THE EFFECTS OF SELECTED RICE AND SOYBEAN PESTICIDES ON THE EGGS OF *PSOROPHORA COLUMBIAE*[1]

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ABSTRACT. Twenty-five pesticides used in the production of rice and soybeans in Texas were tested in the laboratory to determine their toxicity to the eggs of *Psorophora columbiae*. A reduction in hatching rate occurred when eggs were treated with a herbicide formulation containing thiobencarb and with one containing a tank mixture of propanil and molinate. A carbaryl formulation induced hatching of eggs prior to their exposure to the hatching stimulus. Reduced survival to second instar of larvae hatching from treated eggs was observed with insecticide formulations of acephate, carbofuran, malathion, methyl parathion and toxaphene; a fungicide formulation of triphenyltin hydroxide and the tank mixture of the herbicides, propanil and molinate.

INTRODUCTION

Eggs deposited by *Psorophora columbiae* (Dyar and Knab) in southern riceland systems stand to be exposed to a variety of agricultural chemicals used in the culturing of rice and crops alternated with this grain. However, prior to the study described herein, little information existed on the effect of such chemicals on mosquitoes while they are in the egg stage. Powers and Headlee (1939) have reported that certain petroleum oils are toxic to the eggs of *Aedes aegypti* (Linn.).

Certain agricultural chemicals are known to affect the larval stages of mosquitoes occurring in riceland habitats. Lancaster and Tugwell (1969) and Gifford et al. (1969) recorded mortality in rice field populations of *Ps. columbiae* larvae as a result of carbamate and organophosphate insecticide applications for control of the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel. Chambers et al. (1981) observed control of mosquito larvae in rice fields resulting from applications of the herbicide molinate. Resurgence of mosquito populations following an initial reduction in numbers of both mosquito larvae and their predators has been observed in rice fields treated with the herbicide, thiobencarb (Ishibashi and Itoh 1981), and the fungicide, triphenyltin hydroxide (also known as fentin hydroxide) (Schaefer et al. 1981).

The purpose of this laboratory study was to examine the potential effects of various commercial formulations of agricultural pesticides to which eggs of *Ps. columbiae* might be exposed in a rice field habitat. Soybean pesticide formulations were also considered, since soybeans are commonly rotated with rice and soybean fields are important sources of oviposition sites for *Ps. columbiae* in Texas riceland systems[4]. Compounds and/or formulations of these compounds that were potentially toxic to the eggs of *Ps. columbiae* were identified from among those screened and will be subject to further investigation in subsequent field studies.

MATERIALS AND METHODS

The sources of eggs used in this study were *Ps. columbiae* females collected from riceland habitats in Chambers and Jefferson counties (TX) during May and June, 1981 and June and July, 1982. The females were induced to lay their eggs on moist strips of folded cheesecloth. The eggs were subsequently removed from the cheesecloth strips, placed on damp filter paper squares resting on moist cellucotton pads in petri dishes and stored under these conditions in an incubator (26° C, 14:10 light:dark regimen, 90–100% RH) for at least 2 weeks to allow for embryonation to be completed or until such time that the eggs were used in the treatments described below.

Prior to each test, eggs were counted out of the stock egg colony into lots of 10 and each lot was placed into a 2 cm square of filter paper. Five squares of eggs were then arranged on a moist cellucotton pad in each of 8 petri dishes (i.e., 50 eggs/dish). The eggs in 4 petri dishes were treated with a given pesticide formulation and the eggs in the other 4 dishes were treated with deionized water and served as controls.

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[4] Welch, J. B. 1983. The Use of Aerial Color Infrared Photography as a Survey Technique for *Psorophora columbiae* (Dyar and Knab) (Diptera: Culicidae) Oviposition Habitats in Texas Riceland Agroecosystems. Ph.D. dissertation. Texas A&M. Univ., College Station, TX 226 pp.

Egg treatments were conducted on the floor (1.86 m^2) of a wood-framed chamber lined and covered with polyethylene plastic sheeting. Uncovered petri dishes of eggs were placed on the floor of the chamber and then either the pesticide formulation to be tested or deionized water was applied to the dishes of eggs using a Herbi® ultra low volume applicator (Micron Corp., Houston, TX).[5] This applicator is a hand-held, wand-like device equipped with a battery-powered, spinning-disk atomizer. Solutions of the commercial pesticide formulations were gravity-fed to the atomizer assembly via a flexible tube leading from a 2.5 liter tank on the handle of the applicator to the atomizer at tip of the device. The commercial formulation of each pesticide listed in Table 1 was applied at maximum labelled rates by sweeping the applicator back and forth over the floor of the test chamber until the total volume of the diluted formulation was dispensed. In the case of control egg lots, they were treated with a volume of deionized water equal to that of the given diluted pesticide formulation tested.

Immediately after treatment, the petri dishes were covered and returned to the incubator. The eggs were not transferred to clean filter paper squares or petri dishes, since residual effects as well as immediate effects of the given pesticide formulation were to be assessed together, thus, more closely duplicating conditions that could occur under actual field conditions.

At intervals of 12, 24, 48, 96 and 336 hours posttreatment, a square of eggs (10 eggs/square) was taken from each of the 4 treated and 4 control petri dishes. The eggs were either individually removed from each square with jeweler's forceps or washed from the filter paper using deionized water and subsequently drawn up into a Pasteur pipette, placed into hatching tubes (each a 2 cm long, closed-bottomed tube constructed from a Pasteur pipette and placed in a 4-dram shell vial), and subjected to a hatching stimulus of nutrient broth following the procedures described by Novak and Shroyer (1978). Thus, at each posttreatment interval, 40 treated and 40 control eggs were subjected to the hatching stimulus.

Larvae that hatched were allowed to continue their development in the hatching tubes to the second instar. Eggs that failed to hatch were broken open with jeweler's forceps and examined under a stereomicroscope. The eggs were counted as "viable" (at the time of treatment) if a fully-developed embryo was present. If no embryonic development was discernable, or if the embryo was only partially developed, the egg was counted as "unviable" at the time of treatment. The unviable eggs were not included in the final analysis of the results of a given test. Records were kept on each square of eggs as to the total number of viable eggs, the total number of eggs hatching and number of larvae surviving to second instar.

Hatching and survival rates of treated and control insects were compared using logistic regression (Anderson 1980). The analysis also determined whether an observed effect was due to time or to the toxin used and tested the possibility of an interaction between the two. A test of a given pesticide formulation was repeated if reductions in hatching and/or first instar survival rates in the treated mosquito populations were significantly greater ($p = 0.05$) than that occurring in the controls.

Certain of the pesticide formulations determined via the filter paper trials to have significant effects on the egg hatching or larval survival rates of *Ps. columbiae* were retested against eggs placed on a moist soil substrate which more closely approximated field conditions. In these tests, a 7 mm layer of Morey silt loam obtained from the Texas A&M Agricultural Research and Extension Center-Beaumont, TX was placed in the bottom of a petri dish. The soil was moistened to approximately 75% field capacity (Box and Bennett 1959, Olson and Meek 1977) and 150 mosquito eggs were placed on the soil surface. One such dish was treated with the given pesticide formulation and another with deionized water using application procedures previously described for the filter paper trials. Following treatment, the petri dishes of soil and eggs were again stored in the incubator. At each of the same posttreatment time intervals used in the filter paper trials, 30 eggs were removed from each, the treated and control, dish containing soil and subjected to the same hatching and rearing procedures described for the filter paper trials. Records were again kept on the total number of viable eggs, number of eggs hatching and number of hatching larvae surviving to the second instar for each batch of eggs examined. The only pesticide formulations tested in this manner were ones whose effects observed during the filter paper trials were determined not to involve time/toxin interaction.

RESULTS AND DISCUSSION

The herbicide, thiobencarb, and a tank mix of the herbicides, propanil and molinate, appeared to be the only ones among the 25 commercial pesticide formulations tested (Table 1)

[5] Mention of a commercial or proprietary product does not constitute an endorsement by the USDA, TAES or Texas A&M University.

which had any ovicidal effect on *Ps. columbiae* eggs. The mean percent hatch of eggs for all posttreatment time intervals combined in the case of the first test involving thiobencarb was 77% for treated eggs and 94% for control eggs (significantly different, p = 0.01). In the second thiobencarb test, 70% of the treated eggs and 93% of the control eggs hatched (significantly different, p = 0.01). The mean percent hatch of eggs treated with the propanil/molinate mix was 93% for the treated eggs as compared to 99% for the controls in the first test. The statistical analysis performed on these results indicated a significant difference (p = 0.05). A more pronounced difference occurred for the second test involving this mix where the mean percent hatch was 49% for treated eggs as compared to 90% for the controls (significantly different, p = 0.001).

A peculiar phenomenon was observed in the case of tests involving the insecticidal formulation containing carbaryl. Treatment of mosquito eggs with this particular formulation resultd in a rather large number of eggs hatching on the filter paper squares before they were exposed to the hatching stimulus (Table 2). The filter paper test involving the carbaryl formulation was repeated 3 times and the same effect was observed each time. The difference between the carbaryl-treated eggs and the control eggs in regard to abrupt hatching on the filter paper was significant (p = 0.001). Abrupt hatching also occurred to some degree in carbaryl-treated egg samples resting on moist soil (Table 2), but did not occur in those egg samples placed on dry soil. This suggests the ready availability of moisture may play a role in the induction of abrupt hatching by the carbaryl formulation. It is not certain whether it was the carbaryl itself or other additives in the formulation that caused this abrupt hatching of mosquito eggs in the presence of moisture. However, this particular formulation was the only one of the 25 pesticide formulations tested that caused such an effect and further investigation of this phenomenon is warranted.

In regard to survival of larvae hatching from treated eggs, a number of pesticide formulations were found to reduce larval survival rates to the second instar (Table 3). These formulations included ones containing the fungicidal agent, triphenyltin hydroxide, the tank mix containing propanil and molinate and all the ones containing insecticidal agents save for those having carbaryl and methomyl in them. The formulation containing the herbicide, pendimethalin, showed a time/toxin interaction effect in both trials involving this agent, leaving unclear the exact role played by this formulation in reducing survival of larvae hatching from eggs to which it had been applied. A "time/toxin interaction" was determined to have occurred when the observed reduction in egg hatching or larval survival rates was not constant through time. In such instances, it could not be stated that the observed effect was due solely to the toxic properties of the pesticide formulation and the length of exposure

Table 1. Active ingredient(s) in the commercial formulations of rice and soybean pesticides applied at maximum labelled rates to eggs of *Psorophora columbiae* in laboratory tests.*

Active ingredient	Formulation trade name	Active ingredient	Formulation trade name
Herbicide		*Insecticide*	
acifluorfen (S)	Blazer®	acephate (R, S)	Orthene® 75S
alachlor (S)	Lasso®	carbaryl (R, S)	Sevin® XLR
bentazon (R)	Basagran®	carbofuran (R, S)	Furadan® 4 Flowable
bifenox (R)	Modown®	malathion (R)	Cythion®
butachlor (S)	Machete®	methomyl (S)	Lannate® L
linuron (S)	Lorox®	methyl parathion (R, S)	Penncap-M®
MCPA (R)	Rhomene®	toxaphene (R, S)	Attac® 6
Metolachlor (S)	Dual® 8E		
metribuzin (S)	Lexone® 4L	*Fungicide*	
molinate (R, S)	Ordam® 8E	triphenyltin	
oxadiazon (R)	Ronstar®	hydroxide (R)	Super Tin® 4L
pendimethalin (S)	Prowl®		
propanil (R, S)	STAM® M-4	*Tank mix****	
2, 4-D (R)	2, 4-D Amine #4**	propanil/molinate (R)	STAM M-4/Ordam
silvex (R)	Kuron®		
thiobencarb (R, S)	Bolero® 8E		

* "R" and "S" refer to registration of the compound for use in rice and soybeans, respectively.
** Special solution (4 lb a.i./gal.) supplied by Rhone-Poulenc Chemical Co., Monmouth Junction, NJ.
*** Each formulation in the mix applied at the maximum labelled rate recommended when each is used separately.

Table 2. Instances of abrupt hatching of *Psorophora columbiae* eggs in response to treatment with a carbaryl formulation in laboratory tests. Treatment effect observed was significant at p = 0.001 in each test.

Hours post-treatment	Number of eggs hatching prematurely							
	On filter paper						On moist soil	
	Test 1		Test 2		Test 3			
	Treated	Control	Treated	Control	Treated	Control	Treated	Control
12	83	0	103	0	14	0	1	0
24	58	1	43	0	15	0	7	0
48	15	0	12	1	25	0	0	0
96	2	0	1	0	16	0	8	0
336	0	0	1	0	11	1	2	0

time to the given formulation was, to some degree, playing a part. For brevity, the results of the larval survival tests have been summarized in Table 3 in the form of mean percent survival for 2 test replications, each consisting of 5 posttreatment sampling periods.

The observed reduction in larval survival could possibly have been due to residual contamination on the external surfaces of the treated eggs. Depending on the quality of active ingredient(s) present in the formulation and the amount of material applied, the possible concentration of active ingredient present in the hatching tubes was estimated as ranging from 1 ppm to as high as 25 ppm in some tests.

Of all the pesticide formulations tested, the propanil/molinate mixture was the only one which consistently produced an effect on both parameters chosen for the study of *Ps. columbiae* egg survival in response to pesticides. When tested alone, neither propanil (tested twice) nor molinate (tested 3 times) yielded consistent and reproducible results. A number of other formulations tested also showed effects during one test that were not observed when the test was repeated.

Since this was a laboratory study, more data are needed to determine whether the events reported here will occur under field conditions. If any of these pesticide formulations could be demonstrated to consistently reduce the survival of mosquito eggs or early instar larvae in the field, this information could be incorporated into a mosquito management scheme, or used in predictive modeling by mosquito control workers. Reduction of existing mosquito populations as a side effect of chemical applications for crop protection would certainly be desirable in the sense of adding yet another control factor to those already in force against mosquito populations occurring in southern riceland systems.

The observation that the carbaryl formulation may induce abrupt hatching of floodwater mosquito eggs is of particular interest in that the hatching mechanism for eggs of these kinds of mosquitoes is incompletely understood at present. Physiological studies of this phenome-

Table 3. Summary of test results, showing commercial formulations of compounds causing reduction in survival to second instar of *Psorophora columbiae* larvae hatching from treated eggs. Effects were observed during 2 replications of laboratory tests.

Compound tested	$\bar{x}$ % survival for 2 replications, over all time periods		$\bar{x}$ % reduction in survival for 2 replications, over all time periods
	Treated	Control	
acephate*	66.9	73.9	7.0
carbofuran*	4.8	46.8	42.0
malathion*	12.9	50.5	37.6
methyl parathion (p = .001)	2.2	58.2	56.0
toxaphene*	42.9	78.9	36.0
triphenyltin hydroxide*	23.0	61.1	38.1
propanil/molinate (p = 0.01, 0.001)	58.5	79.4	20.9
pendimethalin**	44.3	64.7	20.4

* Denotes time/toxin interaction effect for one test, the other test showing a treatment effect significant at p = 0.001.

** Denotes time/toxin interaction effect in both tests, thus the observed reduction may not be due solely to the compound.

non might prove to be of value in elucidating this aspect of mosquito biology.

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