The Effect of Mercury Compounds on
Breaking of Diapause in the Eggs of
the False Melon Beetle, *Atrachya*
menestriesi FALDERMANN
(Coleoptera : Chrysomelidae)¹

The false melon beetle, *Atrachya menestriesi* FALDERMANN, one of the most serious pests of ladino clover in the northern part of Japan, deposits eggs on or into the earth in summer. Almost all the eggs arrest development at the appendage-formation stage several days after oviposition and hibernate (MIYA, 1965). Under the natural conditions the hibernated eggs hatch in the following spring. For a successive rearing in the laboratory it is desirable to break the diapause as quickly as possible. A usual method for breaking diapause is to expose eggs to a low temperature. In *A. menestriesi* as already reported by MAKI and ANDO (1967), chilling of eggs at 5°C for more than 60 days was necessary to break diapause. On the other hand, application of chemicals was successful in breaking diapause of some insects (HOGAN, 1961, 1962; SLIFER, 1946). This method may break diapause more quickly than chilling. An experiment was therefore carried out to explore chemicals effective for breaking

diapause of this species, and satisfactory results were obtained by using some mercury compounds.

MATERIALS AND METHODS

The same method as described in a previous paper (MAKI et al., 1966) was used for collecting and preserving the eggs. The eggs were treated with an aqueous solution of some mercury compounds by a filter paper method or a dipping method at the pre-diapause and diapause stages. In the former method the eggs were placed on filter paper saturated with the aqueous solution of a given chemical and held in Petri dishes at 26°C until hatching. In the latter method eggs were dipped in solutions of mercuric chloride (5 and 25 ppm as Hg) for certain periods of time and then washed in distilled water for 30 min. Thereafter they were placed on filter paper saturated with distilled water. The eggs were examined during a 20 day period after the treatment. Unhatched eggs were fixed with CARNOY's fluid and stained by carbol-thionine. Whether the embryo was living or dead was judged by its stainability with the dye.

RESULTS AND DISCUSSION

Effects of Mercury Compounds on Breaking Diapause: The results of filter paper application of various mercury compounds is summarized in Table 1. To break the egg diapause of this species, all of the chemicals used in the test proved to be effective. The optimum concentration of Hg with which the highest hatching ratio was obtained, was approximately 5 ppm (Table 1).

Mercuric chloride (5 ppm as Hg) was also effective in dipping, if the time of dipping ranged from 10 min to 8 hr. The rate of hatching increased by increasing the time of dipping up to 1 hr; however, a further prolongation of the treatment increased those eggs that failed to hatch after reaching the eye-spot stage. Furthermore, when the dipping time became too long, most of the eggs were killed at the diapause stage. A similar trend was obtained with a solution of 25 ppm, though a favourable effect on hatching was found in 5 or 10 min treatments. These results indicated that the dipping time for the highest hatch was related to the concentration of Hg (Table 2).

Table 1. EFFECT OF MERCURY COMPOUNDS
BREAKING EGG DIAPAUSE
OF *A. menestriesi*

Chemicals	Optimum concentration (ppm as Hg)	Percentage hatch
Phenylmercuric acetate	5.5	73.3
Methylmercuric chloride	3.8	81.3
Ethylmercuric phosphate	5.0	85.3
Mercuric acetate	5.0	77.3
p-Chloromercuri- benzoic acid	5.0	88.7
Mercuric chloride	7.4	84.0
Mercuric nitrate	6.4	91.3
Control (distilled water)	—	0

One hundred and fifty 30-day-old diapause eggs were treated with filter paper method.

¹ Appl. Ent. Zool. 4 (3): 149—151 (1969)

Table 2. EFFECT OF AQUEOUS SOLUTION OF MERCURIC CHLORIDE ON BREAKING THE DIAPAUSE OF *A. menestriesi*

Time dipped	5 ppm (Hg content)			25 ppm (Hg content)		
	Percentage hatch	Dead eggs at eye-spot stage (%)	Total	Percentage hatch	Dead eggs at eye-spot stage (%)	Total
1 min	—	—	—	18	1	19
5	2	—	2	66	3	69
10	36	1	37	66	12	78
30	64	2	66	63	21	84
1 hr	74	7	81	10	21	31
2	59	11	70	2	18	20
4	62	14	76	0	3	3
8	56	13	69	0	1	1
16	4	37	41	—	—	—
24	0	16	16	—	—	—

One hundred 30-day-old diapause eggs were used with dipping method.

Effect of Mercury Compounds on Pre-diapause Eggs: The pre-diapause eggs (1-4 days after oviposition at 26°C) were dipped in solutions of mercuric chloride (10 grades in concentration from 0.5 to 30 ppm as Hg). As shown in Table 3, the treatment was effective in averting diapause. The optimum concentration of the chemical for this effect was lower than that for breaking the established diapause. In the treated eggs, development continued steadily without evidence of diapause and hatched normally.

Effect of Mercury Compounds Mixed with Some Sulfhydryl Compounds: From the results mentioned above, it was suggested that the breaking of diapause might be caused by mercury ion itself. Then following experiments were carried out to confirm this point.

Solutions of sulfhydryl compounds (see, Table 4) and of 10 ppm (as Hg) mercuric chloride were mixed in a ratio of 1:1. Then, diapause eggs were treated with the mixed solution by means of the filter paper method. As shown in Table 4, almost all the eggs did not hatch, though they were surviving. These results can be explained by assuming

that the ionized Hg in the solution of mercury compounds became associated with the SH group of the sulfhydryl compounds and formed the so-called mercaptides, which did not have diapause breaking action. It might be expected that other water-soluble Hg compounds, which were not used in the present studies, will also be effective.

A successive rearing of this species is now established by application of Hg compounds for a practical purpose.

The authors wish to express their gratitude

Table 3. EFFECT OF MERCURIC CHLORIDE FOR PRE-DIAPAUSE EGGS OF *A. menestriesi* (FILTER PAPER METHOD)

Days after oviposition	Number of eggs examined	Optimum concentration for hatching (ppm as Hg) without diapause	Percentage hatch	Days required for hatching after treatment
1	98	2.5	69.4	12.4
2	109	2.5	80.7	11.5
3	87	2.5	67.8	10.7
4	135	5.0	53.3	11.5
5*	91	5.0	71.4	12.6
6*	117	5.0	70.9	13.1
8*	63	5.0	68.3	12.9
30*	49	10.0	75.5	12.8
Control**	74	—	76.5	12.2

* All of the eggs reached at the diapause stage.

** Non-diapause eggs without Hg-treatment.

Table 4. EFFECT OF MIXED SOLUTIONS OF MERCURIC CHLORIDE AND SULFHYDRYL COMPOUNDS ON THE DIAPAUSE EGGS OF *A. menestriesi*

Chemicals mixed with mercuric chloride	Concentration (M)	Diapause* breaking (%)	Surviving** eggs (%)
Glutathione (reduced)	0.01	0	65
Cysteine hydrochloride	0.01	2	77
α -Lipoic acid	0.005	0	82
β -Mercaptoethanol	0.04	9	60
Control (distilled water)	—	91	—

One hundred eggs were examined each experiment.

* Represented by hatched eggs and dead eggs reached the eye-spot stage.

** This was confirmed by hatching after re-treatment with mercuric chloride.

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Preliminary Report on the Primary Culture of Smaller Brown Planthopper Cells *in Vitro* (Hemiptera : Delphacidae)¹

Since MITSUHASHI and MARAMOROSCH (1964) established the method for the cultivation of leafhopper cells *in vitro*, the cells from several species of leafhoppers have been cultivated successfully in primary cultures. The culture medium NCM-2B (MITSUHASHI, 1969) and the like supported well the outgrowth of the embryonic cells from *Macrostelus fascifrons*, *Agallia constricta*, *Dalbulus maidis*, *Nephotettix cincticeps*, *N. apicalis*, and *Inazuma dorsalis* (MITSUHASHI and MARAMOROSCH, 1964; MITSUHASHI, 1965, 1969; CHIU et al., 1966). All the above leafhoppers are vectors of plant pathogenic microbes and the cultures of their cells had been done for the purpose of investigat-

ing those pathogenic microbes. With the same aim the present author has attempted to cultivate the cells of the smaller brown planthopper, *Laodelphax striatellus* FALLÉN, which is the vector of rice stripe virus, rice black streaked virus, and northern mosaic virus of barley, wheat and oat, and the results hitherto obtained are reported here.

For the cultivation of *L. striatellus* embryonic cells, the same technique and the same culture medium as those used in the leafhopper cell cultures were employed (MITSUHASHI and MARAMOROSCH, 1964). But almost no outgrowth of cells was obtained by this method. Then each process of setting up cultures and the composition of the medium were examined for their adequacy for *L. striatellus* cell culture.

Firstly, the stages of embryos to be used as explants were examined. The embryos around blastokinetic stage have been reported to be the most suitable explants in the case of leafhopper

¹ Appl. Ent. Zool. **4** (3) : 151-153 (1969)