Journal of the American Mosquito Control Association, 9(4):426–430, 1993

EFFECTS OF THE INSECT GROWTH REGULATORS OMS 2017 AND DIFLUBENZURON ON THE REPRODUCTIVE POTENTIAL OF *AEDES AEGYPTI*

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ABSTRACT. The effects of 2 benzoylphenylurea insect growth regulators, OMS 2017 and diflubenzuron, on 4th instar larvae of *Aedes aegypti* were investigated in the laboratory. Apart from delayed lethal action, possible effects on the reproductive potential were also studied. A baseline concentration of each compound causing 30% emergence inhibition was determined. At this concentration, OMS 2017 affected fecundity of the mosquito but diflubenzuron did not. The fertility of females that survived OMS 2017 exposure also was decreased; 23% of the eggs reached the 4th larval instar. Diflubenzuron did not cause any reduction in fertility. The basal follicle number showed great variability after both treatments as it was alternatively equal, lower, or higher than in the control.

INTRODUCTION

Insect growth regulators (IGRs) are considered a new generation of insecticides having great prospects for insect control. These compounds are specific in activity against arthropods, particularly insects. They are biodegradable and thus do not accumulate in environment. They are safe to a large number of nontarget organisms including mammals (Beadles et al. 1975, Apperson et al. 1978).

Best control of insects through the use of IGRs is possible because of their interactions with hormonal control of growth and development. Target tissues for these compounds are the same as for juvenile hormones or ecdysones. The IGRs that seem to act on ecdysone-dependent mechanisms, such as diflubenzuron, are listed as inhibitors of insect molting. Several hypotheses have been proposed to explain their mode of action, such as inhibition of chitin synthesis (Post et al. 1974) by inhibiting chitine synthetase (Van Eck 1979), but also through increase of chitinase activity (Ishaaya and Casida 1974). However, the mode of action of IGRs appears more complex because they probably act on several physiological systems and also differently depending on the compound.

The activity of IGRs generally results in the reduction of adult emergence of the target insect. However, they seem to have side effects, particularly on female reproduction, as indicated by several studies utilizing molt inhibitors as well as juvenile hormone mimics, the other main group of IGRs (Arias and Mulla 1975). For example, effects on fecundity (increase or diminution of the number of eggs laid) and on fertility (reduction of hatchability or viability of eggs) of mosquitoes following larval IGR treatments were documented (Miura et al. 1976, Kelada et al. 1981).

This study investigated effects of 2 benzoylphenylurea molt inhibitors, OMS 2017 and diflubenzuron, on fecundity and fertility of females of *Aedes aegypti* (Linn.) that survived larval exposure to the IGRs. The effects of both compounds on the basal follicle number developed by the surviving females was also studied to understand disturbances of fecundity.

MATERIALS AND METHODS

Mosquito treatment: The IGRs, OMS 2017 WP 25%, [1-(3,5-dichloro-4-(2,2-dichlorocyclopropyl-methoxy)-phenyl)-3-(2,6 difluorobenzoyl) urea] and diflubenzuron (DFB) WP 25%, [1-(4-chlorophenyl)-3-(2,6 difluorobenzoyl) urea], used in the present study were provided by World Health Organization. The bioassays were conducted on the GKEP strain of *Ae. aegypti*, originally collected from Ghana and maintained since 1985. The larval colonies, kept at 28 ± 1°C, were fed a mouse biscuit powder. Adults were maintained at 26 ± 1°C and 65% RH; they were provided a 10% glucose solution and females a periodic blood meal on a guinea pig.

Early 4th instar larvae were exposed to a range of concentrations of OMS 2017 and diflubenzuron according to the procedure of Mulla et al. (1974). Four replicates of 25 larvae for each concentration and corresponding controls were maintained for each IGR evaluated on 3 different occasions. During the exposure period of 24 h, larvae were not supplied with food. After 24-h exposure, larvae were washed thoroughly with tap water and transferred to a pan containing 2 liters of tap water. Each pan, one per concentration, contained 100 larvae. Pupae, when appeared, were caged for emergence. Dead individuals (larvae, pupae, and adults) were recorded daily. Concentrations that induced 30% emer-

Table 1. Concentrations (mg/liter) of the IGRs OMS 2017 and diflubenzuron inducing 30, 50, and 90% of emergence inhibition (EI) of *Aedes aegypti* in the laboratory.

Compound	EI_{30}	EI_{50}	EI_{90}
OMS 2017	0.0029 (0.0019–0.004)[1]	0.0078 (0.0058–0.0104)	0.085 (0.045–0.259)
Diflubenzuron	0.00026 (0.00019–0.00033)	0.0005 (0.00042–0.00061)	0.0035 (0.0026–0.005)

[1] 95% CI in parentheses.

gence reduction (EI_{30}) were then determined for each compound by probit analysis. Subsequently, 4th instar larvae were exposed to these concentrations of each compound and surviving adults from each treatment were collected to evaluate the effects on fecundity, fertility, and basal follicle number.

Fecundity and fertility of surviving females: Four to 5 days after emergence, surviving females were fed on a guinea pig. They were then placed individually in vials for oviposition, which occurred 48 h after the blood meal. The mean number of eggs laid was noted. Eight to 10 days later, 100 eggs were placed in water to evaluate the impact of treatments on the egg hatch.

Basal follicle number in surviving females: Ovaries of surviving females were dissected under a stereoscopic microscope. The last abdominal segment was pulled away with the ovaries attached to determine the number of basal follicles. Dissections were made in saline solution. Ovaries were transferred in a clear droplet of saline solution on the same slide. Adhering tissues were removed, ovarioles were separated, and the basal follicle number was determined. Sutherland et al. (1967) stated that the basal follicle number is numerically equal to the "ovariole number" provided each ovariole contains a basal follicle.

The OMS 2017-treated females were dissected at different periods: 24 h after emergence, 24 h after a blood meal that occurred 4–5 days after emergence, and 5 days after oviposition. For each period, larval treatments were repeated several times. Dissections of DFB-treated females occurred only 24 h after a blood meal with 2 replicates. Differences between treated and untreated females were analyzed by a Student *t*-test.

RESULTS AND DISCUSSION

Diflubenzuron caused higher larval and pupal mortality than OMS 2017, which induced more delayed effects. We observed 5% larval and pupal mortality at 0.001 mg/liter of OMS 2017; the same concentration of DFB induced 17% larval and 21% pupal mortalities. Laboratory bioassays revealed that diflubenzuron was more effective than OMS 2017 (Table 1). Concentrations chosen for next treatments with both IGRs were those that induced 30% emergence inhibition (EI_{30}).

Effects on fecundity and fertility: Females that survived the OMS 2017 treatment laid 30% less eggs compared to controls and this difference was statistically significant ($P < 0.001$). Our results were based only on the surviving population and so displayed a real reduction of the reproductive capacity after the OMS 2017 application. Fecundity of DFB-treated females was not affected. The variability of the effects of IGRs on reproduction was confirmed by different authors. Ke-

Table 2. Effects of the IGRs OMS 2017 at 0.0029 mg/liter[1] and diflubenzuron at 0.00026 mg/liter[1] on fecundity and fertility of female *Aedes aegypti* surviving larval treatments in the laboratory.

Compound	Emergence inhibition (%)	No. of fed females	Average no. of eggs laid ± SD	F_1 4th instar larvae (%)
OMS 2017				
Control	8.6	32	80 ± 37	65
Treated	32.8	25	56 ± 23[2]	23
Diflubenzuron				
Control	11.0	28	68 ± 21	86
Treated	24.0	21	68 ± 30[3]	83

[1] Concentration producing 30% emergence inhibition (EI_{30}).
[2] Significant difference ($P < 0.001$)
[3] No significant difference.

Table 3. Effects of the IGR OMS 2017 on the basal follicle number developed by female *Aedes aegypti* that survived several larval treatments.

	Time of dissection					
	24 h after emergence		24 h after blood meal		5 days after oviposition	
	EI[1] (%)	BFN[2] (±SD)	EI[1] (%)	BFN[2] (±SD)	EI[1] (%)	BFN[2] (±SD)
Control	4.7	94 ± 14	6	93 ± 22	8.7	90 ± 20
Treated	33.5	99 ± 23[3]	56	107 ± 23[4]	41.5	91 ± 14[3]
Control	8	96 ± 19	8.8	82 ± 19	7.4	91 ± 19
Treated	73	100 ± 23[3]	60.5	82 ± 19[3]	36.4	80 ± 12[4]
Control	3.4	109 ± 16	3.3	84 ± 13	6	88 ± 15
Treated	14.5	96 ± 23[4]	13.7	78 ± 13[4]	32.4	93 ± 16[3]
Control	11.4	115 ± 16	8.8	103 ± 14		
Treated	14.7	93 ± 14[4]	28.7	104 ± 15[3]		

[1] Emergence inhibition.
[2] Mean of basal follicle number.
[3] No significant difference.
[4] Significant difference ($P \leq 0.01$).

lada et al. (1981) tested several IGRs, including methoprene (Altosid®) and diflubenzuron (TH6040), against *Culex pipiens* Linn. larvae for their effects on the reproductive potential of the surviving adults and one following generation. Results varied according to the compound and the applied concentration. The average number of eggs deposited was affected by treatment. The numbers increased with methoprene in both generations. After treatment with diflubenzuron, fecundity increased only in the second generation. Diflubenzuron had no effect upon the treated generation and caused an increase or a decrease that was not dose-dependent.

OMS 2017 caused a significant reduction of fertility of surviving females ($P \leq 0.0001$): only 23% of the eggs reached the 4th instar (Table 2). Fifty-eight percent of the treated eggs had still not hatched 12 h after the immersion vs. only 36% in the control. This difference was statistically significant ($P \geq 0.0001$). The presence of an embryo had been observed and so there was probably additional larval mortality during the first stages. Diflubenzuron had no effect on the fertility of the surviving females.

Effects on the development of ovarioles: The basal follicle number showed wide variability from the effects of both IGRs (Tables 3 and 4). In surviving females, it was equal, lower, or higher than that observed in the control. In the assays, the emergence inhibition was not always at 30% as expected; it varied from 73 to 15% and 23 to 39% for OMS 2017- and DFB-treated adults, respectively. It was then possible to define a correlation between the basal follicle number and the emergence inhibition. With OMS 2017 treatment (Table 3), when the emergence inhibition was lower than 30%, the basal follicle number tended to be lower than the control: we observed that with 14.7% EI, treated females dissected 24 h after emergence had 93 ovarioles and the control had 115 ovarioles. With diflubenzuron, results were the same (Table 4); when EI was 23%, treated females had significantly fewer ovarioles than the control (86 and 111, respectively). In *Cx. pipiens* females that survived larval treatment by methoprene and diflubenzuron, the basal follicle number increased (Gaaboub 1976) without any dose-dependent effect. This variability might be based either on the actual ingested amount of compound, possibly evaluated by the emergence inhibition, or on the physiological state of larvae at the time of treatments, which was difficult to determine because synchronously reared larvae were difficult to obtain. Our results led to the hypothesis that, if treatment induced higher mortality, weaker individuals were eliminated and survivors were those

Table 4. Effects of the IGR diflubenzuron on the basal follicle number developed by surviving female *Aedes aegypti*, dissected 24 h after the blood meal.

	Emergence inhibition (%)	Mean basal follicle no. ± SD
Control	2.5	99 ± 14
Treated	39.3	103 ± 10[1]
Control	9.5	111 ± 15
Treated	23.0	86 ± 16[2]

[1] No significant difference.
[2] Significant difference ($P < 0.001$).

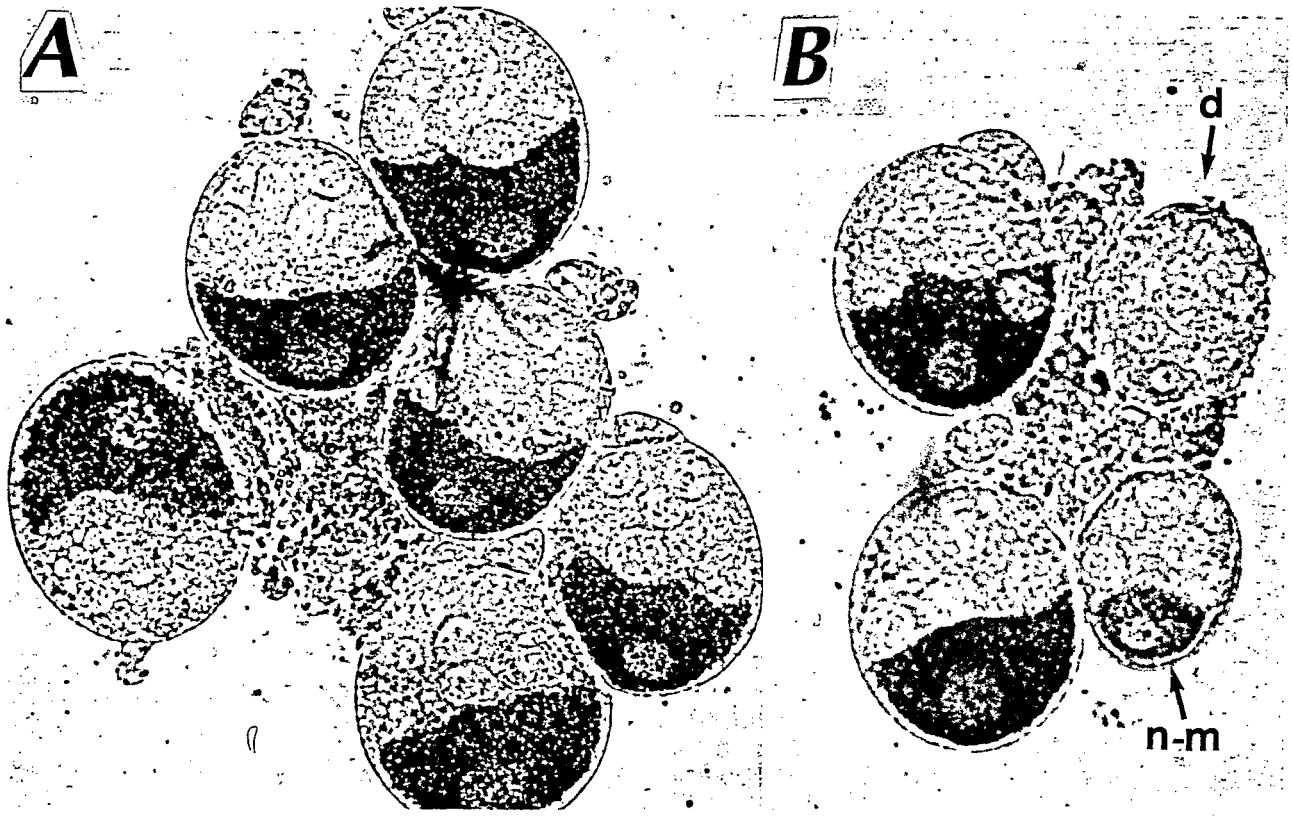

Fig. 1. *Aedes aegypti* follicles at 24 h post-blood meal. A. Normal untreated follicles (100×); B. OMS 2017-treated follicles showing degeneration (d) and nonmatured follicle (n-m) (100×).

that presented a higher reproductive capacity than the control. On the other hand, with a lower pressure, the decrease of the reproductive capacity showed the physiological effect of the treatments. According to Gaaboub (1976), treatment may cause a stimulus before or at the time the number of ovarioles is determined. Rudiments of ovaries appear in mosquito females at the 3rd larval stage (Parks 1955[1]) and if the organization of the gonads becomes obvious at the beginning of the 4th larval stage, ovarioles only differentiate at the pupal stage. Then stimulus of the treatment could induce the maturation of some rudimentary ovarioles or the inhibition of other ones. According to the last hypothesis, OMS 2017 treatment could inhibit the development of some ovarioles in larvae.

Degenerating and nonmatured follicles were observed during dissections (Fig. 1). They were more abundant in DFB-treated females than in OMS 2017-treated ones ($P < 0.02$). As the reproductive potential of surviving females was less affected after diflubenzuron treatment, it suggests that diflubenzuron could act later than OMS 2017, during oogenesis in adults. This hypothesis agrees with the observations of Behan and Hagedorn (1978) showing that larval fat body is not transformed at the time of metamorphosis. If diflubenzuron has an effect on the larval fat body, some of the consequences might be observed at the adult stage during vitellogenin synthesis. The IGRs used might have dual effects on the determination of the basal follicle number, which occurred during the larval life, and the ovarial maturation after the blood meal.

The decrease of fecundity observed after a sublethal larval treatment by OMS 2017 may be explained by a decrease of the basal follicle number developed. Larval treatments often have delayed effects and epidermal cells and reproductive tissues can be considered possible targets of this compound. Results obtained with diflubenzuron show that the mode of action of this compound can be different or that, at the applied concentration and at the time of the treatment, ovaries of *Ae. aegypti* are more susceptible to OMS 2017 than to diflubenzuron.

The entire results obtained with OMS 2017 showed that even if the treatment did not cause 100% adult reduction, the size of the residual cage population was reduced. Field studies are needed to confirm these observations.

[1] Parks, J. J. 1955. An anatomical and histological study of the female reproductive system and follicular development in *Aedes aegypti* (L.). M.Sc. thesis. University of Minnesota, St. Paul.

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