Journal of the American Mosquito Control Association, 10(1):64–66, 1994

EFFECTS OF HOST RESISTANCE AND INJURY ON THE SUSCEPTIBILITY OF *AEDES TAENIORHYNCHUS* TO MOSQUITO IRIDESCENT VIRUS

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ABSTRACT. An iridescent virus is found at low prevalence in populations of *Aedes taeniorhynchus*. Attempts at experimental transmission produced low levels of infection, regardless of the dosage applied. In a test for genetic resistance in colonized *Ae. taeniorhynchus*, the mean infection rates ± SD for groups of randomly selected and sibling larvae were compared. The standard deviation of the sibling groups was not higher than the random groups (4.0 ± 3.1% and 3.0 ± 2.1%), rendering genetic resistance unlikely. Injury to the larvae by feeding silicon carbide fibers consistently caused higher infection rates (4.8 ± 2.0% by virus alone and 17.5 ± 5.3% by virus and fibers concurrently). Similar results were obtained for vertical transmission. These results support the hypothesis that this virus has no active means of penetration, invading only through random breaks in the cuticle or peritrophic membrane.

INTRODUCTION

The first report of a mosquito iridescent virus (MIV) was by Clark et al. (1965). This MIV occurred at low incidences of infection in the black salt marsh mosquito, *Aedes taeniorhynchus* (Wied.). Transmission studies have shown it to be host specific, infecting only *Ae. taeniorhynchus* (Walker) and *Aedes sollicitans* (Woodard and Chapman 1968). Infection rates increased with dosage, reaching a plateau below about 16% (Linley and Nielsen 1968a, Woodard and Chapman 1968, Matta and Lowe 1970, Hembree and Lowe 1976).

Regardless of the dosage level, experimental infection rates with MIV remain low. Stoltz and Summers (1971) found no evidence that MIV penetrated the peritrophic membrane and that the virus was degraded in the midgut. Other host-specific, obligate pathogens encounter and overcome such host defense mechanisms. These pathogens, considered to be well adapted to their specific hosts, have active means of penetration and experimental dose–response curves reach 100% infection. Results of MIV transmission studies suggest that, instead of low susceptibility, a small percentage of the population of *Ae. taeniorhynchus* larvae are susceptible and the rest are refractory.

Two hypotheses are tested concerning the non-susceptibility of most of the host population: 1) genetic resistant to the virus; and 2) the MIV has no specific means of penetration, entering only through randomly occurring breaks in the cuticle or peritrophic membrane.

MATERIALS AND METHODS

To test the genetic resistance hypothesis, a comparison was made between the variability in percentage infection of 2 treatment sets: one set of larvae randomly selected from the entire mosquito colony and another set of larvae consisting of the progeny of individual females. For the random set, a large number of eggs from a laboratory colony of *Ae. taeniorhynchus* were hatched and 18 groups of 150 larvae each were counted into 100 ml containers. The other set of 18 groups was established by obtaining eggs from 18 individual mosquitoes. The eggs were hatched in 100-ml containers. Two days posthatch, the larvae (1st–2nd instar) were placed in 10 ml of 0.5% NaCl in deionized water in 50-mm petri dishes. A dosage of MIV equivalent to the number of virions from 5 infected 4th-instar larvae (5 LEQ) and a small amount of powdered alfalfa pellet (Solar Co., Lynbrook, NY) were added to each container. After a 5-h exposure at 23°C, the larvae were transferred to 18 × 28-cm enamel pans containing 500 ml of the saline solution and fed 3 ml of a 20% alfalfa suspension in water. At 2-day intervals thereafter, an aqueous mixture of 3 parts liver powder : 2 parts powdered hog chow supplement was added to each pan. Five days postexposure, the incidence of disease in the 4th-instar larvae was scored by examination of the larvae against a black background where the iridescence of infected larvae was easily visible. If resistance is inherited, infected larvae would be clustered in sibling groups. This would appear as a greater variability (standard deviation) in the percentage infections in the set of siblings than in the set of randomly selected groups of larvae; mean percentage infection should be the same.

The second hypothesis was tested by comparing incidences of disease in "injured" and "uninjured" larvae. Silicon carbide fibers (SILAR SC-9), used to punch holes in dechorionized insect eggs for insertion of nucleic acid sequences

(Kaeppler et al. 1990), were used to injure groups of *Ae. taeniorhynchus* larvae. Two types of injury were attempted. 1) Ingestion—groups of 100 1st-instar larvae were placed in 50-mm petri dishes containing either 5 LEQ MIV or 5 LEQ MIV + *ca.* 10 μg of fibers in 10 ml 0.5% NaCl in deionized water. This experiment was replicated 6 times and analyzed using a *t*-test for differences of means. 2) External injury—3 groups of 100 larvae in 10 ml saline and 10 μg fibers were placed in a 40-ml round-bottomed centrifuge tube and shaken on a vortex mixer, set on its highest speed, for 1 min. The larvae were transferred to the small petri dishes and the MIV was added. Eighteen hours later the contents of the petri dishes were transferred to 500 ml of dilute saline in 18 × 28-cm enamel pans and reared as described above. As there was no way to assure that fibers were not ingested during the external injury tests, both were subjected to the same exposure regimen except for the agitation. Only an incidence of infection higher than that caused by feeding would be considered as a positive indication of infection through external injury.

In a single test, 100 late 3rd–early 4th-instar larvae were exposed to either MIV alone or MIV + the fibers. The larvae were reared as above and the pupae collected and placed in emergence cages. Adults were fed beef blood, warmed to 37°C, through a membrane. Eggs were collected on wet sphagnum, then hatched 2 wk later by flooding with saline. After oviposition, all females were counted; no attempt was made to determine their infection rate. The larvae were reared in 50 × 37-cm plastic trays with 3 liters deionized water and the 4th-instar larvae were checked for signs of infection as described above.

RESULTS

The mean infection rates of the 18 groups of randomly selected larvae ranged between 0 and 13.7% with a mean ± SD of 4.0 ± 3.1%. The groups of sibling larvae had infection rates between 0 and 9.4%, with a mean of 3.0 ± 2.1%. A *t*-test showed no difference between means. The standard deviation of the sibling groups was not higher than the randomly selected groups.

Overall infection rates ($n = 6$) were 4.8 ± 2.0% by MIV alone and 17.5 ± 5.3% when the fibers and MIV were fed concurrently. The difference was significant at the $P = 0.05\%$ level. Survival was significantly lower for the fiber-fed larvae (57.8 ± 11.3%) than for those fed just the virus (75.2 ± 11.6%). Shaking the larvae with the fibers did not significantly increase percentage infection ($n = 3$; 7.8 ± 2.0% infection) or mortality (67 ± 12.7%) above that observed in the MIV + fiber group.

Survival of larvae exposed as late instars mirrored the results of the early instar experiments. Of the larvae fed the MIV + fibers, 74.0% survived to pupation, whereas 93.0% of those fed only the MIV pupated. Only 1.4% of the 1,006 4th-instar larvae progeny produced by the 41 females exposed to the MIV as late-instar larvae were infected. In the group that had been fed both MIV and fibers, 30 females survived to oviposition. Of their 317 4th-instar progeny, 5.4% were infected.

DISCUSSION

The low and variable rates of infection observed here are consistent with previous studies. Infections initiated in early instar *Ae. taeniorhynchus* caused typical signs of infection and death in the 4th instar (Linley and Nielsen 1968a, Matta and Lowe 1970, Hall and Anthony 1971). Early instar larvae are more susceptible than the late instars, which survive to imaginal stages that produce infected eggs (Linley and Nielsen 1968b, Hall and Anthony 1971). There has been no evidence that nonpatent individuals from experimentally exposed groups were latently infected. Virus applied by injection into adult *Ae. taeniorhynchus* is also known to infect all F_1 progeny (Fukuda and Clark 1975). In another study (Hall and Anthony 1971) all of the progeny of an infected adult female were also infected and died as 4th-instar larvae. Thus, the rate of infection among progeny provides an estimate of the rate of infection in the parental group.

The rates of infection among sibling and randomly selected groups, exposed to MIV as 2nd-instar larvae, were not significantly different. This suggests that there is no simple genetic factor governing resistance in our colony of *Ae. taeniorhynchus*. That high percentages of larvae can be infected by injection of the virus through the external cuticle (Fukuda and Clark 1975) indicates that the cuticle is the primary barrier to infection. Mechanical injury by ingestion of the fibers increases mortality, possibly by injury to the peritrophic membrane. Vigorously shaking early instar larvae in a suspension of fibers neither injured them nor increased their susceptibility to MIV, suggesting that in these experiments, entry through the external cuticle was not likely.

The exposure of 3rd–4th-instar larvae to MIV resulted in vertical transmission to their progeny. Although the results of this single run (1.4% with MIV alone to 5.4% with MIV + fibers) cannot be statistically analyzed, they reflect the results of the early instar tests; increased infection rate and decreased survival in the fiber-treatment group. Infection of late-instar larvae is impor-

tant. Linley and Nielsen (1968b) concluded that the most common mode of transmission in nature is infection of late-instar larvae by infectious virions released from virus-killed larvae of the same generation. Consequently, eggs of the F_1 generation would constitute the primary repository of the virus. This is a reasonable hypothesis for a pathogen of mosquitoes with eggs that persist on the ground for considerable periods of time and then develop synchronously after they are hatched by a flood.

There is no agreement on the site of invasion by MIV. Our results suggest entry through the gut. Stoltz and Summers (1971) found no evidence that MIV penetrated the peritrophic membrane; they could not find virus particles within or in contact with midgut epithelial cells. They also found the virus to be rapidly degraded in the midgut, concluding that if the virus infected through the gut, its only opportunity would be at the anterior end. Hembree and Anthony (1980) reported virus-like particles outside the peritrophic membrane, against the microvilli of the midgut epithelium and within an epithelial cell. Although they observed this 5 h after exposure of the larvae to MIV, they could not prove that it was the MIV or that the larvae would have become infected. Infection can be initiated by injection of virus into the insect (Fukuda and Clark 1975), indicating the possibility for infection through the external cuticle. There was no indication that shaking the larvae with the fibers caused any injury or infection beyond that caused by ingestion of the fibers.

We propose that this obligate, host-specific pathogen has no active means of crossing the protective cuticle or peritrophic membrane of the mosquito larva. The barrier to infection seems to be a mechanical one, the cuticle and peritrophic membrane. Increased mortality rates in fiber-fed larvae provided evidence of injury, probably by abrasion and puncture of the peritrophic membrane. Although percentage infection in the injured groups was still nowhere near 100%, injury would increase the opportunities for both virus entry and mortality. Injury treatment would leave some members of the group uninjured, others injured to a degree that they are susceptible, and some too severely injured to survive. The percentage infection obtained in such experiments would depend upon both the degree of injury needed to allow virus entry and how much injury the larvae can tolerate and still survive. This injury window could be quite small.

To obtain a high incidence of infection, a technique must be developed that will cause a uniform number of breaks, in just the right location for optimal entry by the virus, and that minimizes mortality. Although this might be accomplished in the laboratory, it is unlikely to be practical in the field. A formulation containing an agent that damages the gut might yield high infection rates but would not improve infection rates in subsequent generations. Such a microbial insecticide is impractical, considering the current costs of producing the virus. Therefore, with our current knowledge, this MIV is an unlikely candidate for biological control of mosquitoes.

ACKNOWLEDGMENTS

We are grateful to James Becnel for his advice on this project and to Susan White for her assistance with statistical analysis.

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