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EFFECTS OF TWO INSECT GROWTH REGULATORS ON THE SUSCEPTIBILITY OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE) TO *MOLINEMA DESSETAE* (NEMATODA: FILARIOIDEA)

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ABSTRACT. The effects of 2 growth regulators, diflubenzuron (DFB) and OMS 2017, on the ability of females *Aedes aegypti* to become infected with *Molinema dessetae* was studied under laboratory conditions. OMS 2017 and DFB had no effect on either the amount of blood ingested or the microfilarial load. The infective potential of females that survived DFB treatment was significantly greater than untreated females, but there was no difference between OMS 2017-treated and control females. The percentage of infective larvae in the head after OMS 2017 and DFB treatments was significantly greater than for control females. Insect growth regulators appear to affect the vectorial competence of mosquitoes, and these results indicate the need for preliminary studies before these compounds are used in large-scale control programs.

INTRODUCTION

The capacity of insects surviving insecticidal treatment to transmit parasites is important in understanding the epidemiological impact of control. The impact of sublethal concentrations of insect growth regulators (IGRs) on the parasite–vector relationship remains poorly understood. Insect growth regulators affect hormonal control of mosquito growth and development. The main effect of IGRs is the reduction of adult emergence, but reproduction and ecdysteroid production in surviving females also are affected (Fournet et al. 1993, 1995). The vectorial process may be affected in different ways. The vector may show morphological or physiological changes that alter its capacity to transmit the parasite. Gaaboub and Busvine (1976), for example, showed that application of the IGR PH60:40 (diflubenzuron [DFB]) on larval *Aedes aegypti* (Linn.) modified the potential of a refractory strain (T8) to transmit *Brugia pahangi.* Treatments broke down the refractory nature of the T8 strain significantly, so that 12–16% of the female mosquitoes developed mature filaria larvae. In addition, treatments may affect the development of certain nematode parasites in mosquitoes (Gwadz and Spielman 1974).

In the present research, we studied the effect of sublethal larval treatments by 2 molt inhibitors, OMS 2017 and DFB, on the capacity of surviving *Ae. aegypti* to transmit *Molinema dessetae* (Bain). The objective was to determine if these treatments affected the uptake of microfilariae (mf) and the proportion that developed into 3rd-stage larvae. The use of an experimental filariasis model allowed the study of the mechanisms that manage the vectorial processes. Elissa (1990[1]) used this experimental model to study the effect of larval treatment with deltamethrin on the capacity of *Ae. aegypti* to transmit *M. dessetae,* and showed that vectorial competence decreased. Biological data about the chronology of the life cycle, microfilarial and adult survival, and the periodicity of the mf have been reported by Gayral et al. (1982).

MATERIALS AND METHODS

Mosquitoes: Trials were conducted using the GKEP strain of *Ae. aegypti,* originally collected in Ghana, which has been reared in our laboratory since 1985. Larvae, kept at 28 ± 1°C, were fed finely screened mouse biscuit powder. Adults were maintained at 26 ± 1°C, 65% RH, and were fed a 10% sugar solution; females periodically received a blood meal on a guinea pig.

Parasites: Molinema dessetae was isolated in 1973 from a rodent (*Proechimys oris*) collected in Brazil (Bain 1973, 1974). The natural vector of *M. dessetae* is unknown (Gantier and Gayral 1979), but *Ae. aegypti* is an efficient laboratory host (Petit et al. 1977).

Mosquito treatment: OMS 2017 (1-(3,5-dichloro-4-(2,2-dichlorocyclopropyl-methoxy)-phenyl)-3-(2,6 difluorobenzoyl)urea) and DFB (1-(4-chlorophenyl)-3-(2,6 difluorobenzoyl)urea) were provided by the World Health Organization in April 1983 and in April 1989, respectively. The efficacy of the two IGRs on the 4th instars of *Ae. aegypti* was evaluated according to methods described by Mulla et al. (1974) and Saleh (1985) to determine the concentrations that caused 30% emergence inhibition (EI_{30}) (Fournet et al. 1993). Concentrations of 0.003 mg/liter for OMS 2017 and 0.0004 mg/liter for DFB were applied to a sufficient number of 4th instars to produce 600 surviving adults. Each of 3 cages was stocked with 100 males and 100 female survivors of the OMS 2017 or diflubenzuron treatment or with untreated adults.

Infection of mosquitoes: Infected *P. oris* were

[1] Elissa, N. 1990. Nouvelle approche de la lutte contre les maladies transmissibles: étude de l'effet de certains insecticides sur le développement de *Plasmodium yoelii yoelii* et de *Dipetalonema dessetae* dans leurs vecteurs. Thèse de Doctorat de l'Université Paris–Sud, France.

provided by Dr. Ph. Gayral and Dr. C. Bories, Laboratoire de Parasitologie, Faculté de Pharmacie, 92 296 Châtenay-Malabry, France. Two infected *P. oris* were used. After injection subcutaneously with 200 3rd-stage larvae of *M. dessetae,* rodents exhibited a microfilaremia after 90 days (Gantier and Gayral 1979, Gayral et al. 1982). After day 150 postinfection, the microfilaremia stabilized for a 2-month period. The microfilaria density in blood taken from the ocular sinus of infected rodents was determined before the infection of mosquitoes, which occurred 15 days after insecticidal treatments. In each cage, 100 5- to 7-day-old females were fed on the same rodent for 30 min. Unfed females were discarded. Ten females from each cage were weighed before and 30 min after the infected blood meal to determine the amount of ingested blood. Before weighing, engorged females were placed at 0°C because Brengues and Bain (1972) showed that at this temperature, fewer mf left the midgut for 30 min after ingesting the infected blood meal. Females then were dissected to determine the number of ingested mf. During the 10 days following the infectious blood meal, 3 additional blood meals were given on noninfected guinea pigs. Petit et al. (1977) showed the importance of additional blood meals in the maturation of these filariid larvae, which occurred in the fat body of the mosquito. Females were dissected 21–25 days after the infective blood meal and the number of larvae (L1, L2, and L3) observed in the abdomen, thorax, and head were recorded.

Statistical analysis: Parameters calculated included the overall infection rate (number of infected females/number of dissected females), the infectivity rate (number of females with L3 in all locations/number of dissected females), and the infective potential (number of females with L3 in head location/number of dissected females). We also included the infective head ratio, or the proportion of all L3 that reached the heads of dissected females. For all these parameters, differences were analyzed by a chi-square test.

RESULTS AND DISCUSSION

The microfilaremia (number of mf per 10 μl of blood) from the rodent used for the infection of untreated and DFB-treated females was 172 ± 13 mf; that of the rodent used for OMS 2017-treated females was 117 ± 5 mf. As the difference was not significant ($P > 0.1$), the controls were paired with the OMS treatment. There was no difference between the amount of blood ingested; both treated and control females ingested 2.19 ± 0.57 mm^3 of blood. Untreated females ingested an average of 13 mf/mg of blood and DFB-treated females ingested only 9 mf/mg. This difference was probably due to variation of the microfilaremia during the blood meal. Females treated with OMS 2017 ingested 12 mf/mg of blood.

Table 1. Overall infection rate, infectivity rate, infective potential, and infective head ratio of female *Aedes aegypti* dissected 21–25 days after the infective blood meal.

Treatment	No. dissected females	Infection rate[1]	Infectivity rate[2]	Infective potential[3]	Infective head ratio[4]
Control	70	80.0	77.1	37.1	52.0
OMS 2017	73	76.7	72.6	30.1	62.0[5]
Diflubenzuron	74	83.8	75.5	51.4[6]	60.5[5]

[1] Number of infected females/number of dissected females × 100.
[2] Number of females with L3/number of dissected females × 100.
[3] Number of females with intracephalic L3/number of dissected females × 100.
[4] Number of L3 in head/total number of L3 × 100.
[5] $P \leq 0.006$.
[6] $P \leq 0.04$.

When untreated, OMS 2017-treated, and DFB-treated females were dissected, there was no significant difference between the infection rates of control, OMS 2017-treated, and DFB-treated females (Table 1). The infectivity rates also were similar. No significant difference between the infective potential of OMS 2017-treated females and untreated females was observed. In OMS 2017-treated and untreated females, 22.6 and 23.8% of ingested mf reached the infective stage. However, there was a statistical difference between the infective potential of DFB-treated and untreated females ($P \leq 0.04$). In DFB-treated females, 38.1% of the ingested mf reached the infective L3 vs. 23.8% in control females. This difference was significant ($P \leq 0.01$), that is, DFB-treated females developed a higher rate of L3 than did control females. In OMS 2017-treated and in DFB-treated females, the number of L3 localized in the head was significantly higher than in untreated females ($P \leq 0.006$), showing that treated females were potentially more infective than untreated females.

Sublethal concentrations of IGRs seem to operate late in the infection process, because the filarial loads in surviving females were not affected. The IGRs had no effect either on blood ingestion or on the midgut infection barrier, but the passage of the L3 from the thorax to the head seemed to be more efficient than in untreated females. The passage of infective larvae to the head of the female mosquito was facilitated in insecticidal treatment conditions. The immune response of the mosquito against the parasite may be inhibited by sublethal insecticidal treatments. In female adults, ecdysteroids are produced by the ovaries to induce vitellogenin synthesis by the fat body. Petit and Spitalier-Kaveh (1979) observed that *M. dessetae* induced a chronic activation of the fat body similar to β-ecdysone. OMS 2017 and DFB interfered with ecdysteroid produc-

tion during the last larval instar (Fournet et al. 1995) and although the effect on the adult fat body has not been investigated during this study, a possible interaction cannot be eliminated.

The vectorial competence of *Ae. aegypti* for *M. dessetae* is not markedly affected by sublethal IGR treatment, but some survivors may be more efficient vectors. Insecticidal treatments appear to have different effects on vectorial competence, possibly according to the compound. Deltamethrin treatment inhibited the development of *Plasmodium yoelii* in *Anopheles stephensi* Liston (Elissa 1990[1]), and the oocyst index in *Anopheles gambiae s.l.* Giles infected with *Plasmodium falciparum* is decreased by treatment with *Bacillus thuringiensis* var. *israelensis* (Robert et al. 1987). In contrast, the proportion of females of a refractory strain of *Ae. aegypti* that developed *Brugia pahangi* (Buckley and Edeson) filariae increased after sublethal applications of DDT (Gaaboub and Busvine 1975). These results show that an evaluation of mosquito populations surviving insecticidal treatment may be necessary.

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