were inseminated (Table 1). This is lower than the 55% found during the following year in the same forest by Scholl et al. (1979b). As Thompson (1979) has shown that venereal transmission of LAC virus occurs at a significantly higher rate in females that have taken a blood meal before mating with infected males, the high proportion of uninseminated nulliparous females in biting collections is of epidemiological relevance.

Examination of Malpighian tubules revealed that 39% of females were infected with *Ascogregarina barretti* (Table 1). The gregarine was more prevalent, however, in nulliparous than in parous females, this being especially so in the case of virgin females. Fifty percent of 191 virgin nulliparous females contained oocysts compared to 36% of 96 inseminated nulliparous females and 32% of 264 parous females. Some oocysts persist in infected females of *Ae. triseriatus* after their first and even after their second oviposition (30%, n = 37). On the basis of the high natural incidence of *A. barretti* found in this study, Miller and DeFoliart (1979) restudied *Ae. triseriatus* larval susceptibility to infection from ingested LAC virus (Miller et al. 1978) and found that *A. barretti* infection does not increase larval susceptibility, nor do the spores serve as a vehicle for the virus.

This research was supported by the Research Division, College of Agricultural and Life Sciences, University of Wisconsin-Madison, and by National Institutes of Health Grant AI-07453.

References Cited

Burkot, T. R. and G. R. DeFoliart. 1982. Bloodmeal sources of *Aedes triseriatus* and *Aedes vexans* in a southern Wisconsin forest endemic for La Crosse encephalitis virus. Am. J. Trop. Med. Hyg. 31:376–381.

DeFoliart, G. R. 1983. *Aedes triseriatus:* Vector biology in relationship to the persistence of La Crosse virus in endemic foci. p. 89–104. *In:* Calisher, C. H. and W. H. Thompson (eds.), California Serogroup Viruses. Alan R. Liss, Inc., New York.

Detinova, T. S. 1962. Age-grouping methods in Diptera of medical importance. W.H.O. Monogr. Ser. 47. Geneva. 216 p.

Mather, T. N. and G. R. DeFoliart. 1983. Effect of host blood source on the gonotrophic cycle of *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 32:189–193.

Miller, B. R. and G. R. DeFoliart. 1979. Infection rates of *Ascocystis*-infected *Aedes triseriatus* following ingestion of La Crosse virus by the larvae. Am. J. Trop. Med. Hyg. 28:1064–1066.

Miller, B. R., G. R. DeFoliart, W. R. Hansen and T. M. Yuill. 1978. Infection rates of *Aedes triseriatus* following ingestion of La Crosse virus by the larvae. Am. J. Trop. Med. Hyg. 27:605–608.

Miller, B. R., G. R. DeFoliart and T. M. Yuill. 1979. *Aedes triseriatus* and La Crosse virus: Lack of infection in eggs of the first ovarian cycle following oral infection of females. Am. J. Trop. Med. Hyg. 28:897–901.

Scholl, P. J., C. H. Porter and G. R. DeFoliart. 1979a. *Aedes triseriatus:* Persistence of nulliparous females under field conditions. Mosq. News 39:368–371.

Scholl, P. J., G. R. DeFoliart and P. B. Nemanyi. 1979b. Vertical distribution of biting activity by *Aedes triseriatus*. Ann. Entomol. Soc. Am. 72:537–539.

Thompson, W. H. 1979. Higher venereal infection and transmission rates with La Crosse virus in *Aedes triseriatus* engorged before mating. Am. J. Trop. Med. Hyg. 28:890–896.

EFFICACY OF THREE INSECT GROWTH REGULATORS ON THE DEVELOPMENT OF *AEDES AEGYPTI*

FRED R. S. NELSON, ABDUL K. MOHAMED AND P. VATTIKUTTI

Department of Biology, Jackson State University, Jackson, MS 39217

The basic strategy in utilizing insect growth regulators (IGRs) is to interrupt the development and growth processes of the target organism and thus inhibit emergence of an adult population. A number of IGRs such as methoprene (Altosid) (Mulla and Darwazeh 1975), diflubenzuron (Mulla et al. 1974) and Mon-585 (Jakob 1972) have shown exceptional high levels of activity against mosquitoes or chironomid midges. The growth regulating activities of benzyl, 1,3, benzodioxole derivatives and benzylphenols were reported by Nelson and Tehrani (1982) on the yellow fever mosquito, *Aedes aegypti* (L.), and Dame and Jurd (1983) on three mosquito species, *Anopheles quadrimaculatus* Say, *An. albimanus* (Wied.) and *Ae. taeniorhynchus* (Wied.). This study reports the inhibitory or ovicidal activities of J2532, J2645 and J2644 on *Aedes aegypti.*

The *Aedes aegypti* stock used was from an established laboratory colony. Adults were maintained on 10% sucrose solution supplemented with a blood meal for the females from a restrained mouse. Larval diet consisted of finely ground Purina dog chow. The technical grade compounds J2532 (2, 4-bis [1,1-dimethylethyl]-6-[4-methoxyphenylmethyl]-methoxybenzene), J2645 (2, 6-bis [1,1-dimethylethyl]-4-[4-methoxyphenylmethyl] phenol), and J2644 (2, 4-bis [1,1-dimethylethyl]-6-[4-methoxyphenylmethyl] phenol), were supplied by Dr. Leonard Jurd, Western Regional Research Center, ARS, USDA, and Dr.

S. C. Rawlins, Screwworm Research, SEA, USDA, Mission, Texas. All experiments were conducted at 27 ± 2°C and were replicated four times. The concentrations tested in this study were obtained by the preparation of stock solutions in acetone and subsequent water dilutions.

Tests on the effects of J2532 and J2645 on adult emergence were conducted in 250 × 80 mm glass dishes. The two IGR-compounds were tested at 0.003, 0.01, 0.5 or 1.0 ppm. Each dish contained 1000 ml solution of the desired concentration of either J2532 or J2645 and 100 early fourth-stage larvae. Untreated larvae were included in all tests as controls. Larval diet consisting of finely ground Purina dog chow was provided (ca. 2 shakes/dish from a salt shaker) on day 1 and 3–4 days later.

Each dish was capped with fine white cloth netting (ca. 40 mesh) to prevent adults from escaping. Dishes were monitored at intervals of 24 hr and dead or moribund larvae, pupae and adults were removed. Those adults that appeared extremely weak were recorded as dead, while those observed resting on the cloth netting or flying within the dishes were transferred to a cage (30 × 30 × 30 cm diam), counted and scored as normal adults, but were not offered food. The results of this test were analyzed by ANOVA and the Duncan's (1955) New Multiple Range Test (P = 0.05).

To evaluate the ovicidal activity of the 3 IGRs, eggs less than 18 hr old were used in all tests. A number of newly emerged males and females were caged together for 3 days and on day 4 they were allowed to feed for ca. 3 hr on a restrained mouse. Then, 20 females (12 to 14 hr post-blood meal) were transferred singly to separate 1-pt. (0.47 liter) paper cups with water, lined with strips of paper 5.1 cm high and capped with cloth netting. Egg papers were removed randomly from five of the oviposition cups and the papers were then air dried to remove excess moisture.

Each egg paper was cut into three pieces which were immersed in 3 ml of 0.01, 1.00 or 5.00 ppm solution of the appropriate IGR for 5 or 30 min. The control egg papers were immersed in water and acetone only. Subsequent to the two exposure periods, treated and untreated egg masses were rinsed three times and transferred to separate petri dishes containing distilled water. Six days after exposure, egg hatch was determined under a stereomicroscope as described by Miura and Takahashi (1979).

INHIBITORY EFFECTS. Table 1, summarizes the effects of J2532 and J2645 on the development of *Ae. aegypti*. There was only slight reduction (<10%) in the larval population at application rates with both compounds. Thus, the inability of the IGRs to induce high levels of larval mortality, resulted in a large number (>90%) of these larvae reaching the pupal stage. The highest level of mortality (80.8%) occurred in the pupal population. Statistical analysis of these data revealed no significant differences (P=0.05) between the controls, larvae and adults at dosage levels tested.

The IGR J2532 produced 75.6% pupal mortality at 0.5 ppm and 77.2% at 1.0 ppm, while J2645 yielded 74.6% at 0.5 ppm and 80.8% at 1.0 ppm. Comparatively, mortalities in the untreated checks were 6.2 and 5.4% (Table 1). At 0.003 ppm there was no significant effect on pupal survival, whereas at 0.01 ppm an intermediate level of control was observed. Pupal mortality at 0.01, 0.5, and 1.0 ppm levels of both IGRs was significantly (P=0.05) greater than the controls. Inhibition of adult emergence reached a maximum of 86% at 1.0 ppm with both IGR-compounds. The levels of activity detected in this study with these compounds were less than those reported for methoprene, diflubenzuron and Mon-585. Also, the IGR activity of the two compounds parallel each other as indicated in these tests.

OVICIDAL ACTIVITY. The length of exposure (Table 2) revealed erratic effects on the percentage hatch with increasing concentration levels in some treatments. These were probably due partially to variability in the rate of egg hatch. However, a 5 ppm dose of J2532 (30 min) yielded the highest reduction in hatchability (67%). In all except one case, (74% at 0.01 ppm of J2532), the percentages of eggs hatched in relation to exposure periods (30 and 5 min) did not vary markedly with concentration levels of J2532, J2645 or J2544. The com-

Table 1. Average percent mortality of *Aedes aegypti* in larval stage with insect growth regulators.

IGR	Dosage (ppm)	Larvae	Pupae	Adults	E/I[2]
J2532	0.003	1.2 Aa[1]	4.4 Aa	0.0 Aa	5
	0.01	1.2 Aa	47.6 Bb	0.6 Aa	49
	0.50	6.2 Aa	75.6 Bb	1.0 Aa	82
	1.00	8.8 Aa	77.2 Bb	0.2 Aa	86
	0.00	0.6 Aa	6.2 Aa	0.2 Aa	7
J2645	0.003	5.0 Aa	9.4 Aa	0.8 Aa	15
	0.01	2.6 Aa	60.0 Bb	0.8 Aa	63
	0.50	4.2 Aa	74.6 Bb	0.6 Aa	79
	1.00	5.4 Aa	80.8 Bb	0.4 Aa	86
	0.00	3.4 Aa	5.4 Aa	0.2 Aa	9

[1] Means not followed by a capital letter vertically and small letter horizontally differ significantly (P = 0.05) according to Duncan's New Multiple Range Test.

[2] E/I = Emergence inhibition.

Table 2. Ovicidal activity of three insect growth regulators on *Aedes aegypti* eggs at indicated periods.

Insect growth regulator	Concentration (ppm)	Exposure (min)	Percent hatch
Control (water + acetone)	0.00	5	91
Control (water + acetone)	0.00	30	81
J2532	0.01	5	74
	0.01	30	36
	1.00	5	34
	1.00	30	56
	5.00	5	40
	5.00	30	33
J2645	0.01	5	44
	0.01	30	53
	1.00	5	52
	1.00	30	48
	5.00	5	39
	5.00	30	38
J2644	0.01	5	56
	0.01	30	51
	1.00	5	43
	1.00	30	41
	5.00	5	42
	5.00	30	39

parative ovicidal activities of the three IGRs closely paralleled each other.

In contrast, concentration levels of 0.1 and 1.0 ppm of the IGR-inhibitor, SIR 8514 (2-Chloro-N-[4-triflurомethoxy)phenyl] amino]-carbonyl] benzamide) induced high percentages of abnormally (longitudinally split egg shells or partially hatched eggs) hatched *Culex quinquefasciatus* Say eggs (Miura and Takahashi 1979). At similar dose levels in our tests only minimal numbers (ca. 1%) of longitudinal split egg shells were observed. The dissimilarity in the level of activity observed in our study and that of Miura and Takahashi may be due to the differences in the age of the egg or species tested. Those workers reported that eggs exposed to IGRs 24 hr post-oviposition were more tolerant than those exposed at earlier ages. Nelson et al. (1982, 1983a, 1983b) studied the growth regulating and sterilizing activities of several related IGRs against *Ae aegypti* and *Cx. quinquefasciatus*. Of the six IGRs tested, J2706 (2, 4-bis(1,1-dimethylethyl)-6-(phenylethyl) phenol) (A13-70736) and J2931 (2, 4-bis(1,1-dimethylethyl)-6-(4-methoxyphenylmethyl)-methoxybenzene) showed the greatest promise (>90%) in inhibiting the emergence of adults. *Culex quinquefasciatus* was the most susceptible of the two species to the IGRs tested. Unlike the activity of methoprene (Aris and Mulla 1975) no morphogenetic aberrations (larval-pupal-intermediates or pupal-adult-intermediates) were observed in the treatment replicates. However, in our current study, treatments of eggs with J2532, J2645 and J2644 resulted in an insignificant number of hatched eggs with longitudinal splits. Nelson and Tehrani (1982) evaluated the sterilizing effects of the IGRs, J2644 and J2581 (5-ethoxy-6-(4-methoxyphenylmethyl)-1,3-benzodioxole against *Ae. aegypti*. The authors found that the two IGRs did not significantly affect the fertility of either sex of the species or reduced hatchability of eggs from treated females mated to normal males.

ACKNOWLEDGMENTS

The study reported herein was supported by Grant No. RR08047 from the National Institutes of Health. Thanks are also extended to Drs. S. C. Rawlins and L. Jurd for supplying the IGR-compounds.

References Cited

Aris, J. R. and M. S. Mulla. 1975. Morphogenetic aberrations induced by a juvenile hormone analogue in the mosquito, *Culex tarsalis*. J. Med. Entomol. 12:309.

Dame, D. A. and L. Jurd. 1983. Assessment of benzyl and cinnamyl substituted phenol and 1,3-benzodioxole derivatives as IGRs against mosquitoes. Mosq. News 43:50–54.

Duncan, D. B. 1955. Multiple range and multiple F. tests. Biometrics 11:1–42.

Jakob, E. L. 1972. Mosquito larvicide studies with Mon-585 a juvenile hormone mimic. Mosq. News 32:6–10.

Miura, T. and R. M. Takahashi. 1979. Effects of the insect growth inhibitor SIR 8514 on hatching of southern house mosquito eggs. J. Econ. Entomol. 72:692–694.

Mulla, M. S., R. Norland, T. Ikeshoji and W. L. Kramer. 1974. Insect growth regulators for the control of aquatic midges. J. Econ. Entomol. 67:167–170.

Mulla, M. S. and H. A. Darwazeh. 1975. Evaluation of insect growth regulators against *Psorophora confinnis* in southern California. Mosq. News 35:281–285.

Nelson, F. R. S. and B. Tehrani. 1982. Effects of benzylphenol and benzyl-1, 3-benzodioxole derivatives on fertility and longevity of the yellow fever mosquito. J. Econ. Entomol. 75:877–878.

Nelson, F. R. S., A. Rejali and A. K. Mohamed. 1983a. Performance of an insect growth regulator on two species of mosquitoes. J. Georgia Entomol. Soc. 18:359–363.

Nelson, F. R. S., A. Rejali and A. K. Mohamed. 1983b. Susceptibility of *Aedes aegypti* and *Culex quinquefasciatus* to a new insect growth regulator. J. Florida Anti-Mosq. Assoc. 54:8–10.