CHEMOSTERILIZATION OF OULEMA MELANOPA (L.) WITH APHOLATE AND TRIPHENYL TIN HYDROXIDE

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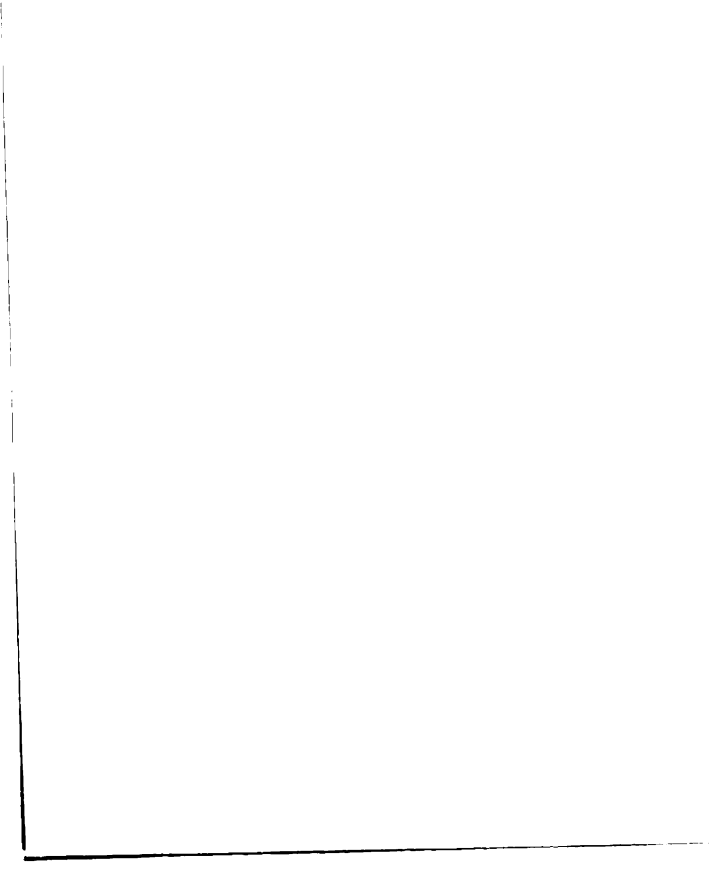

ABSTRACT

CHEMOSTERILIZATION OF OULEMA MELANOPA (L.)
WITH APHOLATE AND TRIPHENYL TIN HYDROXIDE

by M. Ikechuku Ezueh

Apholate (2,2,4,4,6,6, hexakis (1-aziridinyl) 2,2,4, 4,6,6, hexahydro 2,4,6,1,3,5, triphosphatriazarine) was an effective chemosterilant for the cereal leaf beetle, Oulema melanopa (L.). A 0.05% aqueous concentration, when given to both sexes, caused 100% sterility. When only males were treated a 0.1% solution was needed to effect complete sterility. Longevity of treated males was reduced at the sterilizing dosage, death occuring mostly 10 days after treatment. Treated males were found to reta in their normal mating behavior but early mortality seemed to reduce their mating competitiveness in comparison with the normal males. A ratio of treated males to untreated males of about 12:1 would be required to reduce egg viability to zero percent. The early mortality of the treated males would necessitate more frequent release of sterilized beetles.

Histological sections revealed no damage to the testis of males treated with 0.1% apholate. Motile sperms were found in treated males 21 days after treatment.

An exploratory study of the sterilizing properties of Dowco-186 (Triphenyl tin hydroxide) indicated that it

was most effective when both sexes were treated, and as long as the beetle fed on treated plants. Concentrations of 2.0% and 3.0% caused lethargy, manifested by inactivity and starvation. At lower concentrations, these effects were progressively less pronounced.

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By

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INTRODUCTION

Since its introduction into this country, the cereal leaf beetle, Oulema melanopa (L.) has spread over much of Michigan, some parts of Ohio, Indiana and Illinois. This growing infestation has stimulated an active search for an effective means of control. Continuous efforts have been made in the past through research and field operations to achieve some control by conventional methods. Complete reliance on the use of insecticides for control or eradication programs has been on the wane due to the possible development of insect resistance to insecticides and also due to the potential hazards to public health of accumulating chemical residues in the natural environment. Research efforts have therefore, been directed to exploring other strategies for insect control, which would preclude undesirable side-effects inherent in the widespread use of insecticides.

The dramatic eradication of the screw-worm fly, Cochliomyia hominivorax (Coq.) from the island of Curacao and later from Florida and other southeastern states by sustained release of radiosterilized males, revolutionized the entire approach to insect control (Bushland and Hopkins, 1951, 1953; Baumhover et al., 1955; Knipling, 1955). However, the deleterious effects of radiation on the longevity and general vigor of insects, coupled with the

problems of mass-rearing and dissemination, often render this method impractical when extended to other pests. It is essential that the sterilized males remain in all biological aspects fully competitive with the normal males (Chamberlain, 1962; Borkovec, 1964; Knipling, 1964). The discovery of radiomimetic chemicals which induced sterility without adversely affecting sexual behavior and life-span opened a new approach in the "sterile male" concept. Research entomologists are investigating the potentialities of chemosterilization in order to establish the theorized superiority of such chemicals over ionizing radiations and conventional insecticides.

The most currently used chemosterilants are aziridine alkylating agents. Apholate, (2,2,4,4,6,6, hexakis (1-aziridinyl) 2,2,4,4,6,6,hexahydro 2,4,6,1,3,5, triazatriphosphorine) was used in the present studies. Dowco 186 (Triphenyl tin hydroxide) represents a new class of chemosterilants that have been shown to produce sterility in the house fly, Musca domestica, the flour beetle Tribolium confusum Jacquelin du Val, and the German cockroach Blatella germanica (L.) (Kenaga, 1965). Preliminary studies of its effects on the reproduction of the cereal leaf beetle were also carried out.

The purpose of this work was to evaluate the possibility of controlling the cereal leaf beetle by use of chemosterilized males. The main objectives were:

1. To determine the optimum dosage of apholate needed to sterilize the males and its effects on the longevity and mating competitiveness of such sterilized males.

2. To evaluate some characteristics of Dowco 186 as a candidate chemosterilant for this pest.

LITERATURE REVIEW

The earliest recorded effects of radiation on insects was demonstrated on a coleopteran insect, the cigarette beetle, Lasioderma serricorne (F.) (Runner, 1916). Exposing these beetles to Roentgen rays, he caused them to lay large numbers of infertile eggs. Müller (1927, 1928, 1940) made a momentous discovery when he described the effects of X-rays on Drosophila melanogaster and used the term "dominant lethality" to account for the chromosome changes which led to the production of non-viable eggs. Hanson (1928) also reported the effects of X-rays on productivity and sex ratio in Drosophila melanogaster. Since then, extensive work on the effects of ionizing radiations has been done with a variety of insects. Not until the work of Bushland and Hopkins (1951, 1953) on the sterilization of the screw-worm flies was this ingenious discovery redeemed from the realm of scientific curiosity to the more practical. Knipling (1955) first proposed the idea of controlling insects by use of genetically deficient individuals of the same species. This principle was validated by the successful eradication of the screw-worm fly on the island of Curacao (Baumhover et al., 1955). The sterilization of insects by chemicals is a recent corollary to the radiation-sterilization technique. There are some classical references as to biological

effects of chemicals. Sternberg et al., (1958) cited the work of Gabriel and von Hirsch in 1896 as the first illustration of selective systemic damage by alkylating agents. In 1898, Paul Ehrlich recognized the extraordinary pharmocological properties of ethylene imine and ethylene oxide. However the idea that certain chemicals mimiced the biological effects of ionizing radiations developed from the war time research on vesicant poison gases (Alexander, 1960). Mustard gas (2,2'-dichloro-diethyl sulfide) was the first chemical found in these efforts to exhibit radiomimetic characteristics (Auerbach and Robson, 1943, Koller, 1947). Rapoport (1946) provided the final evidence along these lines when he described the mutagenic action of an alkylating agent, diethyl sulfide.

Various chemicals have been reported over many years as being capable of reducing the reproductive potential in insects and a list of these has been presented in the literature review by Smith, LaBrecque and Borkovec (1964). The aziridine derivatives constitute the most important and extensively used group of chemosterilants. Their sterilizing property depends on the aziridine ring which attacks the genetic material or some other compound with biologically significant functional groups, by a process of biological alkylation involving nucleophilic substitution (Borkovec 1962). Alexander (1960) pointed out that there were other potential nucleophilic centers in the biological system on which alkylation may occur such as proteins and

vitamins. He however, observed that the consistency of the genetic effects of these compounds, suggested that they attacked the genetic material-DNA. Several theories have been put forward about the detailed cytological effects of aziridine compounds. Among these, the cross-linking theory of Stacy et al. (1958), had gained popular credence.

LaBrecque et al. (1960) were the first to show the sterilizing property of apholate on house fly reproduction. Later, the screw-worm fly was sterilized with the same compound, by dipping larvae and prepupae, by dusting prepaupae and adults and by feeding adults 0.5% and 1.0% (Chamberlain). Similar tests were done by Crystal (1963), Crystal and LaChance (1963) on the house fly with thiotepa, tretamine, apholate and other aziridine derivatives. Lindquist et al. (1964) reported that out of 50 potential sterilants, only apholate consistently sterilized the boll weevil.

One of the acclaimed advantages of Chemosterilants over high energy radiation was that they induced sterility without significantly reducing the longevity and sexual vigor of the treated insects. It is however, noteworthy that the screw-worm flies which were used in the classical demonstration of the sterile-male principle manifested no loss of sexual vigor or vitality when irradiated as pupae (Bushland and Hopkins, 1953). This response has been associated with several other members of the Diptera, irradiated in the pupal stage. Henneberry and McGovern (1963) using Drosaphila melanegaster and Steiner and Christenson (1956)

using Oriental fruit fly, Dacus dorsalis Hendel observed that the irradiated pupae gave rise to adults which were quite competitive. Earlier work on Coleoptera indicated a high rate of survival when these insects were treated with gamma-radiation. Jaynes and Godwin (1957) found that 5,000-10,000 roentgen (r) caused sterility in the pine weevil, Pisodes strobi without reduction in longevity. Cork(1957) also stated that gamma-irradiated flour beetles lived longer when given several small daily doses. These accounts would appear to challenge the theoretical advantages of chemosterilants over ionizing radiations, but comparative and singular studies have clearly shown that chemosterilants are the better choice for the sterilization of many insects. Weidhass and Schmidt (1963) irradiated Aedes aegypti (L.) and observed a reduction in mating behavior, but chemically sterilized males retained normal sexual vigor. Davis et al. (1959) studied the effects of gamma-radiation on mating behavior of the common malaria mosquito, Anopheles quadrimaculatus Say and reported a remarkable loss in mating competitiveness by the treated males. The comparative studies of Schmidt et al. (1964) very strongly stressed the merits of chemosterilization. They treated house fly pupae 31-54hr. prior to eclosion with 2850r and fed 1% apholate to 1-day-old adults. Mixed ratios of treated and untreated males were allowed to compete for virgin females in several cages. Chemosterilized males either equalled or surpassed the radiosterilized

ones and besides greater permanency was exhibited in chemically treated house flies than in the irradiated ones. Apholate sterilized adults of Culex pipiens quinquefasciatus Say were highly competitive (Dame and Ford 1964). LaBrecque, Meifert and Smith (1962) sterilized male house flies on diet containing 1% apholate, and these were found to be as successful as normal males in competition for mates. Adult males of green sheep blow fly, Lucilia sericata Meigen treated topically with apholate were reported to be equally competitive as the untreated males (Millar, 1965). Among the Coleoptera, efforts to sterilize the boll weevil, Anthonomus grandis Boheman with gamma-irradiation revealed that the sterilizing dose reduced longevity drastically and sterility was not permanent. Mayer and Brazzel (1966) irradiated 12- and 36-hr. old adult boll weevils with 8,000r and found that although they mated normally for 10 days, longevity was highly reduced. Lindquist et al. (1964) conducted sterility studies on the boll weevil with apholate concentrations of 0.5%, 1.0% and 2.0%. Mortality of the treated males was high particularly at 11 and 20 days after treatment and they were not as competitive as normal males. Lindquist (unpublished data, 1965) observed however, that apholate sterilization was less deleterious to the insect than radiation. Male azuki bean weevils sterilized by apholate topically applied in acetone were as competitive as normal males in mating (Nagasawa and Shinohara, 1965).

The triphenyl tin compounds have only recently been evaluated by Kenaga(1965) and he showed that these chemicals sterilized at dosages which did not affect the biological functions of the insect adversely. Japanese workers, Nagasawa, Shinohara and Shiba studied the sterilizing effect of Dowco-186 on Callosobruchus chinensis but did not report mating or longevity observations.

Hoopingarner et al. (1965) indicated that X-rays were detrimental to adult cereal leaf beetle. No mating tests were undertaken in these studies as there was no known sexing method. The high rate of mortality reported, above 90% in 14 days at 5,000r, necessitated the present studies with chemosterilants in the hope of finding a more practical sterilizing technique for this pest.

MATERIALS AND METHODS

The beetles used in these studies were either field-collected or laboratory-reared adults. Pre-diapause beetles were obtained from wheat and oat farms as summer adults. Post-diapause beetles were obtained as spring adults or by cold-treating laboratory-reared adults at 40°F for at least 8 weeks. Post-diapause beetles were stored in plastic containers at 40°F until needed, while pre-diapause beetles were maintained on young wheat plants in cages of about 15"x17"x18".

Apholate Treatment

A fresh stock of 2% aqueous solution of apholate (technical) w/v was made up for each testing period. 0.2% of Triton X-100 was added to the solution as a wetting agent. Series of desired dilutions were made from this stock preparation. The beetles were anaesthesized with ether and treated by a modified dipping technique of Sawicki and Farnham (1964). The apparatus consisted of a sintered glass Buchner funnel fitted to 1-litre Buchner flask. Suction pressure was provided by a vacuum tap. Twenty-five randomly selected beetles were used in the dosage assay because a reliable sexing method was not initially available. These were tipped into the funnel after anaesthesia and the appropriate apholate concentration was immediately added. Each

treatment lasted for 30 seconds. Solutions of 2% to 0.001% were screened for the optimum sterilizing dosage. The beetles were reared on young potted wheat plants in glass chimney cages kept in a Sherer controlled environment chamber at 80°F, relative humidity of about 50-60% and 16-hour photoperiod. Temperature and humidity of chamber were recorded by means of a Friez hygro-thermograph model 594. Mortality was checked daily and dead beetles were removed. Fresh plants were supplied when necessary and all transfer of beetles was done with an aspirator. Eggs were kept in covered petri dishes layered with wet filter papers and allowed 4-7 days to hatch. Tests lasted from 9-25 days. All counts of eggs and larvae were made with a manual tally counter. Viability was determined on the basis of egg hatchability. A control cage was set up for each test. Mortality data was corrected by a modification of Abbott's formula (Hinman, 1947).

Mating Competitiveness and Longevity Tests

At this stage a reasonably reliable sexing method was found, based on the morphological character of the inverted "V"-shaped intercoxal portion of the first abdominal sternite. Only post-diapause beetles were used in these tests. Four proportions of treated and untreated males were used in the ratios of treated males:to normal males:to normal females of 2:1:1, 4:1:1, 6:1:1 and 9:1:1. The sterilizing dosage used was 0.1% apholate as this was found to induce 100% dominant lethals in males after a single dipping

treatment. Four cages were set up in the usual manner and in addition, one cage consisting of treated males only and virgin females was used as check on the adequacy of the treatment. A control cage was used in order to estimate the natural egg viability. Normal males were differentiated from treated males by clipping off the elytron at the posterior margin with very fine scissors. This facilitated the recording of mortality data and did not hinder the normal activities of the beetles so deformed. Observations were made daily for dead beetles and eggs were incubated as described before. Ratios were maintained as much as possible by replenishing from separate colonies of each category. Mortality data were statistically checked by the Fourfold Contingency tests for unequal samples at the 5% significance level (Mainland and Murray, 1952).

Effect of Early Mortality on the Mating Competition Tests

Four replications of the 2:1:1 ratio of treated males, normal males, and normal females were established and daily mortality recorded of both treated and normal males. Two control cages were used with these tests. Eggs were harvested every 48 hours and incubated as described above for 5 days. Percent viability of each egg batch was noted in order to establish a trend in viability over an entire test period of 21 days and to relate this to the mortality rates of both treated and normal males.

Dowco-186 (Triphenyl Tin Hydroxide)

(a) Dipping method: 0.1% w/v solution of the chemical was made up with 70% v/v mixture of acetone and water. The beetles were slowed down with ice and then treated with 0.1% and 0.01% concentrations of the chemical by the dipping technique used in previous tests. Treatment was for one minute and rearing was carried out as before in a Sherer controlled chamber.

(b) Oral treatment--males only: The chemical was administered orally by treating wheat plants on which the beetles fed. 3%-0.1% concentrations w/w were applied by spraying, using Bentonite as the carrier. The sterilant was first dissolved in acetone and then mixed with a known weight of Bentonite. The solvent was driven off with an airstream in an evaporating dish placed in a ventilating hood. Each preparation was emulsified with 2 ml. of Triton X-100 by Lourdes Instrument model mm-1 using approximately 3 times its own weight of water. These were stored in the refrigerator until required for application. An improvised sprayer was used in the spraying of the plants. All spraying was done in protected areas. Male beetles were allowed to feed on treated plants for 7 days after which females were introduced. Rearing and observations were carried out routinely. The test lasted for 18 days.

(c) Oral treatment--both sexes: 3.0%-0,1% concentrations of the triphehyl tin compound were used. Ten males and ten females were introduced on the treated wheat plants

and allowed to feed for 10 days. The plants were changed every two days because of the phytotoxicity of the chemical. They were subsequently transfered to untreated plants for mating and oviposition. Egg batches were collected while they fed on treated plants and after transfer to the untreated plants. The harvested eggs were incubated and checked for viability.

Cytological Technique (Apholate Treated Males)

Feulgen-fast green staining of 10u sections of testes from treated males were made for cytological observations. Microphotographs were taken with Kodak pony II camera and Wild Heerburg phase microscope. The final photographs represent 2700 X, 1100 X, and 540 X magnifications. Panatomic X high contrast film was used. Microscopic examination of spermatozoa from treated males was also carried out 21 days after treatment. Carlson's Drosophila sperm media, with the omission of penicillin, was prepared for this work. All disections were done in the sperm media and the testis was carefully teased out in a drop of the preparation for sperm motility observations. Testes sizes were visually estimated for gross comparisons.

RESULTS

Screening for Dosage Level

Preliminary assay for optimum dosage level was carried out with randomly chosen beetles. Results obtained with treated pre-diapause beetles were quite inconsistent and so most of the tests were done with post-diapause beetles only. Apholate concentrations of 0.1%, 0.25%, and 0.5% suppressed oviposition and caused high mortality. There was apparently some difference in survival between treated pre-diapause and post-diapause beetles (see Tables 1 and 2). In the pre-diapause beetles there was over 80% mortality at the above concentrations in 12 days. At corresponding dosage levels, post-diapause beetles ranged from 48-76% in mortality. Apholate was therefore more toxic to pre-diapause than post-diapause beetles. 0.05% concentration induced complete sterility but at lower concentrations, adult survival and percent hatch of eggs were as high as in the controls (Table 3). 0.05% was the lowest concentration that caused sterility to both sexes.

Male Optimum Sterilizing Concentration

After a suitable sexing technique became available and the technique was perfected, male beetles were assayed for the required sterilizing concentration using 0.1% and 0.05% levels. Eggs resulting from the matings of 0.1%

TABLE 1--Sterilizing effect of 30 sec. apholate dip on cereal leaf beetles (pre-diapause adults)--two replications.

% Concentration Apholate	Average % Mortality in 12 days[a]	Eggs Laid	Percent Hatch
1.0	96.7	None	----
0.5	80.0	None	----
0.25	80.0	None	----
Control	0	425	58.5

TABLE 2--Effects of 30 sec. single apholate dip on post-diapause cereal leaf beetles--2 replications.

% Concentration Apholate	% Mortality[a]	Number of Eggs	Percent Hatch
1.0	76*	----	----
0.5	72*	----	----
0.25	52*	----	----
0.1	48*	----	----
Control	0*	310	56.0
0.05	36.4**	98	0
0.025	4.5**	120	30.8
0.01	4.5**	180	43.3
0.001	0**	182	68.7
Control	0**	328	57

[a]Corrected by Hinman's (1947) modification of Abbott's formula.

*In twenty-one days.

**In eighteen days.

TABLE 3--Sterility of male cereal leaf beetles treated (by 30 sec. dipping) at 0.05% and 0.1% apholate concentrations.

Concentration of Apholate (%)	No. of Beetles Treated Males	Females	Average Eggs/ Female	% Hatch
0.1 I	25	25	14.4	0
II	15	15	13.9	0
0.05 (once)	25	25	9.0	20.5
Control	25	25	26.6	49.8

treated males did not hatch. Some egg hatch resulted from the matings of beetles treated with 0.05% concentration. It would appear that 0.1% was an effective sterilizing dosage for the male cereal leaf beetle, since subsequent trials produced consistent effects. This concentration was used in subsequent tests. In some of the later tests however, about 5-10% viability was observed at this dosage level, but this was attributed to the chemical which had degenerated in storage over a long period of time.

Mating Competitiveness

In these tests, percent viability conformed very closely with theoretical expectations based on the ratios of treated and untreated males. Chi^2 probabilities in all ratios except the 9:1:1 ratio indicated no significant variation between observed and expected results (Table 4). At this ratio probability was between .05 and .02. Mating activity appeared quite normal in all treatments and there

was no reduction in fecundity when results from the control were compared. These results indicated that the treated males were at least as competitive as the normal males as expected viabilities were observed in most cases. The graph (No. 1) pictorially relates these data.

TABLE 4--Mating Competitiveness of male cereal leaf beetles dipped once in 0.1% apholate.

Sex Ratio[a] T. M. : N. M. : N. F.	Number of Eggs Laid	% Viability Expected	% Viability Observed
9 : 1 : 1	86	6.3	3.5
6 : 1 : 1	180	8.6	7.2
4 : 1 : 1	173	12.0	10.9
2 : 1 : 1	222	20.1	15.3
1 : 0 : 1	607	0	0
0 : 1 : 1	325	----	60.3

T. M. = treated males

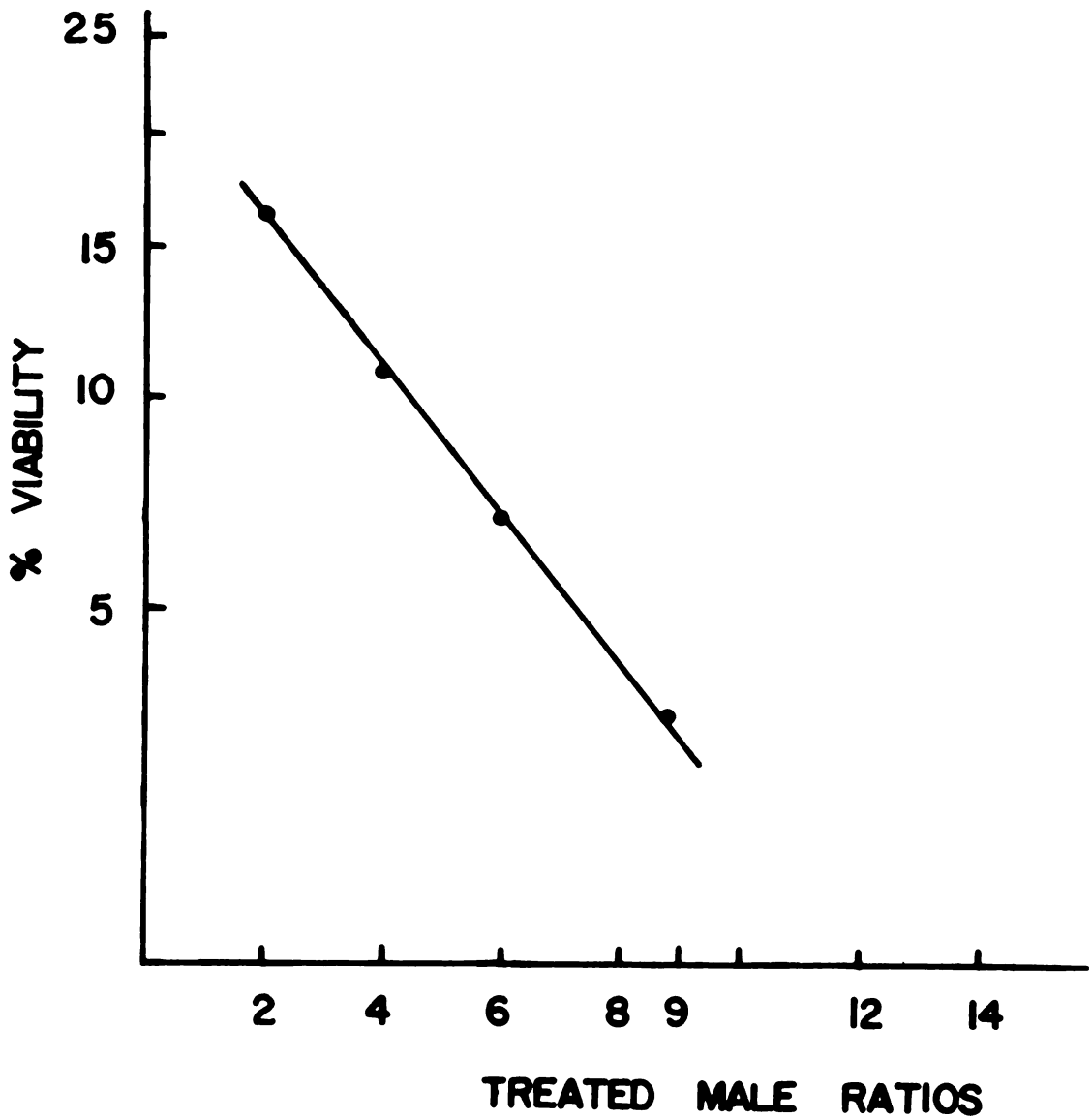
N. M. = normal males

N. F. = normal females

[a]Multiples of 4 and 8. Chi^2 probabilities for all ratios were greater than 0.2 except in the 9:1:1 ratio. In this ratio P = 0.02-0.05. Significance was calculated at the .05 and .01 fidicual limits.

Treated Male Longevity

Normal males lived longer than apholate-treated males (see Table 5), as a 0.1% concentration caused about 90% mortality in 28 days. Significant mortality occurred at about 10 days after treatment (Analysed by the Fourfold



Graph No. 1.--Graph showing the relationship between increasing ratios of treated males and egg viabilities.

TABLE 5--The effect of 0.1% apholate on longevity of male cereal leaf beetle dipped once for 30 seconds.

Treatment	Mean No. of Beetles/Treatment	Percent Mortality (Avg. of 3 Replicates)				
		Days After Treatment				
		7	10	14	21	28
Apholate 0.1%	28	14	43	57	82	90
	16	12.5	37.5	69	75	88
Control	15	0	0	7	20	20

Contingency tests of Mainland and Murray, 1952). Normal males averaged 20-28% mortality in 28 days, with little or no deaths occurring in the first 10 days after treatment. Apholate reduced male longevity in the cereal leaf beetle.

Effect of Early Mortality on Mating Competitiveness of Treated Male Cereal Leaf Beetles

Actual figures obtained from four replications were somewhat variable, but a general trend was established. In all cases, there was initial reduction in egg viability very close to that expected from the ratio studies of treated males. The expected viability was 20 percent for the 2:1:1 ratio used in this study. Viability progressively increased with time as the number of competing sterile males was decreased by death. Data presented (Table 6) represents the average results obtained from the replications (see graph No. 2). Early mortality of treated males clearly affected the egg viability. Since more than

TABLE 6--The effect of early mortality of treated males on the mating competition tests.

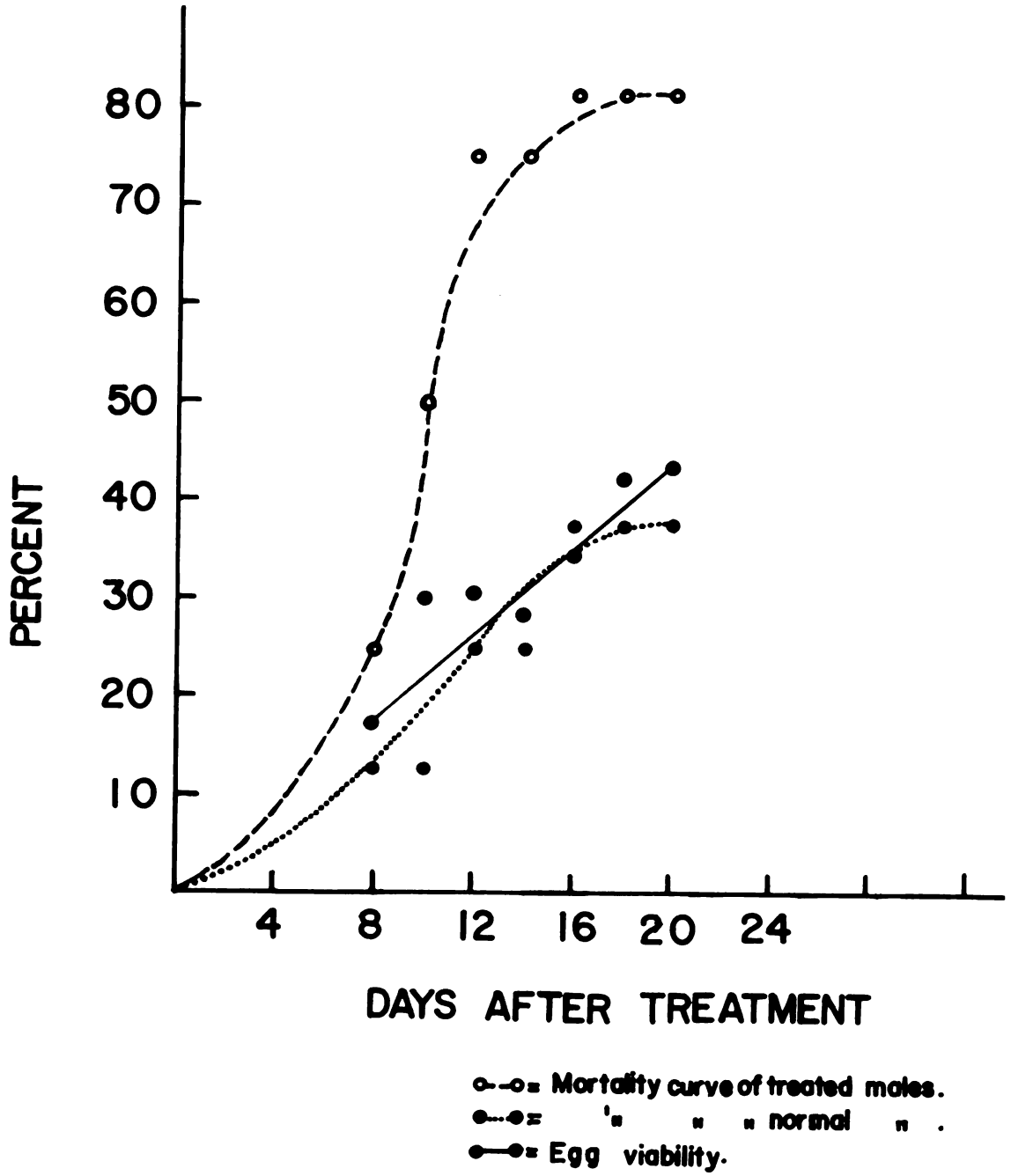
Days After Treatment	Avg. No. of Males Alive Treated	Normal	Avg. No. of Eggs Per Treatment	Avg. % Hatch	Range
0	16	8			
7-8	12	7	71	17.9*	15.2-20.0
9-10	8	7	86.5	30.2	13.7-38.0
11-12	4	6	140.8	30.7	21.2-40.5
13-14	4	6	179.8	28.7	21.9-41.2
15-16	3	5	158.5	34.9	25.0-50.0
17-18	3	5	116.5	42.3	30.0-59.8
19-20	3	5	133.0	43.5	35.0-59.2

*Expected egg viability - 20%.

50% of treated males were dead by the 10th day at which time oviposition generally began, this factor weighted the ratios in favor of the untreated males.

Dowco-186 Trials (Oral treatment--males only)

The results were astonishing in that there was no indication of any reproduction control (see Kenaga, 1965). Egg viabilities in all treatments were either normal or surpassed that of the control (Table 7). Mortality was quite low throughout the entire 18-day test period. Since only one test was done these results cannot be conclusive, however, there was an indication that this chemical was not a male sterilant for cereal leaf beetles.



Graph No. 2.--Graph showing the effect of early mortality on the mating competitiveness of the treated males.

TABLE 7--Effect of Dowco-186 on post-diapause cereal leaf beetles when males only were fed on treated plants for 7 days (15 males x 15 females).

%Concentration	No. of Males Alive	Eggs	% Hatch
3.0	14	62	85.5
2.0	15	139	68.5
1.0	14	190	61.0
0.1	13	136	56.6
Control	14	212	58.5

Dowco-186 (Dipping test-males only)

In the dipping test, 0.1% Dowco-186 in 70% aqueous acetone caused 100% mortality of the beetles in 24 hours. 0.01% had no effect on either survival or reproduction. Viability of eggs was close to that of the control. The dipping technique does not appear to be suitable with this chemical for the cereal leaf beetles.

Oral Treatment--both sexes

Feeding was allowed on the treated plants for about 10 days in order to ensure that sufficient Dowco-186 was obtained by the beetles. Only at 0.1% treatment was there normal feeding and some oviposition while on the treated plants. Some degree of reproduction control was obtained at this level during this peroid. A complete recovery of fertility followed as soon as the beetles were transfered to untreated plants.

TABLE 8--Effect of dipping males of post-diapause cereal leaf beetles in 0.1% and 0.01% concentrations of Dowco-186 for 1 minute.

% Concentration	Males	Females	% Mort. at 24 hrs.	% Mort. at 9 days	% Egg Hatch
0.1	15	15	100	100	----
0.01	15	15	---	27.0	53.3
Control	15	15	---	20.0	55.0

At 2.0% and 3.0% levels most of the beetles were either dead or extremely lethargic. No mating or oviposition occurred at these concentrations before and after the introduction of untreated plants (see Table 9-B). 1.0% caused some lethargy but some of the beetles regained activity as soon as a fresh untreated plant was introduced. Initial oviposition was low and viability was only 4.0%. The second batch of eggs increased in number considerably and hatchability was in the normal range. Continuous feeding on treated plants appeared necessary before any sterility could be achieved. 2.0% and 3.0% were probably above sterilizing dose.

Cytological Observations (On apholate treatment)

Gross comparison of testes from treated males with those from normal males showed no difference in size. There appeared to be no regression due to the effect of the chemical at the 0.1% level. 10u sections stained in Feulgen-fast green, showed no tissue degeneration. At the

TABLE 9--Effect of feeding both sexes of cereal leaf beetle on plants treated with Dowco-186 (10 days duration).

Dowco-186	Treated Plants				
	1st Egg Batch		2nd Egg Batch		% Mort. at
% Concn.	Eggs	% Hatch	Eggs	% Hatch	20 Days
0.1	41	14.6	52	38	
1.0	--	----	--	--	
2.0	--	----	--	--	
3.0	--	----	--	--	
Control	37	51.4	52	69	
	Untreated Plants				
	3rd Egg Batch		4th Egg Batch		
0.1	104	63.5	59	40.7	50
1.0	46	4.0	242	56.2	60
2.0	---	----	---	----	75
3.0	---	----	---	----	80
Control	48	41.7	---	----	36

cellular level nuclei appeared normal and a few cells caught at the prophase stage portrayed no abnormalities (see figure 1). Testis was filled with spermatozoa which were morphologically as normal as those of untreated males (see figures 2 and 3). Motile spermatozoa were found in treated males 21 days after treatment. Sperm motility was therefore not hampered by the treatment.

Figure 1.--Cross-section of testis of male cereal leaf beetle treated with 0.1% apholate, showing spermatozoa and some nuclei in prophase.
No abnormalties noticed.

Stain--Feulgen-fast green; Magnification.....2700 X.

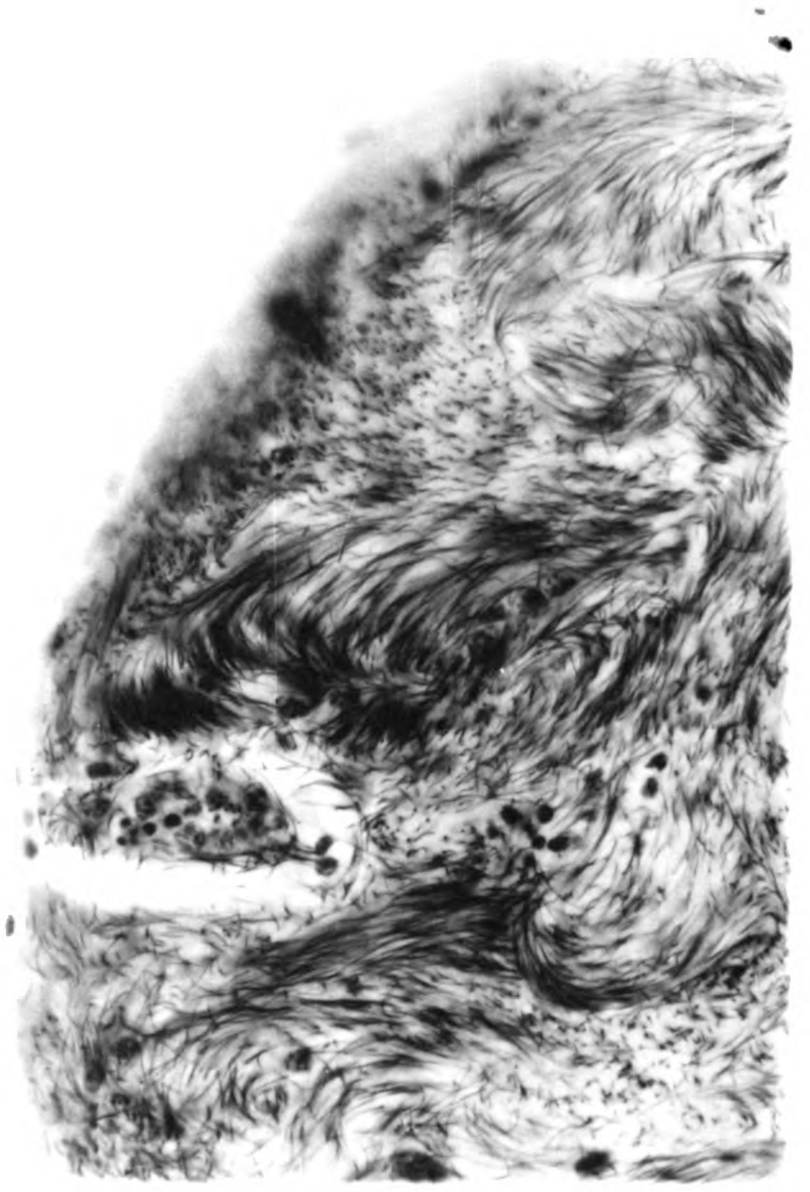


Figure 2.--Part of the cross-section of the testis of male cereal leaf beetle treated with 0.1% apholate, showing abundant spermatozoa in normal conditions.

Stain--Feulgen-fast green; Magnification.....540 X.

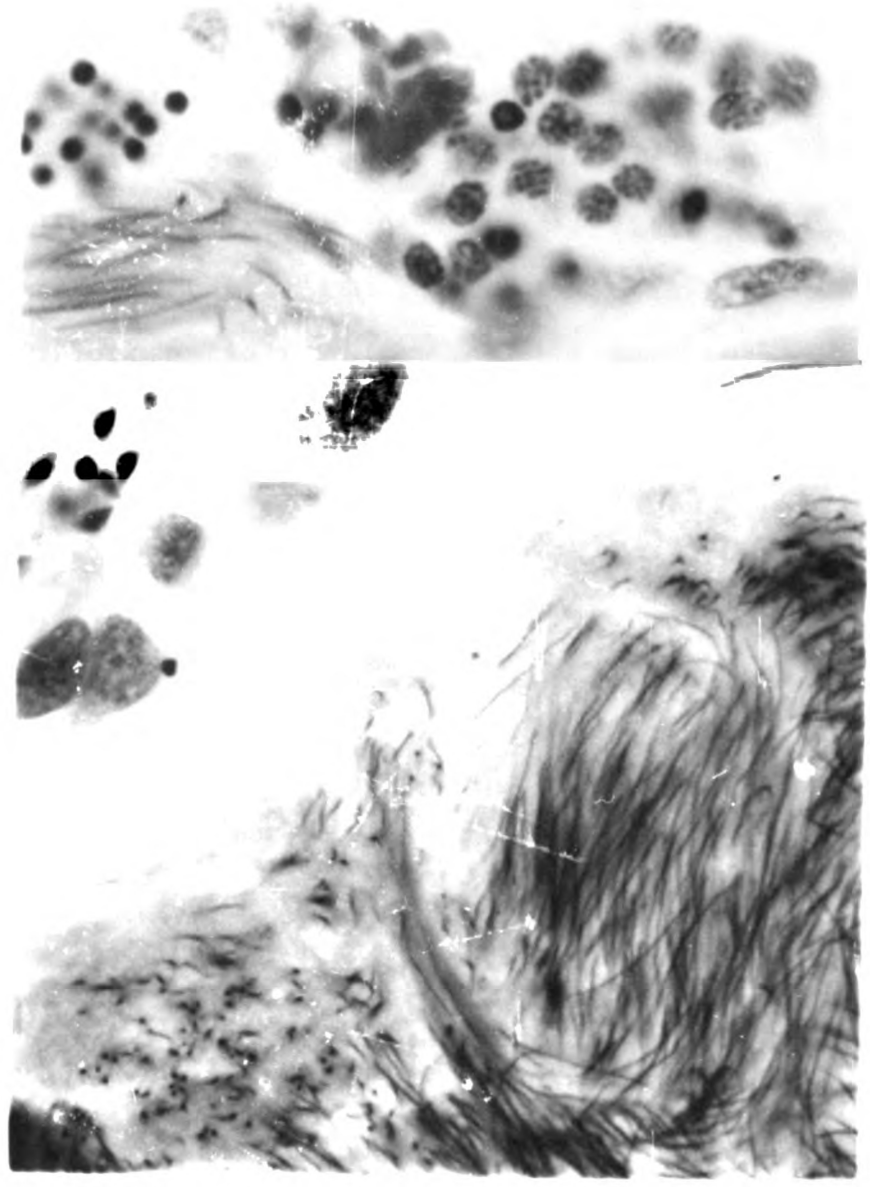


Figure 3.--Cross-section of testis of normal male post-diapause cereal leaf beetle.

Stain--Feulgen-fast green; Magnification.....1100 X.

DISCUSSION

Apholate successfully sterilized adult cereal leaf beetles under laboratory conditions. It does not seem however, that it is a very practical chemosterilant for this pest in terms of actual field operations. In order to realize the full potentials of the sterile-male technique in insect control, the survival of sterilized males must be high enough to sustain the desired competition in the natural population. In present investigation apholate significantly reduced the life-span of the male cereal leaf beetle at the dosage required for sterilization. Mortality was found to be high particularly at 10 days after treatment. Hoopingarner et al. (1965) found that radiosterilized adults suffered a mortality of over 90% in 14 days. Mortality of apholate-treated adults for a corresponding period of time was found to be about 36%. Treated male mortality ranged from 60-70%. These figures indicate a greater degree of survival in apholate-treated beetles than in the irradiated ones. However, since mortality of apholate-sterilized males exceeded that of normal males (0-7% in 14 days) the use of this chemical for the sterilization of the cereal leaf beetle promises no clear advantage over irradiation.

Apholate-sterilized cereal leaf beetle males were as competitive as normal males (see Table 4), but the occurence

of early mortality among the treated males obscured the influence of this factor in actual results. It is suspected that in this insect continual insemination might be essential for adequate production of eggs. If this is so, the advantage to normal males of this early mortality is very evident. For, if the normal males continued to mate after the reduction in the number of the competing sterile males, the situation would allow for a preponderance of normal spermatozoa over spermatozoa with dominant lethals in the spermatheca. In a strictly monogamous species or even in a polygamous species in which only the first few matings are relevant, sterile male mortality might not be very influential in mating competitiveness since all the dominant lethal spermatozoa required to compete would have been introduced before any significant death occurred, provided the sterile males were competitive. Murray and Bickley (1964) reported that in Culex pipiens quiquefasciatus the sperm of the first mating was responsible for zygote formation. LaBrecque (1961) also found that in the house fly, the first male to copulate with the female influenced egg viability. In these cases, early mortality would not upset the expectations to a great extent. But, in Drosophila melanogaster Henneberry (1963) reported that females which mated first with treated males produced sterile eggs, but when subsequently mated with untreated males they produced viable eggs. Similar findings were reported with fruit flies (Steiner and Christenson, 1956). In these examples,

early death of the sterile males would favor normal males. Multiple matings in this instance poses a problem. If the sterile-male method were to be used for this pest, it would entail more frequent releases in order to maintain the desired ratio of untreated to treated males. This would of course involve large-scale rearing procedures which might be unattainable if research resources were inadequate.

A graph relating egg viabilities with ratios of treated males was presented earlier (see Graph No. 1) on which a few theoretical inferences could be based. The graph shows that a ratio of treated males to untreated males of about 12:1 would be required to reduce egg viability to zero percent. This would of course entail raising a large number of insects for treatment and release. Judging from the difficulties at the moment of maintaining a large colony of these beetles in the laboratory, a twelve-fold release seems quite unattainable and therefore unadvisable. In the mating competitiveness studies reported elsewhere in this work, a 4:1 ratio was found to have resulted in an egg hatch of 10%, which corresponds to about 50% reduction in normal egg viability. If this technique were to be employed for the eradication of the cereal leaf beetle, this ratio would be sufficient to initiate an effective downward trend in the natural population. Moreover, from a practical point of view, it is a more attainable ratio even with the limited facilities that are now existing.

Although apholate-sterilization of the cereal leaf beetle appears to have poor prospects for use in the

sterile-male release method, the search should continue for a chemical with a wider margin of safety. Chamberlain (1962) remarked that a potential chemosterilant should not be discarded merely on the basis of some toxicity. If rearing methods are improved sufficiently to ensure a large production of insects, it might be possible to adopt frequent releases in order to off-set the problems of early mortality of treated males. If a chemical were found that could be applied as a contact residue baited with some specific attractant in the natural population, male mortality might not be a major problem if the males retained their normal mating behavior after autosterilizing themselves. In this case male and female sterilization might occur which should facilitate realization of the desired effect.

Preliminary trials with Dowco-186 produced no conclusive results but a few observations appear to be quite relevant for future work with it. It was found that this compound may not be a male sterilant since females mated with treated males produced viable eggs at all concentrations assayed. It might be necessary therefore to treat both sexes in order to produce sterility. Kenaga (1965) in his pioneer reports on the triphenyl tins pointed out that they were less effective on males than on females of those insects studied. Concentrations of 0.1 and 1.0% did not seriously affect the feeding behavior and general vigor of the beetles. At these two concentrations, some temporary reproduction control was obtained which was lost

as soon as the insects were transfered to untreated plants. It does seem that feeding on the sterilant must not be broken in order to ensure complete sterility. High mortality observed at concentrations of 2% and 3% resulted mainly from starvation, because the beetles were very lethargic and could not carry out normal functions. Flint (1965) described similar conditions in the eye gnats which were treated with high doses of radiation. Grosch (1956) had earlier described an identical occurrence as a "radiation induced lethargy." The potentialities of Dowco-186 as a chemosterilant for this pest cannot be fully appraised until a reliable method of administering it to the insect is found. The method of application used in these studies posed feeding problems and retarded progress with the investigation.

Histological sections of the testis of apholate-treated males showed no tissue damage or cellular disruption. The testis was full of spermatozoa which appeared morphologically normal. Motile sperms were observed in the testis of males up to 21 days after treatment. Damage to reproductive tissue has been reported in the boll weevil Anthonomus grandis treated with apholate at 15 ppm. (Lindquist et al., 1964). Rai (1964) also stated that at 0.05% concentration, apholate caused degeneration of the testis in Drosophila melanogaster. These authors treated younger stages of the insects and it is probable that in such early formative stages the reproductive organs might be more

susceptible to damage. The male post-diapause cereal leaf beetles were at a mature stage and any damage would have been less easily discernible.

SUMMARY

1. Apholate sterilized post-diapause adults of the cereal leaf beetle. Both sexes were sterilized by 0.05% while 0.1% sterilized the males.

2. Treated males did not live as long as normal males, and mortality was high from 10 days after treatment.

3. Mating behavior of treated males was not affected by the sterilizing dosage.

4. A 12:1 ratio of treated males to untreated males would be required to reduce egg viability to zero percent. The early mortality of the treated males would necessitate more frequent release of sterilized beetles.

5. Dowco-186 produced some sterility while both sexes fed on treated plants. This sterility was lost as soon as the beetles were returned to untreated plants.

6. High doses of Dowco-186 caused lethargy which eventually led to death.

7. No tissue damage or cellular disruption occured in the reproductive organs of post-diapause males treated with 0.1% apholate. Sperm motility was not affected by this dosage level.

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