present in medial basal hypothalamic (MBH) membranes and several hypothalamic metabolic functions change after melatonin treatment, including protein synthesis, serotonin and  $\gamma$ -aminobutyric content, neurotransmitter uptake and neurohormone release (for references, see Cardinali<sup>4</sup>). Some of the actions of melatonin on MBH probably involve effects on cyclic nucleotide levels; melatonin decreases in physiological concentrations, cyclic AMP accumulation of rat MBH in vitro<sup>5</sup>. The present study aimed to examine cyclic AMP accumulation in MBH explants obtained from rats subjected to pinealectomy (Px) or

superior cervical ganglionectomy (Gx). The latter procedure removes almost all functionally relevant pineal innervation, and interrupts the peripheral sympathetic innervation to the CNS.

**Material and methods.** Adult male Wistar rats were kept under light between 07.00 h and 21.00 h daily and were given access to Purina chow and water ad libitum. Px, Gx or sham-operation were performed under light ether anesthesia as described before<sup>6</sup>. The rats were killed 3 or 7 days after Px; Gx was performed 24 h before sacrifice. The day before Gx the animals were kept for 24 h under light; this procedure is known to induce postsynaptic supersensitivity of  $\beta$ -adrenoceptors in one of the superior cervical ganglia territories at least, i.e. the pineal gland<sup>7</sup>. The rats were killed by decapitation and the brains were quickly removed. Individual MBH (about 4 mg wet weight) were incubated at 37 °C in 0.2 ml Krebs Ringer bicarbonate buffer, pH 7.4, containing glucose (1 mg/ml) and 1 mM theophylline, as described elsewhere<sup>5</sup>. Cyclic AMP was extracted by homogenization of tissue in 5% trichloroacetic

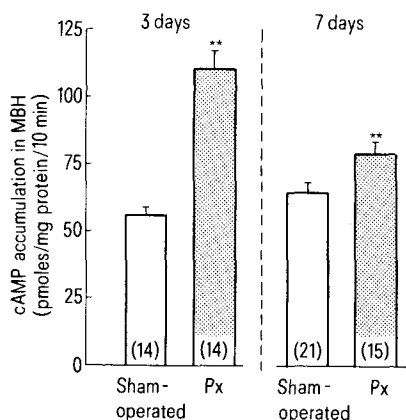


Figure 1. Effect of pinealectomy (Px) performed 3 or 7 days before sacrifice on cyclic AMP accumulation by rat medial basal hypothalamic (MBH) explants. Shown are the means  $\pm$  SEM (n). \*\*  $p < 0.01$ , Student's t-test.

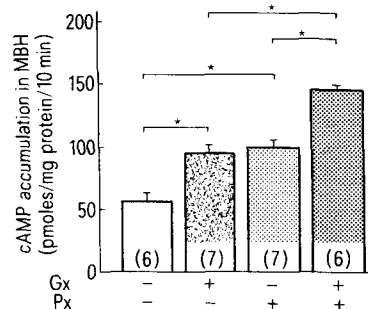


Figure 2. Effect of pinealectomy (Px) and/or superior cervical ganglionectomy (Gx) on cAMP accumulation by rat medial basal hypothalamic (MBH) explants. Gx was performed 24 h before sacrifice while Px was performed 3 days before sacrifice. Shown are the means  $\pm$  SEM (n). \*  $p < 0.05$ , analysis of variance, Scheffé's test.