Journal of the American Mosquito Control Association, 13(3):292–295, 1997

PHOTOSENSITIZATION OF *ERETMAPODITES QUINQUEVITTATUS* THEOBALD (DIPTERA: CULICIDAE) EGGS AND LARVAE WITH PHOTOFRIN II®

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ABSTRACT. The susceptibility of *Eretmapodites quinquevittatus* eggs and larvae to photosensitization with Photofrin II® (PII) was determined. Hatching rates of eggs (5 min to 2 h after oviposition with no PII exposure) exposed to solutions of PII (90 μg/ml) in the dark for 12 h, before photoirradiation for 72 h, were very similar to those of untreated controls ($P > 0.05$). Hatching rates of eggs (5 min after oviposition with no PII exposure) exposed to PII (90 μg/ml) and photoirradiated simultaneously for 72 h were significantly lower than for untreated controls ($P < 0.05$). Permeability of the egg to PII diminishes as the eggs age and the serosal cuticle becomes mature. *Eretmapodites quinquevittatus* larvae were killed after exposure to PII (90 μg/ml) that had been previously photoirradiated for 1–11 days. The effect was age and size related. First-instar to early 3rd-instar larvae were killed when exposed to PII (90 μg/ml) with no photoirradiation.

Mosquitoes transmit pathogens and present a serious health hazard in many parts of the world. Chemical insecticides are used to control these vectors, yet these chemicals can be highly toxic to nontarget species in the environment and some mosquitoes have become resistant to them. Photosensitizers may provide a safe way of controlling insect pests (Service 1995, Waggoner 1995).

Photosensitization involves activation of light-sensitive compounds, producing chemical reactions that damage or destroy cells; in some cases the excited photosensitizer is converted into a toxic photoproduct (Spikes 1984, 1985). Photosensitizers kill *Anopheles* and *Culex* larvae in the presence of ultraviolet or visible light (Barbieri 1928, Schildmacker 1950). Photoreactive compounds have been successful at killing mosquito, fly, and ant larvae (Graham 1972, Sakurai and Heitz 1982, Kagan et al. 1983, Hartberg and Judy 1990). The latest review of porphyric insecticide action is provided by Rebeiz (1995). We have found no reports of photosensitive effects observed on mosquito eggs. Furthermore, little is known of how long photoreactive compounds persist in the environment.

The mosquito eggshell includes 2 layers covering the oocyte. The secondary membrane forms the hard, proteinaceous chorion and consists of two layers called the endochorion and the exochorion (Clements 1992). Most species of mosquitoes require from 1 to 2 h for sclerotization of the endochorion (McGrane et al. 1988). The chorion is highly permeable to water and certain solutes during the first 1–2 h after oviposition and eggs are more tolerant to increasing salt concentrations with age (Morris et al. 1989). *Aedes aegypti* eggs become less permeable to water as the endochorion sclerotizes (McGrane et al. 1988). Water permeability is believed to be influenced by a waxy layer associated with the serosal cuticle. Eggs of *Aedes* sp. develop maximum resistance to dehydration when the serosal cuticle has developed (Harwood and Horsfall 1959). We make these comparisons to *Aedes* because *Aedes* and *Eretmapodites* are closely related genera (Edwards 1941, Hartberg and Faircloth 1983).

We determined the susceptibility of eggs of *Eretmapodites quinquevittatus* to Photofrin II® (PII), a photosensitizer that, after ingestion and activation by light, kills *Er. quinquevittatus* larvae (Hartberg and Judy 1990; Helleck and Hartberg, unpublished). *Eretmapodites quinquevittatus* was used because we have studied its bionomics more thoroughly than any other species in our laboratory (Helleck 1992). Our primary question is, "Can some form of Photofrin II® cross the chorion and, following photoirradiation, kill the developing embryo?" For this to happen, light will have to pass through the chorion and PII will have to pass through the serosal cuticle. Beament (1989) stated the serosal cuticle of *Culex* eggs is virtually impermeable to most chemicals in solution 12 min after oviposition. This study also examines the residual toxic effects of photoirradiated PII, in solution, on *Er. quinquevittatus* larvae.

Laboratory cultures of *Er. quinquevittatus* (EQ-MIXED strain, established by W. K. Hartberg from larvae collected in Dar es Salaam, Tanzania) were maintained at 25°C. 80 ± 5% relative humidity, and a 14:10 light : dark photoperiod in the Medical Entomology Laboratory at Baylor University. Eggs (mixed autogenous and anautogenous) were oviposited on moist, bleached paper towels (egg papers) lining the inside walls of glass jars (279.3 ml) placed in the colony cages. Eggs were not induced to hatch, as *Er. quinquevittatus* eggs do not diapause. Eggs were kept on moist egg papers and hatched after 48–50 h.

Jars containing egg papers and tap water were collected from colony cages after 5–10 min of exposure to ovipositing females and replaced with an-

other jar containing an egg paper and tap water. Each egg paper was examined using a dissecting microscope (10×) and those with eggs attached were cut into strips and replaced in the jars. The jars were then covered with glass petri dish tops. Eggs were incubated at 25°C for 0 (= control), 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, and 135 min. The chorionic incubation period (110–120 min) of *Er. quinquevittatus* eggs (10 replicates; approximately 25 eggs/replicate) was discovered by observing, at 5-min intervals after oviposition, the change from an opaque unsclerotized egg to a black completely sclerotized egg (Helleck and Hartberg, unpublished data). Egg age is the period referring to the length of time eggs from each group developed in the absence of PII. One collection of eggs was used for each egg age group (including controls) for a given batch of eggs from the EQ-MIX-ED colony. Four more collections, from different egg batches, were used for each egg age group.

Two different controls were used in these experiments. Control 1 refers to eggs collected and photoirradiated, but not exposed to PII. Control 2 refers to eggs collected and immediately exposed to PII, but not photoirradiated.

A PII solution typically consists of hematoporphyrin and hydroxyethylvinyldeuteroporphyrin monomers (15%) and ester-linked (10–20%), ether-linked (20–30%), and acid-resistant (35–50%) oligomers of porphyrin rings (Byrne et al. 1990). For each egg age group, egg strips were placed in a solution of PII (90 μg/ml) and placed in total darkness for 12 h. After treatment, egg strips were rinsed 7 times with distilled water, replaced in jars (with petri dish covers) containing tap water, and exposed to white light (1.12 mW/cm^2, 350–800 nm) for 72 h. Approximately 5 min elapsed while rinsing and transferring eggs to the light source. Eight 15-W Philips® fluorescent light bulbs in parallel array, placed approximately 25 cm above the egg strips, were used to photoirradiate the eggs. All eggs were photoirradiated with the same light source and handled identically, except control 1 was not exposed to PII and control 2 was not photoirradiated.

After egg aging for the periods previously described, other egg strips were placed in PII solution (90 μg/ml) and immediately exposed to white light (1.12 mW/cm^2, 350–800 nm) for 72 h. All eggs were photoirradiated with the same light source and handled identically, except control 1 was not exposed to PII and control 2 was not photoirradiated.

Eggs were collected, hatched, and larvae were reared to the 1st, 2nd, 3rd, or 4th instar under normal environmental chamber conditions before being exposed to PII. Larvae (n = 25/instar) were placed in separate solutions of PII (90 μg/ml) that had initially been photoirradiated for 1–11 days and then continuously photoirradiated until all larval stages of pupation occurred. Control 1 larvae were simultaneously reared under the same environmental chamber conditions and photoirradiated, but were not exposed to PII. Control 2 larvae were also simultaneously reared under the same conditions while being exposed to PII, but were not photoirradiated. Dead larvae were counted and removed at 24-h intervals.

The proportion of eggs hatched (5 replicates/experimental group/light treatment and 5 replicates/control group/light treatment) was analyzed by the Kruskal–Wallis nonparametric, multiple comparisons test and the Tukey-type test for individual comparisons (alpha = 0.05) (Zar 1984). Larval mortality analysis consisted of comparison of numbers surviving under control and experimental conditions.

No significant differences in hatching rate were found between experimental and control groups of eggs exposed to PII solution in the dark for 12 h followed by photoirradiation (Table 1, P = 0.8757 corrected for ties). The PII entering the eggs could not be photoactivated as no light was provided during the initial 12 h of egg development. Sufficient light intensity for photosensitization could not penetrate the chorion and activate PII during photoirradiation immediately after the 12 h dark treatment period because the eggs had fully sclerotized after 2 h.

The percent hatch for each experimental group after treatment with PII solution followed by immediate photoirradiation (no darkness) is shown in Table 1. The Kruskal–Wallis multiple comparison yielded a nonsignificant probability of P = 0.0727, This was so close to our significance level of P = 0.05 that we investigated the individual comparisons for any significant differences. Hatching rates of eggs in the 5-min group were significantly different (57%, SE = 0.01, $q_{0.05,\infty,12}$ = 4.72) from control 1. The 5-min period left after the 5-min (group 5) egg aging group must have been long enough for PII to enter, be activated by light, and destroy enough tissue to reduce hatching rate for this group. An incubation period of 10 min or more appeared to allow insufficient time for uptake of enough PII to be photoactivated to cause serious tissue damage and significantly reduce hatching rates. This would support earlier work by Beament (1989) concerning egg permeability and we propose a similar period of permeability of the serosal cuticle for *Er. quinquevittatus* that might be exploited using chemical control methods.

All larval instars were killed when exposed to previously photoirradiated PII (Table 2). Photofrin II photoirradiated for 11 days before larval treatment killed all 1st-instar larvae within 24 h, all 2nd-instar larvae within 48 h, all 3rd-instar larvae within 72 h, and all 4th-instar larvae within 96 h. Photofrin II photoirradiated for <10 days killed all larval instars in less time. First- through 3rd-instar larvae were killed by PII even when not photoirradiated, yet late 3rd- and 4th-instar larvae were not affected by nonphotoirradiated PII (control 2).

Table 1. Percent (%) hatch of eggs laid by *Eretmapodites quinquevittatus* after treatment with Photofrin II® (PII) solution (90 μg/ml), in darkness for 12 h, followed immediately by photoirradiation and percent (%) hatch of eggs laid after treatment with PII solution (90 μg/ml) and photoirradiation (no darkness) simultaneously for 72 h. Standard errors are in parentheses; 5 replicates/treatment. Control 1 = no PII, photoirradiation; control 2 = PII, no photoirradiation; w/o = without.

Minutes aged w/o PII (egg age group)	No. eggs	% hatch
	Darkness	
5	34	91.2 (0.1)
10	87	94.3 (0.0013)
15	55	96.4 (0.002)
30	59	88.1 (0.1)
45	99	94.9 (0.1)
60	57	93.0 (0.004)
75	67	91.0 (0.1)
90	72	83.3 (0.1)
105	82	87.8 (0.003)
120	47	89.4 (0.1)
135	61	95.1 (0.002)
Control 1	48	95.8 (0.003)
Control 2	51	90.0 (0.003)
	No darkness	
5	84	57.1 (0.1)[1]
10	118	87.3 (0.001)
15	109	89.9 (0.1)
30	84	92.9 (0.002)
45	64	93.8 (0.003)
60	110	91.8 (0.003)
75	66	84.8 (0.1)
90	101	95.0 (0.003)
105	69	85.5 (0.005)
120	85	83.5 (0.003)
135	76	82.9 (0.004)
Control 1	73	93.2 (0.1)
Control 2	70	88.0 (0.004)

[1] Significantly different from controls (egg age group 0) at $\alpha = 0.05$ using Kruskall–Wallis multiple comparisons test, and Tukey-type a posteriori individual comparisons test to check for significant differences in individual contrasts ($q_{0.05,\infty,12} = 4.72$).

Table 2. Survivorship of *Eretmapodites quinquevittatus* larvae in photoirradiated solutions of Photofrin II® (PII) (90 μg/ml). Control 1 = no PII, photoirradiation; control 2 = PII, no photoirradiation.[1]

No. days solution photoirradiated	Larval instar	No. surviving at 24 h	48 h	72 h	96 h
1–7	All	0			
8	1st	0			
	2nd	0			
	3rd	3	0		
	4th	5	0		
9	1st	0			
	2nd	0			
	3rd	6	0		
	4th	8	0		
10	1st	0			
	2nd	1	0		
	3rd	12	2	0	
	4th	16	7	2	0
11	1st	0			
	2nd	6	0		
	3rd	17	8	0	
	4th	21	16	9	0
Control 1	1st	25	25	25	24
	2nd	25	25	25	25
	3rd	25	24	23	23
	4th	24	23	23	23
Control 2	1st	0			
	2nd	0			
	3rd (early)	4	0		
	3rd (late)	25	25	25	25
	4th	25	25	25	25

[1] n = 25 larvae/instar.

These results suggest a common larvicidal chemical species is generated photochemically from the molecular species in PII (Spikes 1984). The results of control 2 also suggest that PII is toxic even when not photoactivated, yet longer exposures and greater concentrations are required.

We conclude that PII will kill mosquito eggs if they are subjected to PII immediately after oviposition. This could be accomplished by maintaining a minimum concentration of PII in water. It is not known, however, what effect PII might have on other organisms residing in aquatic communities. Exposure to sublethal levels of PII might cause damage that adversely affects later generations of mosquitoes. We saw such effects in preliminary work (Helleck and Hartberg 1993) in which 4th-instar larvae of *Er. quinquevittatus* were exposed to PII solutions (≤20 μg/ml). The adults that emerged laid significantly fewer eggs compared to adults developing from untreated larvae. The long-term effects of PII and other photoreactive compounds must be investigated further before they can be recommended as mosquito control agents.

We thank QuadraLogic Technology, of Vancouver, British Columbia, Canada, for providing the Photofrin II®. We also extend our thanks to M. M. Judy, Baylor Research Institute in Dallas; Robert Novak, Center for Economic Entomology, University of Illinois; and Constantine Rebeiz, Laboratory of Plant Pigment Biochemistry and Photobiology, University of Illinois, for their critiques of this manuscript.

REFERENCES CITED

Barbieri, A. 1928. Sensibilizadores fluorescentes como larvicidas. Acción fotdynámica de la luz. Riv. Malariol. 7:456.

Beament, J. 1989. Eggs—the neglected insects. J. R. Army Med. Corps 135:49–56.

Byrne, C. J., L. V. Marshallsay and A. D. Ward. 1990. The composition of Photofrin II®. J. Photochem. Photobiol. 6(1–2):13–27.

Clements, A. N. 1992. The biology of mosquitoes, Vol-

ume 1. Development, nutrition, and reproduction. Chapman & Hall, London, UK.
Edwards, F. W. 1941. Mosquitoes of the Ethiopian region III: culicine adults and pupae. British Museum (Natural History), London, UK.
Graham, K. 1972. Entomological, ecological, and evolutionary implications of photodynamic action. Can. J. Zool. 50:1631.
Hartberg, W. K. and W. H. Faircloth. 1983. Mitotic chromosomes of the mosquito *Eretmapodites quinquevittatus* Theobald. Mosq. Syst. 15(4):325–329.
Hartberg, W. K. and M. M. Judy. 1990. Photosensitization of mosquito larvae with DHE. Proc. Tex. Mosq. Control Assoc.
Harwood, R. F. and W. R. Horsfall. 1959. Development, structure, and function of coverings of eggs of flood water mosquitoes. III. Functions of coverings. Ann. Entomol. Soc. Am. 52:113–116.
Helleck, A. M. 1992. Selected bionomic observations of *Eretmapodites quinquevittatus* Theobald (Diptera: Culicidae). Master's thesis. Baylor University, Waco, TX.
Helleck, A. M. and W. K. Hartberg. 1993. Preliminary observations on the effect of non-lethal concentrations of DHE on mosquito larvae. Proc. Tex. Mosq. Control Assoc. 37:3.
Kagan, J., J.-P. Beny, G. Chan, S. N. Dhawan, J. A. Jaworski, E. D. Kagan, P. D. Kassner, M. Murphy and J. A. Rogers. 1983. The phototoxicity of some 1,3-butadienes and related thiophenes against larvae of the mosquito *Aedes aegypti* and of the fruit fly *Drosophila melanogaster*. Insect Sci. Appl. 4:377.
McGrane, V., J. O. Carlson, B. R. Miller and B. J. Beatty. 1988. Microinjection of DNA into *Aedes triseriatus* ova and detection of integration. Am. J. Trop. Med. Hyg. 39:502–510.
Morris, A. C., P. Eggleston and J. M. Crampton. 1989. Genetic transformation of the mosquito *Aedes aegypti* by micro-injection of DNA. Med. Vet. Entomol. 3:1–7.
Rebeiz, C. A., L. J. Gut, L. Keywan, J. A. Juvik, C. C. Rebeiz and C. E. Bouton. 1995. Photodynamics of porphyric insecticides. Crit. Rev. Plant Sci. 14(4):329–366.
Sakurai, H. and J. R. Heitz. 1982. Growth inhibition and photooxidative toxicity in the house fly *Musca domestica* L., caused by xanthene dye in larval growth medium and after injection. Environ. Entomol. 11:467–470.
Schildmacker, H. 1950. Über photosensibilisierung von stechmuckenlarven durch fluoreszierende farbstoffe. Biol. Zentralbe. 69:468.
Service, R. F. 1995. New compounds make light of pests. Science. 268:806.
Spikes, J. D. 1984. Photobiology of porphyrins, pp. 19–39. *In:* Porphyrin localization and treatment of tumors. Alan R. Liss, New York.
Spikes, J. D. 1985. The historical development of ideas on applications of photosensitized reactions in the health sciences. R. V. Bensasson, E. J. Land, G. Jori and T. G. Truscott (eds.), pp. 124–144. *In:* Primary photoprocesses in biology and medicine. Plenum Press, New York.
Waggoner, J. 1995. Light plus food coloring sends pests to their reward. Biophotonics International. Sept./Oct. 1995:88–89.
Zar, J. H. 1984. Biostatistical analysis, 2nd ed. Prentice-Hall, Englewood Cliffs, NJ.