原　著

Development of Sterility Through Pupal Treatments in *Musca domestica nebulo* Fabr.
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18. イエバエ *Musca domestica nebulo* Fabr. 蛹に施用した不妊剤の効力発現

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いろいろな発育時期のイエバエ *Musca domestica nebulo* の蛹を apholate, tepa, metepa, hempa, hemel のアルコール溶液に浸漬し，その蛹から羽化した成虫の不妊性を調べた．不妊効力は不妊剤の種類・濃度によって異なった．すなわち，4% tepa で処理された蛹から羽化した成虫は，産卵が行なわれなかった．Apholate および metepa 4%液での処理では完全に不妊となったが，hempa および hemel 4%液処理では，濃度，蛹の発育時期に拘らず，不妊効力は弱かった．イエバエの蛹を不妊剤で処理するには，aziridine 剤が alkylating 剤よりもすぐれている．

The reduction of a natural population of an insect through the release of sterile males in the field largely depends on laboratory colonization of the species, sterilization techniques and releasing procedure. Unlike the larvae and adults, pupae seldom move about and can be readily dipped in solutions of chemosterilants. Chamberlain (1962) succeeded in inducing a complete sterility in *Cochiliomyia hominivorax* by dipping the pupae in solutions of apholate while Bushland and Hopkins (1953) found that the screw-worm fly was most sensitive to sterilization by gamma radiation when treatments were made in the pupal stage. Shaw and Riviello (1965) also observed 100.0 percent sterility in the Mexican fruit fly, *Anastrepha luderis* when the pupae were dipped for 60 seconds in 5.0 percent solution of tepa in ethanol.

Sterility could also be induced in houseflies when four day old pupae were immersed for 30 minutes in equal parts of acetone and water saturated with a known chemosterilant (2, 2'-dichloro-N-methyldiethyl amine) (Piquett and Keller, 1962). Gouck (1964) reported sterility in *M. d. domestica* on dipping the pupae in ethanol solutions of apholate, tepa and metepa at concentrations of 2.5 to 5.0 percent for 30 to 300 seconds. Similar results were obtained by Labrecque *et al.* (1966) and Combiesco and Enesco (1968) with hempa and thiotepa when the pupae were dipped in solutions of these chemicals.

The above findings led the author to investigate if *M. d. nebulo* could be sterilized by dipping the pupae of different ages in ethanol solutions of apholate, tepa, metepa, hempa and hemel.

Materials and Methods

One to three day old pupae were sorted out from the rearing jars and dipped in the desired solution of the chemosterilant for 30, 60, 120 and 240 seconds. In each test 100 pupae were used.

The pupae were kept in an aluminium spoon and held in place by a piece of screen wire bent over the bowl of the spoon and clamped to the handle. The spoon containing the pupae was submerged in the sterilant solution and the pupae thus treated were placed on a blotting paper for ten to fifteen minutes before being transferred to a clean petri dish. The beaker, spoon and the screen were cleaned with acetone and washed with water after each dipping. On emergence the adults were released in cloth cages and were fed on regular fly food. Each test was continued for twenty days and hatching of the eggs was determined by collecting random sample of 100 eggs on black moist cloth. After twenty four hours the eggs were examined and percent sterility and net sterility was calculated. The empty pupae were counted after ten days and the number of flies emerged was determined.

Results

The results (Tables 1-5) clearly show that

M. d. nebulo can be sterilized by dipping the pupae in ethanol solutions of apholate, tepa, metepa, hempa and hemel. The degree of sterility greatly varied with each chemosterilant and of the five chemicals tested, tepa induced a much higher degree of sterility at all concentrations tested. Oviposition was completely inhibited when pupae of various age groups were

Table 1. Percentage emergence and sterility obtained by dipping 1 to 3 day old pupae in ethanol solutions of apholate.

Age of pupae (Days)	Concentration (%)	Time in seconds 30		60		120		240	
		% flies emerged	% net sterility	% flies emerged	% net sterility	% flies emerged	% net sterility	% flies emerged	% net sterility
1	0.5	52.0	64.5	45.0	87.4	43.0	88.5	36.0	80.9
	1.0	54.0	85.1	49.0	96.4	38.0	97.4	32.0	98.7
	2.0	51.0	92.9	40.0	96.7	41.0	99.4	39.0	99.7
	4.0	48.0	100.0	37.0	100.0	32.0	100.0	34.0	100.0
	Control	76.0	22.9	69.0	20.2	64.0	20.5	58.0	14.5
2	0.5	58.0	67.9	52.0	81.5	49.0	80.6	37.0	88.1
	1.0	54.0	89.2	46.0	91.3	40.0	91.5	34.0	95.6
	2.0	61.0	94.5	55.0	98.3	51.0	98.6	45.0	99.1
	4.0	54.0	97.2	46.0	98.9	39.0	100.0	36.0	100.0
	Control	80.0	22.9	75.0	20.2	63.0	20.5	60.0	14.5
3	0.5	68.0	60.3	59.0	78.9	47.0	83.8	45.0	86.1
	1.0	70.0	86.1	53.0	98.8	44.0	90.4	30.0	93.4
	2.0	65.0	92.2	48.0	97.6	46.0	100.0	40.0	100.0
	4.0	64.0	95.07	70.0	99.3	42.0	100.0	39.0	100.0
	Control	78.0	22.9	75.0	20.2	68.0	20.5	61.0	14.5

Table 2. Percentage emergence and sterility obtained by dipping 1 to 3 day old pupae in ethanol solutions of tepa.

Age of pupae (Days)	Concentration (%)	Time in seconds 30		60		120		240	
		% flies emerged	% net sterility	% flies emerged	% net sterility	% flies emerged	% net sterility	% flies emerged	% net sterility
1	0.25	61.0	72.6	56.0	82.08	53.0	86.9	48.0	88.4
	0.5	59.0	85.6	53.0	91.6	49.0	93.3	32.0	97.8
	1.0	58.0	97.01	55.0	98.1	52.0	100.0	43.0	100.0
	2.0	51.0	100.0	45.0	100.0	43.0	**	32.0	**
	4.0	49.0	**	42.0	**	37.0	**	35.0	**
	Control	76.0	22.9	69.0	20.2	64.0	20.5	58.0	14.5
2	0.25	59.0	68.1	58.0	76.4	51.0	84.2	47.0	88.3
	0.5	58.0	79.8	54.0	91.6	39.0	94.08	36.0	97.4
	1.0	60.0	93.7	51.0	97.4	43.0	99.7	36.0	100.0
	2.0	59.0	100.0	53.0	100.0	45.0	**	41.0	**
	4.0	51.0	**	48.0	**	41.0	**	38.0	**
	Ccntrol	80.0	22.9	75.0	20.2	63.0	20.5	60.0	14.5
3	0.25	63.0	64.4	59.0	75.4	52.0	76.2	46.0	87.6
	0.5	59.0	87.02	55.0	92.3	51.0	93.4	48.0	95.2
	1.0	57.0	96.3	52.0	98.3	48.0	99.3	37.0	100.0
	2.0	56.0	100.0	49.0	100.0	53.0	100.0	45.0	**
	4.0	50.0	**	39.0	**	40.0	**	34.0	**
	Control	78.0	22.9	75.0	20.2	68.0	20.5	61.0	14.5

** The females did not oviposit.

Table 3. Percentage emergence and sterility obtained by dipping 1 to 3 day old pupae in ethanol solutions of metepa.

Age of pupae (Days)	Concentration (%)	Time in seconds 30		60		120		240	
		% flies emerged	% net sterility	% flies emerged	% net sterility	% flies emerged	% net sterility	% flies emerged	% net sterility
1	0.5	62.0	61.9	55.0	71.3	41.0	82.6	57.0	86.1
	1.0	59.0	81.5	51.0	91.8	47.0	44.08	44.0	97.4
	2.0	60.0	89.2	56.0	92.9	53.0	94.2	51.0	98.7
	4.0	61.0	93.7	56.0	98.1	52.0	100.0	37.0	100.0
	Control	76.0	22.9	69.0	20.2	64.0	20.5	58.0	14.5
2	0.5	67.0	58.4	58.0	69.04	52.0	75.9	48.0	83.1
	1.0	65.0	72.4	56.0	91.1	55.0	91.4	46.0	94.9
	2.0	58.0	83.4	55.0	92.3	51.0	92.9	49.0	97.07
	4.0	52.0	84.5	46.0	86.9	48.0	94.3	41.0	97.9
	Control	80.0	22.9	75.0	20.2	63.0	20.5	60.0	14.5
3	0.5	63.0	47.2	58.0	61.7	60.0	77.1	54.0	79.5
	1.0	70.0	71.3	59.0	79.8	58.0	92.3	56.0	95.2
	2.0	60.0	87.6	58.0	89.4	48.0	94.8	41.0	98.7
	4.0	56.0	83.3	51.0	93.4	45.0	95.8	37.0	95.2
	Control	78.0	22.9	75.0	20.2	68.0	20.5	61.0	14.5

Table 4. Percentage emergence and sterility obtained by dipping 1 to 3 day old pupae in ethanol solutions of hempa.

Age of pupae (Days)	Concentration (%)	Time in seconds 30		60		120		240	
		% flies emerged	% net sterility	% flies emerged	% net sterility	% flies emerged	% net sterility	% flies emerged	% net sterility
1	0.5	71.0	1.03	67.0	5.01	67.0	13.8	60.0	20.3
	1.0	55.0	12.06	56.0	17.9	44.0	27.04	48.0	35.9
	2.0	69.0	11.9	66.0	19.2	60.0	27.4	58.0	32.5
	4.0	63.0	29.9	56.0	35.2	40.0	41.7	36.0	53.4
	8.0	52.0	35.9	36.0	43.3	41.0	67.1	32.0	74.03
	16.0	51.0	68.6	49.0	74.4	36.0	78.3	39.0	91.1
	Control	76.0	22.9	69.0	20.2	64.0	20.5	58.0	14.5
2	0.5	69.0	—	68.0	2.7	54.0	6.6	42.0	13.8
	1.0	68.0	7.1	67.0	17.1	56.0	21.6	50.0	27.6
	2.0	76.0	14.7	64.0	23.4	53.0	30.6	44.0	40.2
	4.0	67.0	26.3	61.0	35.08	52.0	45.7	40.0	61.8
	8.0	57.0	53.04	39.0	56.7	35.0	75.5	41.0	84.3
	16.0	61.0	65.3	50.0	69.6	43.0	79.7	35.0	89.05
	Control	80.0	22.9	75.0	20.2	63.0	20.5	60.0	14.5
3	0.5	63.0	1.8	66.0	9.4	58.0	8.5	51.0	13.2
	1.0	69.0	3.1	63.0	4.2	55.0	6.3	52.0	21.9
	2.0	67.0	6.2	56.0	25.8	51.0	26.9	46.0	36.4
	4.0	65.0	28.2	61.0	37.2	39.0	49.3	38.0	63.4
	8.0	60.0	56.4	52.0	65.1	49.0	60.0	43.0	77.5
	16.0	56.0	62.8	41.0	71.4	42.0	78.1	37.0	85.8
	Control	78.0	22.9	75.0	20.2	68.0	20.5	61.0	14.5

Table 5. Percentage emergence and sterility obtained by dipping 1 to 3 day old pupae in ethanol solutions of hemel.

Age of pupae (Days)	Concentration (%)	Time in seconds 30		60		120		240	
		% flies emerged	% net sterility	% flies emerged	% net sterility	% flies emerged	% net sterility	% flies emerged	% net sterility
1	1.0	68.0	—	65.0	8.4	63.0	—	59.0	2.2
	2.0	76.0	3.8	68.0	13.2	69.0	18.3	65.0	24.6
	4.0	68.0	17.1	63.0	28.3	58.0	37.6	54.0	50.9
	Control	76.0	22.9	69.0	20.2	64.0	20.5	58.0	14.5
2	1.0	75.0	3.5	70.0	4.6	73.0	9.8	64.0	8.8
	2.0	72.0	2.7	68.0	9.6	65.0	9.4	58.0	14.7
	4.0	70.0	31.4	62.0	34.3	60.0	38.1	56.0	46.7
	Control	80.0	22.9	75.0	20.2	63.0	20.5	60.0	14.5
3	1.0	74.0	3.5	68.0	8.1	72.0	5.5	69.0	5.7
	2.0	73.0	2.2	62.0	7.2	72.0	10.8	60.0	16.2
	4.0	68.0	10.4	64.0	25.4	61.0	36.6	52.0	40.2
	Control	78.0	22.9	75.0	20.2	68.0	20.5	61.0	14.5

dipped in 4.0 percent solution of tepa for 30, 60, 120 and 240 seconds. Apholate also produced 100.0 percent sterility in flies emerging from puparia that had been dipped in 4.0 percent solution of this chemical for four minutes. Similarly metepa induced 100.0, 97.9 and 95.2 percent sterility in flies that emerged from one to three day old pupae dipped in 4.0 percent solution of the chemical for four minutes. On the other hand hempa failed to produce 100.0 percent steriliry in flies even at a concentration of 16.0 percent and the sterility induced by hemel was also very low. Higher concentrations of hemel could not be tested as it was not possible to dissolve the required quantities of the chemical in ethanol. Taking net sterility as a criterion aziridine compounds were much more effective than the non alkylating agents. 92.9, 100.0 and 89.2 percent sterility was obtained when one day old pupae were dipped for 30 seconds in 2.0 percent solution of apholate, tepa and metepa respectively as against 11.9 and 0.0 percent sterility when they were dipped in solutions of hempa and hemel at the same concentration and for the same period. The age of the pupae did not affect the development of sterility by any of the chemosterilant tested. At all ages, concentrations, and periods, tepa produced highest sterility and this is not far from the findings of Grover *et al.* (1967) who also found tepa to be more effective than apholate or metepa in pupal treatments of *Culex fatigans*.

There was a marked reduction in the rate of eclosion where the dipping period was extended for four minutes. This effect may be due to the solvent itself as pupae dipped in ethanol also showed a much lower emergence. That the age did not affect the rate of eclosion is in conformity with the earlier findings of Labrecque *et al.* (1966) who found that pupal age did not affect the emergence of flies in case of *M. d. domestica*.

The present method for inducing sterility may seem to be very convenient because of the case with which the pupae can be dipped in solutions of chemosterilants. It is not as economical as the feeding method. The susceptibility of the pupae is very low and hence large quantities of the chemical would be required if pupal treatments are adapted for large scale control operations.

Summary

The potentlalities of alkylating and non alkylating compounds were determined by dipping the pupae of various ages in ethanol solutions of chemosterilants for different periods of time. It was found that they were capable of producing sterility in flies that emerged from the pupae that had been dipped in ethanol solutions of apholate, tepa, metepa, hempa and hemel.

However, the degree of sterility was variable with each chemosterilant and the concentration tested. Oviposition was totally inhibited in flies emerged from pupae of various ages dipped in 4.0 percent solution of tepa. Complete sterility was also observed with apholate and metepa at concentration of 4.0 percent of the chemicals while hempa and hemel could not produce high degree of sterility at any concentration and period suggesting that aziridine compounds hold superiority over non alkylating agents in pupal treatments.

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Permanency of Sterility Effects of Chemosterilants in *Musca domestica nebulo* Fabr.

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19. 不妊剤施用イエバエ *Musca domestica nebulo* Fabr. における不妊効力の持続性

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Apholate, tepa, metepa, hempa および hemel をイエバエ *Musca domestica nebulo* の雌雄に施用し，それぞれの不妊効力の持続性を調べた．雄にこれらの不妊剤を施用して，つぎつぎと正常な雌4匹と交尾させ，産卵数，ふ化率を調べた結果，いずれもふ化幼虫数が極めて少なく，不妊効力が持続することがわかった．一方，雌に施用した場合，施用不妊剤の濃度が高いと，その雌から産れた卵のふ化率は最初から終りまで低く，不妊効力の持続を示したが，施用量が少ないと，後から産れる卵のふ化率が高くなり，不妊効力が持続しないことがわかった．

The choice of a chemosterilant would greatly depend on its low toxicity and the permanence of sterility. Knipling (1964) has stressed the necessity for the permanence of sterility in practical application of sterile male release technique but the results obtained by other workers show that this effect is variable from species to species and the chemosterilant used. Morgan and Labrecque (1962, 1964) observed a degeneration in oocytes in the ovarian chambers of chemosterilized houseflies and Weidhaas *et al.* (1961) obtained a much higher sterility in *Aedes aegypti* when the females were fed on a diet treated with alkylating compounds. Dame and Ford (1964) conducted experiments to determine the permanency of sterility effects produced by apholate and tepa in *Aedes aegypti* and reported that males treated with apholate recovered after four series of mating but those treated with tepa retained a high degree of sterility during successive matings.

Kilgore and Painter (1962) reported that recovery of fertility occured when the flies were fed on a diet containing 5-fluorouracil for 36 to 48 hours after emergence. Similar results were obtained by Sacca *et al.* (1964) with tepa in case of *M. d. domestica*. Painter and Kilgore (1964) tested fifteen comounds against *M. d. domestica* and found that only apholate and thiotepa induced permanent sterility and none of the eggs deposited were viable. Lachance *et al.* (1969) reported that the minimum dose of apholate, tepa, metepa and hempa which produced dominant lethal mutation in the sperms also caused 100.0 percent mortality of the gonial cells and no sign of any spermatogenetic activity could be observed in