A study on the effects of apholate on the biology of rice weevil, *Sitophilus oryzae* LINNE

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化學的 不姙誘起物質 Apholate가 쌀바구미(Sitophilus oryzae L.)에 미치는 生物學的 影響

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摘　　要

쌀바구미의 幼蟲을 3期로 區分하여 各種 濃度의 Apholate를 處理하여 그의 致死率 · 發育速度 · 產卵數 · 不姙率 및 그의 持續性 與否를 調査하여 다음과 같은 結果를 얻었다.

(1) 當代 致死率은 濃度가 높아짐에 따라 높아 졌으며, 幼齡期 處理時의 致死率은 老齡期 處理時보다 높았다.

(2) 次代 致死率은 處理된 性에 따라 差가 있었고 全體的으로 雄蟲 處理時가 雌蟲 處理時보다 높아 致死突然變異 誘起率이 雄蟲 處理時에 높아짐을 알 수 있었다.

(3) 0.5% 以上의 處理區에서는 羽化 成蟲 중에 各種 畸形이 出現하였으며, 그 數는 濃度가 높아짐에 따라 컸다.

(4) 產卵數의 減少는 雌蟲 處理時가 雄蟲 處理時보다 甚하였으며, 이것은 Apholate의 卵子形成에 미치는 直接的인 影響이라고 생각된다.

(5) 發育速度는 老齡期 處理時가 幼齡期 處理時보다 늦어지며, 그 效果는 濃度의 增加에 따라 높아진다. 次代의 生育期間은 雌蟲 處理時가 雄蟲 處理時보다 길어졌다.

(6) 化學的 不姙劑는 當代의 影響뿐 아니라 次代에도 그 影響力이 持續된다.

The complete eradiacation of the screw worm fly, *Cochliomyia hominivorax* (Coq.), from State of Florida by mass releasing of radiation induced sterile flies has greatly stimulated the interest of the entomologists to the use of sterile techniques in a pest control.

An induction of sterility could be obtained not only by irradiation, but also by chemicals. In the use of radiation induced sterility, it may require not only large plant to produce large number of insects to irradiate; elavorate radiation facilities, but also it has been known that radiations have greater effects on the biology of the insects than chemosterilants, for example, mating behaviour or longevity. From such, there have been many works on the use of chemosterilants since 1960.

The main advantage of the sterile male techniques over the chemical control seems to be so called 'bonus effects', which was fully explained by KNIPLING · LINQUEST et al. (1964) observed that apholate had not only reduced the number of fertile eggs of *Anthonomus grandis*, but also had increased greatly the mortality during the postembryonic development, and stressed the total control of reproduction. HYUN and SHIM (1965) also reported that tepa treatment had induced lethal mutation of the rice weevils, and had increased mortality during the postembryonic development.

The purpose of this experiment was to investigate the effects of apholate on the biology of the rice weevil, not only on the immediate generation, but also on the next generation.

I. Materials and Methods

The used insects were laboratory strain of *Sitophilus*

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oryzae, which had been reared on polished rice at laboratory condition.

In order to obtain a uniform age group, each of four glass bottles containing 150 gs. of polished rice (moisture content of 14.5%) were infested with about 10,000 weevils. The weevils were removed by sieving them out from the grains after five days. The infested grain was throughly mixed by put them together, and 20 gs. of infested grain was allocated in a small glass tube (2.9×11.8 cm.).

The glass tubes were covered with paper by means of a rubber band, and kept them at temperature of 29±1°C. and at relative humidity of 75%.

Apholate was supplied by Olin Chemical Co. U.S.A., and kept in a desiccator containing $CaCl_2$ in a refrigerator. The purity was 90%. It was diluted with distilled water at the time of use. 0.2 ml. The apholate solution (0.2ml.) was added to the grain in the glass tube, and shaked for the thorough distribution of the materials. 0.2 ml. Disterilled water (0.2ml.) was added to the grain for the control.

The treatments were made right after the separation of the weevils from the grain, four days and eight days after separation of the weevils from the grain. These are approximately correspond to the egg- 1st, 2nd-3rd, and 3-4th instar larvae. These treatment will refer to treated stages I, -II, and -III in follows.

The mortality of the larvae in the grain was examined at intervals of six days after treatment. Two gs. of the grain were taken out from glass tubes and kept them in 50% alcohole. Acid fucshin staining method was used for detecting the infested grain. The infested grain were carefully examined for the death of larvae by dissecting the grain under the stereomicrocope. The living larvae showed creamy white colour with flesh looking, and the dead one showed some brownish colour with shrinkage. There, however, may have been some misinterpretation of the individuals which died shortly before the examination.

In order to examine the biological effects of apholate other than on the mortality of the immadiate generation, individual infested grain was put in a small glass tube before emergence to prevent the mating. They were sexed and mated with the opposite sexes from untreated grain.

Four mating pairs were put in a glass tube, which containing 10 gs. of polished rice. The grain was renewed every day for five days period. The number of eggs in the grain was examined by fucshin staining method.

All the experiments were carried out at temperature of 29°C. and relative humidity of 75% maintained by using saturation of NaCl. Three replicates were used throughout the experiments.

II. Results and Discussion

1. Effects on the immadiate generation

(1) Effects on the mortality in the immediate generation

The mortalities during the postembryonic development, when various larval stages were treated with apholate, are showing in Table 1.

Table 1. The effects of apholate on the mortality during the postembryonic development when the larvae were treated at various developmental stages (corrected for control).

Age	Concentrations						
	0	0.03	0.06	0.12	0.25	0.50	1.0
I	0	26.90	38.24	41.66	47.96	54.86	72.22
II	0	13.23	25.11	39.17	49.17	33.66	52.09
III	0	16.84	18.25	13.02	11.96	25.40	29.19

As shown in Table 1, the mortalities increase with the concentrations, and differ with the treated stages at the same does. Within the same doses the greatest mortalities are always found with the treated stage I, followed by stage II, and the least are found with the treated stage III, except at the dose of 0.03%.

As shown in Table 1, lethal effects of apholate on the postembryonic development of rice weevils seem not to be great except at high doeses. CHAMBERLAIN (1962) reported 100% mortality from application of 59 ppm of apholate to *C. hominivorax* larvae. HYUN and SHIM(1965) reported from 86 to 93% mortality when the rice weevil larvae had treated with tepa. It would be direct effects for *C. hominivorax*, and may be different from the case of rise weevils, in which the chemosterilants would be taken into the body through impregnated endosperm of rice.

The different effects with the treated stages may be interpreted in three ways, the physiological differences of the larvae with the developmental stages, the differences in the uptake of the material with the ages, and the duration of the exposure.

Young larval period is the most actively growing period, and somatic differentiation might be great, and the germ cells come to active later when the somatic cell differentiation has completed considerably.

Since it has been suggested that the alkylating agents react directly with the cell chromosomes by attacking one or more of the reactive loci on the nucleic acid molecule, the effects of apholate on an organism may be closely related with the cell activity. The greater mortalities associated with the younger larval treatment might be resulted from the greater somatic cell activities in the younger larvae.

The differences in an absorption amount of chemosterilant with the developmental stages was reported by DAME and SCHMIDT(1964) from the metapa treatment of *Aedes aegypti*, and ICHINOSE and ISHII(1965) reported that young larvae of *Barathra brassicae* had absorbed more DDT than the old larvae.

HYUN and SHIM(1965) suggested such difference in amount of absorption with the ages might be related with the surface volume relation; the young larva has greater surface area than the older larva in a unit weight, and there might be some physiological differences in the activity of the cells.

The duration of exposure to the material may be another factor to be considered; the young larvae have been exposed longer time than the older larvae.

It, however, would be reasonable to be think of the combined effects of these factors rather than single factor.

(2) Effects on the developmental periods

The average days required for the postembryonic development of the treated generation are showing in Table II.

The developmental periods increase with the doses specially at greater than 0.25%, and differe with the treated stages. The difference in days between control and treatments at does of 1.0% are 1.8 days for stage I, 2.2 days for the stage II, and 3.6 days for stage III. With tepa treatment at various immature stages of rice weevils, HYUN and SHIM(1965) reported longer developmental period with the high doses.

Table 2. Days required for completion of the postembryonic development when various larval stages were treated.

Age	Concentration						
	0	0.03	0.06	0.12	0.25	0.5	1.0
I	32.69	32.9	33.6	33.8	33.9	34.1	34.5
II	32.69	32.8	33.4	32.9	34.1	34.6	34.9
III	32.69	32.9	33.8	33.9	34.3	34.9	36.3

The emerged adults from the treated grain, specially higher doses, showed abnormality; decrease in size, abnormal development of elytra, light colour with decreased size to a half of the normal individual. More abnormal individuals were found with the treated stage I, than that at stage II, and none was found with treated stage III.

Statistical analysis shows that both the doses and the treated stages are significant. ($F, 6, 75, .05 = 2.32 < 6.283$ for does, $F, 6, 75, 05 = 2.32 < 4.816$ for treated stage).

The longer developmental periods and induction of abnormal individual might be resulted by the disturbance of the normal cell differentiation by the treatment. HYUN and SHIM(1965) reported prolonged postembryonic developmental period of the rice weevils from tepa treatment at greater than 0.25%.

It is interesting to note that shorter developmental periods found with younger larval stage treatment. This may be related with the greater selectivity (mortality) in the young larval stage treatments, that is, many of them would die, and few of selected individuals seemed to be remained in younger stage treatment.

The effects of apholate would be some partial retardation of the somatic cell differentiation and caused the prolonged postembryonic development, when the older larvae were treated.

(3) Effects on the oviposition

The four emerged weevils from treated grain were mated with opposite sexes from untreated grain. The average number of eggs laid by four females for five days period are showing in Table 3.

The average number of eggs per female decrease

Table 3. Average number of eggs laid by four female in five days, when adults from treated grain mated with opposite sexes from untreated grain.

Mating	Age	Concentrations						
		0.00	0.03	0.06	0.12	0.25	0.50	1.0
TF× NM	I	31.2	4.5	25.2	23.2	19.5	14.2	10.0
	II		28.9	27.4	19.7	25.2	20.6	12.3
	III		30.6	26.5	26.3	21.7	23.5	17.4
NF× TM	I		24.5	18.2	25.2	26.7	23.8	21.0
	II		31.1	34.8	31.5	34.7	21.4	19.2
	III		31.8	30.7	29.6	26.5	27.3	18.9

T=Treated; N=Normal; F=Female; M=Male.

with the increased doses of apholate, and differ with the treated sexes within the same treatment. The number of egg laid by four females during the period ranged from 10 to 17.4 eggs with female treatment, while it was from 18.9 to 21.0 with male treatment according to the treated stages at doses of 1%.

The reduction in oviposition rate of chemosterilant treated female have been reported by various workers. MORGAN and LABRECQUE(1964) reported some histological effects of tepa treatment on the ovary of house fly, and observed the retardation in egg formation. Such a retardation in egg formation results in reduction of oviposition in case of female treatment, and greater sterility for the male treatment as showing in later.

The decrease in number of eggs per female might be resulted by the direct retardation effects on the egg formation when the females were treated, and the effects would be greater with the higher dose. The decrease in number of eggs per female was not significant with the male treatments, except at higher than 0.5%, and may be related with the mating satisfaction. Because the small size or delayed development might have some effects on the vigor of the mating, this may be indicated by the greater effects with the stage I treatment.

The number of eggs per female was greater when older larvae were the treated. The older larvae may have greater potential for the egg formation. Since the retardation effects of apholate on the somatic cell differentiation might be greater when young larvae were treated, the potential egg formation might be small, when the larvae had been treated at younger stages.

2. Effects of the apholate on the biology of the progeny

(1) Effects on the mortality of the progeny

Four pairs of treated weevils and nontreated opposite sexes, put in 10 gs. of grain which had renewed every day for five days period. The removed grains were examined for the mortality as in previous experiment.

The total mortalities are calculated by reducing the number of emerged adults from the total number of eggs found in the grain at the begining of the experiment. Consequently, the mortalities are including the sterile eggs, and the mortalities during the postembryonic development. They are showing in Table 4.

Table 4. Total control of reproduction in the progeny when adults from the treated grain mated with the opposite sexes from untreated grain (corrected for control).

Mating	Age	Concentrations					
		0.00	0.06	0.12	0.25	0.5	1.0
TF× NM	I	0	47.94	80.41	90.72	96.39	100
	II		63.53	72.47	85.38	86.13	99.32
	III		76.59	88.41	95.87	97.56	98.38
NF× TM	I		83.42	93.78	92.75	96.41	100
	II		76.97	79.65	88.39	97.36	99.85
	III		84.78	89.93	93.65	92.14	100

The rice weevil complete its development in a grain, and it is difficult to separate the sterility from the larval mortality. The larval mortalities were examined by taking two gs. sample grain from the infested grain every six days. The larval mortalities and calculated sterilities are showing in Table 5 and 6.

As shown in Table 5, the total control of reproduction was greater with the increased doses and almost complete control could be obtained at dose of 1%. Male treatment seems to be more effective than female treatment. It is interesting that the least control effects were obtained with stage II treatment.

Table 5. The larval mortality of the progeny when the various larval stages of the previous generation were treated with various concentrations of apholate.

Mating	Age	Concentrations					
		0.00	0.06	0.12	0.25	0.5	1.0
TF× NM	I	0	35.54	32.16	38.69	40.18	41.09
	II		22.81	38.49	39.10	39.51	40.25
	III		16.70	19.87	22.97	25.64	33.77
NF× TM	I	0	45.35	46.76	48.69	52.40	64.14
	II		29.04	37.35	45.80	40.51	37.43
	III		26.87	30.50	41.18	38.24	37.50

Table 6. Calculated sterility of the emerged weevil from treated with apholate at various larval stages.

Mating	Ages	Concentrations					
		0	0.06	0.12	0.25	0.5	1.0
TF× NM	I	0	12.47	48.26	53.08	56.23	59.96
	II		40.66	33.99	56.31	58.63	59.03
	III		59.81	68.55	73.18	72.08	64.65
NF× TM	I		38.08	46.03	44.05	44.06	35.86
	II		47.94	42.30	43.57	54.97	61.41
	III		57.83	59.47	53.06	54.09	62.53

As shown in Table 6, the larval mortality was greater when younger stages were treated, while the sterile effects were greater when the older larvae had been treated. These may mean that the effects of the treatment would be mainly on the somatic cell for the younger stage treatment, while greater effects on the germ cell formation for the older larval treatment. The effects on the germ cell seem to be secondary for the younger stage treatment, but it is direct effects for the older stage treatment.

(2) **Effects on the developmental periods**

The number of emerged progeny weevils were counted every day, and the average postembryonic developmental periods are showing in Table 7.

The developmental periods increase with the higher doses, specially when the females were treated. The greater maternal effects may be caused by the physiological defects of the eggs. The developmental periods increase with the older larval treatment. This may mean that either the physiological defects of eggs

Table 7. Days required for completion of development of the progeny when treated adults mated with untreated adults.

Ages	Matings	Concentrations			
		0	0.25	0.5	1.0
I	TF×NM	32.69	33.86	35.08	34.13
	NF×TM		29.37	30.57	29.88
II	TF×NM		33.13	34.65	35.48
	NF×TM		31.30	33.14	34.15
III	TF×NM		34.16	35.96	37.18
	NF×TM		31.95	34.06	35.13

would be greater with later treatment or the selection through the lethal mutation may be greater with the earlier stage treatment.

Some morphological abnormalities were found among the emerged adult from the treatment of greater than 0.5 %, some adults showing unexpended elytra, small body size, and the head within the prothorax. Such morphologically animal adults were more with the treated stage I than any other stage treatments.

From foregoing discussion, the effects of apholate on the biology of the rice weevils seem to be related closely with the cell activities, and following generalization could be made.

The effects on the somatic cell would be complete death of the individual or partial growth retardation of the individual. Such partial growth retardation results in prolonged larval development, abnormal adults and/or subsequent effects on the germ cell differentiation. The greatest mortality, more abnormal individuals and the greatest reduction in number of eggs with treated stage-I would be related with the active cell division of the young larvae.

The effects on the germ cell would be complete inactivation of the germ cell, and partial effects on the germ cell inducing lethal mutation. The greater reduction effects on the oviposition with higher doses in treated female may be the direct inhibition of the egg formation, and greater stertlity of the egg may be caused by the inactivation of the germ cell.

The mortality during the postembryonic development of the progeny might be considered combined effects of the somatic cell and the genetic effects on the parents.

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