LABORATORY STUDIES ON THE FEASIBILITY OF INTEGRATED MOSQUITO CONTROL USING AN INSECT GROWTH REGULATOR AND A MERMITHID NEMATODE

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ABSTRACT. Laboratory studies showed that the insect growth regulator Altosid 5E®, applied at doses ranging from 5 to 50 ppb did not interfere with the preparasitic, parasitic or postparasitic development of *Romanomermis culicivorax*, a mermithid parasite of mosquitoes. Both Altosid 5E and *R. culicivorax* were separately effective in controlling pupal and larval populations respectively of *Aedes aegypti*. Host mortality was considerably increased when the mermithid and the insect growth regulator were used concurrently against mosquitoes, suggesting a promising future for integrated mosquito control programs based on the use of mermithids and insect juvenile hormone analogs.

INTRODUCTION. Field trials have demonstrated that insect growth regulating compounds such as Altosid 5E® (Schaefer and Wilder 1973) and the mermithid nematode *Romanomermis culicivorax*[3] (Petersen, J. J. 1973, Petersen and Willis 1976, Mitchell et al. 1974) are separately effective in controlling mosquito populations. The development of certain nematodes is known to be affected by a variety of insect juvenile hormone analogs (Davey and Hominick 1973, Dennis 1976) but no studies have been done to ascertain whether such compounds interfere with the development of mermithids. In order to assess the potential of using Altosid 5E together with *R. culicivorax* in integrated mosquito control programs, laboratory experiments were set up to find out whether or not the development of the mermithid was affected by the insect growth regulator.

MATERIALS AND METHODS. Laboratory infections of newly hatched *Aedes aegypti* larvae were set up routinely (Gordon et al. 1974) using a culture of the mermithid's egg and adult stages stored in sand (courtesy of Dr. J. J. Petersen, U.S. Department of Agriculture, Lake Charles, Louisiana).

The effect(s) of Altosid 5E on *R. culicivorax* development (preparasitic, parasitic and postparasitic) was assessed at 26° C for 3 concentrations of the insect growth regulator: 5, 20 and 50 ppb. Stock solutions of Altosid 5E were made up in acetone and appropriate volumes added to the rearing systems using a microsyringe.

To study the effect of Altosid on the latter stages of the mermithid's parasitic development, 4 groups (5 dishes of 100 insects in 250 ml water per group) of mosquito larvae were set up for each of the 3 Altosid concentrations: (a) Altosid treated uninfected larvae (4th instar), exposed (24 hr) to the chemical 4 days after hatching; (b) larvae infected 24 hrs after hatching with *R. culicivorax* (15 per ml) at a high rate of parasitism (92%) and a level of infection of 1 nematode per infected host (Gordon et al. 1974); (c) insects infected as in (b) then exposed (24 hr) 4 days after hatching to the requisite concentration of inactive ingredients (accounting for 34.5% of the Altosid 5E formulation);

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[3] Synonymous with the Louisiana strain of *Reesimermis nielseni* (Ross and Smith 1976).

(d) Altosid-treated infected hosts, combining procedures (a) and (b). To determine whether the Altosid affected early parasitic development, groups (a) to (d) were set up for a single Altosid concentration (20 ppb), applied 2 days rather than 4 days after hatching. Numbers of dead host larvae and pupae as well as numbers of emerging adults were counted from 6 days onwards after hatching. Random samples of mosquito larvae exposed to Altosid 2 days after hatching were dissected at day 4 and the mermithids' development assessed in relation to controls. In all cases nematodes were allowed to emerge from the mosquitoes and postparasites were counted, reared to adults in petri dishes containing distilled water and sand, then sexed.

Since the data from these preliminary experiments showed that Altosid and *R. culicivorax* were individually effective in controlling *Ae. aegypti*, a further experiment was devised, using groups of mosquitoes exposed to a lower concentration of preparasites (8 per ml) and a subsequently lower rate (46%) of parasitism.

The effects of the insect growth regulator on the infectivity of the preparasitic stage of *R. culicivorax* was assessed by exposing infective stages in 250 ml water (12–15 preparasites per ml) for 4 hr and 12 hr to each of the 3 Altosid concentrations (3 replicates per concentration). Three additional dishes were set up containing the same concentration of preparasites, except the requisite quantity of inactive ingredients of the Altosid preparation was added instead of Altosid. One hundred newly hatched mosquito larvae were added to each of the dishes (24 hr) and also to 3 dishes of preparasites in water alone. After 24 hr, 100 mosquito larvae from each group were dissected and the rate of mermithid infection recorded. After 74 hr, a further 100 mosquito larvae from each of the 3 groups were dissected and the presence and progress in development of the nematodes noted. Nematodes were collected from the final experimental group on their emergence after 6 days.

The effect of Altosid 5E on postparasitic development was studied by infecting *Ae. aegypti* and collecting the nematodes which emerged. Within 24 hr of emergence, nematodes were paired (5 male:5 female) in petri dishes containing water and exposed to 5, 20 or 50 ppb Altosid 5E. They were transferred after 24 hr to fresh water and the postparasitic development followed. The 2 control groups consisted of emergent nematodes which were paired as above and either exposed to inactive ingredients for 24 hr or left to complete their development without exposure.

RESULTS. Altosid 5E did not affect the parasitic development of R. *culicivorax* nor did it interfere with the number of postparasites which emerged from the hosts or the sex of the mermithid (Table 1). Altosid was effective at all doses in causing high mortality of the mosquitoes at the pupal stage, while *R. culicivorax* infection (determined to be on average 1 nematode per host) resulted in high rates of mortality of the larval mosquito, most commonly at the last larval instar. Host mortality was increased to approximately 100% when mermithid infected mosquitoes were exposed to even the lowest dose of Altosid 5E employed. When Altosid was applied to mosquitoes at lower (50%) rates of parasitism, host mortality was still increased to approximately 100%.

Exposure of preparasites to Altosid for either 4 hr or 12 hr did not interfere with the infectivity of the mermithid (Table 2). Rates of mermithid parasitism and numbers of postparasites which completed development were not affected by the insect growth regu-

Table 1. Effects of Altosid 5E on *Ae. aegypti* and parasitic development of *R. culicivorax*.

Host group [a]	Time of exposure [b]	% mortality host larvae	% mortality of pupae	Overall % host mortality	Total number post parasites emerged
Altosid 5ppb	4	10.0±1.3	41.3±4.5	48.5±9.9	0
Infected	–	92.5±1.9	1.3±1.3	93.9±1.2	278 (147 ♂ 122 ♀)
Infected+Altosid 5ppb	4	97.4±0.7	2.6±0.2	100.0	258 (133 ♂ 121 ♀)
Altosid 20ppb	4	7.2±1.0	75.2±2.6	82.4±2.3	0
Infected	–	84.0±4.7	0	84.0±4.7	262 (145 ♂ 115 ♀)
Infected+Altosid 20ppb	4	82.2±2.4	17.2±2.8	99.4±0.6	263 (153 ♂ 107 ♀)
Altosid 50ppb	4	13.2±3.0	83.6±1.9	96.8±1.4	0
Infected	–	82.6±4.4	10.5±3.2	93.1±2.0	260 (153 ♂ 100 ♀)
Infected+Altosid 50ppb	4	78.2±2.4	21.8±2.4	100.0	226 (117 ♂ 107 ♀)
Altosid 20ppb	4	9.2±0.3	67.2±3.3	76.4±3.6	0
Infected (50%)	–	41.6±3.8	2.8±0.2	44.4±2.3	123 (71 ♂ 52 ♀)
Infected (50%)+Altosid 20ppb	4	50.0±2.8	42.8±3.6	92.8±4.4	137 (76 ♂ 61 ♀)
Altosid 20ppb	2	37.6±4.5	34.0±7.2	71.6±8.4	0
Infected	–	88.4±2.0	0.4±0.1	88.8±2.1	248 (146 ♂ 85 ♀)
Infected+Altosid 20ppb	2	85.2±2.0	10.4±1.3	95.6±2.2	184 (105 ♂ 70 ♀)

[a] The presence of acetone and inactive ingredients did not affect the results and were not included in the table.
[b] Indicates time after hatching in days that the hosts were subjected to a 24 hr treatment with Altosid 5E or its inert ingredients.

Table 2. Effects of Altosid 5E on infectivity of preparasitic *R. culicivorax*.

Treatment	Rate of mermithid parasitism (%)		No. postparasites emerged	
	4 hr[a]	12 hr[a]	4 hr[a]	12 hr[a]
Altosid 5ppb	85.2±3.2	92.6±1.3	246 (135 ♂ 111 ♀)	267 (156 ♂ 111 ♀)
Inactive ingredients	88.4±1.2	90.2±2.0	253 (151 ♂ 102 ♀)	273 (141 ♂ 132 ♀)
Untreated	88.4±1.5	88.4±3.8	248 (135 ♂ 113 ♀)	268 (147 ♂ 121 ♀)
Altosid 20ppb	90.2±2.4	94.6±1.9	272 (140 ♂ 132 ♀)	285 (158 ♂ 127 ♀)
Inactive ingredients	92.5±0.3	90.4±4.5	276 (146 ♂ 110 ♀)	262 (138 ♂ 124 ♀)
Untreated	88.4±2.8	92.2±3.8	252 (136 ♂ 126 ♀)	277 (142 ♂ 135 ♀)
Altosid 50ppb	93.3±3.1	88.6±4.9	281 (148 ♂ 133 ♀)	278 (153 ♂ 125 ♀)
Inactive ingredients	90.2±4.4	82.2±0.2	275 (159 ♂ 116 ♀)	282 (161 ♂ 121 ♀)
Untreated	96.4±1.3	90.2±2.2	261 (146 ♂ 115 ♀)	269 (159 ♂ 110 ♀)

[a] Duration of treatment of preparasites.

lator or its inactive ingredients. Moreover, dissections showed that the nematodes which were exposed as preparasites to Altosid developed normally.

Altosid did not affect the postparasitic development of *R. culicivorax*. Neither the time taken for the postparasitic molt to occur nor the time taken from emergence to oviposition varied between treatments: 6.0±0.5 days and 9.0±2.0 days respectively. Also there was no significant difference between treatments for proportion of postparasites which completed the molt, the number of eggs laid per female or percent viability of the eggs (Table 3).

DISCUSSION. This study has shown that the insect growth regulator, Altosid 5E, applied at doses ranging from 5 to 50 ppb, does not interfere with the development (preparasitic, parasitic or postparasitic) of the mermithid nematode *R. culicivorax*. The active ingredient of Altosid 5E (150 propyl (2E, 4E)-11-methoxy-3, 7, 11-trimethyl-1, 4-dodecadienoate) is chemically related to but not identical with the insect's authentic juvenile hormone(s). Thus, our data suggest but do not confirm that *R. culicivorax* develops independently of the host's juvenile hormone titer. Numerous investigators have studied the effects of a variety of juvenile hormone analogs on nematodes (Davey and Hominick 1973, Dennis 1976) but only one of

Table 3. Effects of Altosid 5E on development of postparasitic *R. culicivorax*.

Treatment	% postparasites molted	No. eggs per female[a]	% egg viability
Altosid 5ppb	90.2±1.6	185.3±52.5	63.5±12.6
Inactive ingredients	88.6±3.7	166.3±26.9	68.6± 8.2
Untreated	95.1±6.2	163.0±28.6	72.4± 3.7
Altosid 20ppb	86.8±4.7	167.7±62.1	70.2± 4.6
Inactive ingredients	91.4±1.1	134.6±14.1	74.3± 8.2
Untreated	90.3±3.6	148.3±30.3	72.9± 2.8
Altosid 50ppb	92.4±2.8	177.0±16.1	70.6± 6.4
Inactive ingredients	90.2±2.6	198.6±48.2	68.4±10.6
Untreated	92.6±2.2	171.6±11.7	74.8± 9.2

[a] Two days after initiation of oviposition.

these studies involved entomophilic nematodes (Hansen and Buecher 1971). These authors found that compounds with juvenile hormone activity reduced the viability of 1st stage larvae but not oviposition or exsheathment of *Neoaplectana carpocapsae* DD–136, nor was exsheathment of *Neoaplectana glaseri* affected.

Synthetic juvenile hormones such as Altosid 5E offer considerable potential as insect pest control agents and field tests have demonstrated such compounds to be effective against mosquito populations (Schaefer and Wilder 1973, Mulla 1974) without affecting non-target organisms. If larval mosquitoes are exposed to Altosid, larval-pupal intermediates are eventually produced which are usually unable to emerge as adult flies. Insect growth regulators appear to function by interfering with enzymes which mediate the normal catabolic breakdown of the insect's juvenile hormone(s) (Downer et al. 1975). Our laboratory data confirm the effectiveness of Altosid substances in mosquito control, since even low doses of the compound resulted in high levels of pupal mortality. The mermithid *R. culicivorax* is also regarded as a highly promising control agent of mosquitoes (Petersen 1973, Petersen and Willis 1976, Mitchell et al. 1974), but in contrast to Altosid, the mermithid causes mortality of the mosquito host at the last larval instar, just prior to pupation. This is brought about by a steady depletion of the host resources during the developmental phase of the nematode within the host haemocoel, followed by death of the host when the nematode emerges from it on completion of this phase of its development. In all of our experiments, high levels of mortality due to *R. culicivorax* were recorded.

Our data show that host mortality is increased considerably (to ca. 100%) when a combination of the mermithid and Altosid 5E is used against the mosquitoes. Thus, integrated mosquito control programs using mermithids together with insect growth regulators could prove more effective than either control agent used separately, because infected hosts die prior to pupation while Altosid treated hosts are killed as pupae. Insects which are pests only in the adult stages of their life cycle can be controlled at an immature stage, rather than attempting to control an existing adult infestation that is causing problems.

ACKNOWLEDGMENTS. We are grateful to Dr. J. J. Petersen of the United States Department of Agriculture, Lake Charles, Louisiana for supplying the cultures of *R. culicivorax* and to Zoecon Corporation for supplying the samples of Altosid 5E and inactive ingredients. This study was supported by the National Research Council of Canada (operating grant no. A6679; negotiated grant no. D–43) and by the Banting Research Foundation.

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PAROUS RATES OF OVERWINTERING *CULEX PIPIENS PIPIENS* IN NEW JERSEY[1]

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ABSTRACT During the winters of 1974–1975 and 1975–1976, hibernating *Culex pipiens pipiens* L. were collected from a large population at Fort Mott in Salem County, New Jersey and examined for parous rates to determine the percentage which ingested blood prior to diapause. The information was used to help assess the likelihood of *Cx. p. pipiens* harboring arboviruses over the winter in New Jersey. Parity was determined by the Detinova method of ovarian tracheolation as well as the stage of ovarian development in individual ovarioles.

Results for 1974–1975 showed that of 120 mosquitoes examined, 119 were nulliparous, with the single parous specimen taken early in the hibernating period. A further indication of nulliparity was obtained from the examination of individual ovarioles since all those examined were in stage N, the earliest stage in the developmental process. During 1975–1976, only 12 of 820 *Cx. p. pipiens* were parous, 6 of which were found in October. No parous mosquitoes were found during February or March. Data suggest that some parous mosquitoes enter hibernation, but few, if any, survive the winter. Even with a low percentage of the population which might exhibit gonotrophic dissociation, *Cx. p. pipiens* seems to be an unlikely source for overwintering virus.

INTRODUCTION

Human outbreaks of St. Louis encephalitis (SLE) have occurred sporadically in New Jersey during the past 12 years. An outbreak in the Camden area during 1964 resulted in 94 cases with 8 deaths (Goldfield et al. 1965). After a single case in 1965 (Kandle et al. 1967) no SLE was recognized until 1975, when a sizeable epidemic swept across many parts of the United States. A total of 29 cases and one death was recorded in New Jersey (Anonymous 1976). The suspected epizootic and epidemic vector, as in previous outbreaks, was the common house mosquito, *Culex pipiens pipiens* L. (Goldfield et al. 1965, Luby et al. 1969).

The overwintering mechanism of SLE virus has not been determined. Possibilities include latent infections in small rodents, birds, amphibians or reptiles, infection in migratory birds which return with virus in the spring and overwintering adult mosquitoes. *Cx. p. pipiens* is a bird feeder, a known SLE vector, and it hibernates as an adult female, making the mosquito a likely host for overwintering SLE. There is,

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