LABORATORY EVALUATION OF FOUR ORGANOPHOSPHATE COMPOUNDS AS LARVICIDES AGAINST FIELD COLLECTED SALT MARSH *CULICOIDES* SPP. (DIPTERA: CERATOPOGONIDAE)[1]

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ABSTRACT. The relative effectiveness of 4 organophosphate compounds was tested in the laboratory against *Culicoides* spp. larvae obtained from salt marsh areas on the Gulf coast of Florida. Larvae were exposed to the insecticide either in estuarine water or in a combination of estuarine water and dried substrate from the salt marsh habitat. The relative potency of the 4 chemicals varied greatly, with chlorpyrifos being the most toxic. Nine times as much temephos, 22 times as much fenthion, and 176 times as much malathion were required to achieve the 50% mortality level compared to chloryprifos (LC_{50}, 0.5 ppb) in water. Addition of habitat soil did not change the order of effectiveness, but 9 times as much chlorpyrifos, 2.3 times as much temephos, and 1.3 times as much fenthion or malathion were required to cause the same mortality as in estuarine water alone.

INTRODUCTION

Two species of biting midge, *Culicoides furens* (Poey) and *C. mississippiensis* Hoffman are prevalent on the Gulf coast of Florida. Both species are of public health importance because the bites of the females are numerous, annoying, often cause enduring discomfort, and if scratched may result in secondary infection (e.g., cellulitis). Because of movement of adults away from the breeding places, adulticide control measures such as ground and aerial pesticide applications provide only temporary relief and repeated applications must be made as new females emerge. Larval control is therefore particularly attractive since active biting midge larvae can be found in the salt marsh substrate during all seasons. Recent investigations (unpublished data) indicate that the larvae may be seasonally clumped into relatively small vegetative zones. Thus, properly timed larvicidal treatment to selective areas of the marsh could result in a significant reduction of the subsequent adult population.

Treatment of the substrate with insecticides for control of *Culicoides* larvae found in intertidal areas is not a new concept. Curran and Goulding (1950), Labrecque and Goulding (1954), Jamnback et al. (1958), Breeland and Smith (1962), and Wall and Doane (1965) all treated intertidal areas with chlorinated hydrocarbon insecticides. These insecticides are no longer acceptable because of environmental persistence.

In field tests conducted against *Culicoides melleus* (Coquillett) larvae in sandy intertidal plots in Cape Cod, Massachusetts, Wall and Marganian (1971) reported successful control, ranging from excellent to effective, with chlorpyrifos, temephos, fenthion, and malathion, with minimal adverse effects on other intertidal fauna in or adjacent to the treated areas. Ferguson and Cawthorne (1980) showed temephos to be effective against *Culicoides molestus* (Skuse) larvae in Australia. Kay et al. (1973) in Australia and Campbell and Denno (1976) in New Jersey stated that temephos had little disruptive effect on nontarget estuarine organisms.

This paper describes laboratory studies to determine the effectiveness of these organophosphorus insecticides against *Culicoides* spp. larvae found in intertidal study areas on the Gulf coast of Florida. Specific objectives were to determine the relative toxicities of these 4 compounds, and the effect of the substrate soil on their effectiveness.

MATERIALS AND METHODS

Larvae were obtained from known salt-marsh breeding habitats near Yankeetown, Florida. Mixed populations of *C. furens* and *C. mississippiensis*, which share the same habitat, were used in our tests. The 2 species were considered together since it is difficult to identify these species as living larvae. Substrate samples (ca. 10 cm diam X 8 cm deep) were taken with posthole diggers and larvae removed by the agar extraction technique (Kline et al. 1981).

After removal from the substrate sample, the 3rd and 4th instar larvae were selected and 6–8 were transferred with a pipette into each well of

[1] This paper represents the results of research only. Mention of a pesticide in this paper does not constitute a recommendation for use by the U.S. Department of Agriculture nor does it imply registration under FIFRA as amended. Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the USDA.

a black porcelain multi-well spot plate. Use of the black spot plates provided good contrast for handling the white larvae. Ten larvae were transferred with a small angled dissecting probe from the spot plate into 50 ml Nalgene® beakers containing 25 ml of estuarine water. These larvae were then exposed to the insecticide either in filtered estuarine water (w) in 250 ml test glass beakers or in a combination of estuarine water and dried substrate (w/s) from the salt marsh habitat. In the aqueous test, the content of the 50 ml beakers containing the larvae was poured with a swirling action into a 250 ml test beaker which contained 100 ml of filtered estuarine water treated at predetermined discriminating concentrations. In the w/s test, 10 g of dried substrate was added to 100 ml of filtered estuarine water in the test beakers and the mixture stirred vigorously for 5 min. The insecticide solution was then added and this mixture stirred gently for an additional 5 min, and then allowed to settle for 1 hr. The mixture was then filtered through Miracloth® cloth to remove most of the substrate so that the larvae could be seen. The larvae were then added.

Test solutions were initially prepared as stock solutions of the technical grade material in reagent grade acetone containing 1 mg/ml. Subsequent dilutions of the stock solution were made in reagent grade acetone; the final dilution contained sufficient active ingredient so that 1 ml was used to obtain the desired test concentration.

Based on preliminary tests to establish the approximate dose ranges, the exposures were conducted at the following concentrations (ppb): for the treatment in estuarine water only—chlorpyrifos 0.25-1.00, fenthion 10–25, temephos 3–7, and malathion 50–500; in the estuarine water/habitat soil mixture—chlorpyrifos 3–7, fenthion 10–50, temephos 5–25, and malathion 50–1000. Each insecticide combination was tested at 4 or more concentrations. The discriminating solutions were run in duplicate beakers with 10 larvae each, thus exposing 20 larvae to the some concentration level. Tests were replicated at least 3 times so that a minimum of 60 larvae was exposed at each concentration. Duplicate beakers of acetone-water solutions with acetone quantities equal to the largest amount added to the test beakers were set up as checks.

The beakers were held at 25±1°C for 24 hr posttreatment, after which mortality readings were taken. Larvae were considered dead if, when touched with a probe, they did not respond with their characteristic serpentine motion, i.e., rapid lateral flexing of the body. Data from all tests were summed and the dose-response relationship determined by probit analyses of the log-transformed mortality data (Finney 1971) using SAS PROBIT procedure (Ray 1982).

RESULTS AND DISCUSSION

The regression values (slopes), standard errors of the slopes, and LC_{50}'s and LC_{90}'s with corresponding 95% fiducial limits for each chemical are shown in Table 1. Check mortality was less than 1% (0–5%) in beakers with sea water alone and when habitat soil was added. Therefore, no corrections were made for mortality.

Slope comparisons of the 4 chemicals showed 3 separate groupings: malathion (both w and w/s treatments) was not only the least toxic of the chemicals tested but the LC_{50} and LC_{95} response interval included a broad range of concentrations. Similar and intermediate slope values were obtained for both treatments with chlorpyrifos and with fenthion and for the w/s treatment of temephos, even though these chemicals differed widely in toxicity. Similarity

Table 1. Effectiveness of organophosphorus compounds as larvicides against field-collected larvae of salt marsh *Culicoides* spp. from Yankeetown, Florida.

Chemical	No. tests[1]	Treatment	LC_{50} (ppb)	95% Fiducial limits	LC_{90} (ppb)	95% Fiducial limits	Slope[2]	S.E.
temephos	5	water	4.6	4.4– 4.8	8.2	7.5– 9.1	2.85a	0.23
	3	water/soil	10.7	9.8– 11.6	33.8	28.7– 41.8	1.42b	.10
chlorpyrifos	6	water	0.5	0.5– 0.6	1.3	1.0– 2.0	1.81b	.28
	4	water/soil	4.3	3.4– 5.1	10.3	7.8– 21.9	1.91b	.49
fenthion	3	water	10.8	9.3– 12.1	27.6	24.0– 33.6	1.76b	.20
	4	water/soil	13.9	12.3– 15.5	41.0	34.8– 52.0	1.53b	.16
malathion	3	water	87.9	72.0–106.1	453.4	326.0–744.9	1.00c	.12
	4	water/soil	114.6	97.6–134.8	624.1	466.7–924.9	.97c	.08

[1] Each test consisted of at least 4 discriminating concentrations of insecticide; duplicate beakers containing 10 larvae each were used to each concentration.

[2] Numbers followed by the same letter do not differ significantly at the 5% level.

of the slopes for these 3 chemicals probably occurred because they are similar compounds (phosphorothioates) and thus might be expected to have similar modes of action. However, temephos in water only exhibited a different pattern, with the largest slope and also the narrowest concentration interval between LC_{50} to LC_{95} values.

The relative potency of the 4 chemicals varied greatly, with chlorpyrifos the most toxic. Against field-collected larvae, 9 times as much temephos, 22 times as much fenthion, and 176 times as much malathion were required to achieve the 50% mortality level compared to chlorpyrifos in water. Addition of habitat soil did not change the order of effectiveness, but 9 times as much chlorpyrifos, 2.3 times as much temephos, and 1.3 times as much fenthion or malathion were required to cause the same mortality as in estuarine water alone. At the LC_{95} level chlorpyrifos (w) was the most toxic material, followed in order of toxicity by temephos (w), chlorpyrifos (w/s), fenthion (w), temephos (w/s), fenthion (w/s), malathion (w) and malathion (w/s). Each of these materials required approximately 6, 8, 21, 26, 32, 349 and 480 times more material to achieve the LC_{95} level than chlorpyrifos (w), respectively. These toxicity data support the reports in the literature on the relative potency of these 4 insecticides for field-collected *C. furens* larvae evaluated in laboratory tests in Puerto Rico (Fox et al. 1968) and for *C. melleus* evaluated in small field plots in Cape Cod, Massachusetts (Wall and Marganian 1971).

These same organophosphorus insecticides were tested against *C. variipennis* (Coquillett) larvae, a nonestuarine species. Apperson (1975) in California found that malathion (LC_{50}, 0.64 ppm) was the least effective whereas chlorpyrifos-methyl (LC_{50}, 0.026 ppm) and temephos (LC_{50}, 0.025 ppm) were the most biologically active. Chlorpyrifos (LC_{50}, 0.042 ppm) was next in order of effectiveness, followed by fenthion (LC_{50}, 0.058 ppm). In Holbrook's (1981) study in Colorado against field-collected larvae the order of effectiveness of 3 compounds at the LC_{50} level was: chlorpyrifos (0.0037 ppm), fenthion (0.0298 ppm) and temephos (0.0404 ppm).

The above data indicate that these insecticides, with the possible exception of malathion, merit further testing under field conditions.

References Cited

Apperson, C. S. 1975. Biological activity of insecticides against *Culicoides variipennis* (Coquillett) (Diptera: Ceratopogonidae). Proc. Calif. Mosq. Contr. Assoc. 43:118–119.

Breeland, S. G. and J. P. Smith. 1962. Observations on the importance of flight ranges in the control of *Culicoides* in the Panama Canal Zone. Mosq. News 22:147–154.

Campbell, B. C. and R. F. Denno. 1976. The effect of temephos and chlorpyrifos on the aquatic insect community of a New Jersey salt marsh. Environ. Entomol. 5:477–483.

Curran, R. F. and R. L. Goulding, Jr. 1950. The problems and methods involved in sampling salt marsh sandfly populations. Proc. Annu. Mtg. Fla. Anti-Mosq. Assoc. 21:113–117.

Ferguson, K. J. and J. H. Cawthorne. 1980. Boom spray application of insecticide for control of estuary breeding biting midges (*Culicoides*, Ceratopogonidae). Mosq. News 40:217–222.

Finney, D. J. 1971. Probit analysis. Cambridge Univ. Press. 333 pp.

Fox, I., G. A. Rivera and C. C. Umpierre. 1968. Toxicity of various insecticides to *Culicoides furens* larvae in Puerto Rico. Mosq. News 28:68–71.

Holbrook, F. R. 1981. Evaluation of three insecticides against colonized and field-collected larvae of *Culicoides variipennis* (Diptera: Ceratopogonidae). J. Econ. Entomol. 11:736–737.

Jamnback, H. A., W. J. Wall and D. L. Collins. 1958. Control of *Culicoides melleus* (Coq.) (Diptera: Heleidae) in small plots, with brief descriptions of the larvae and pupae of two coastal *Culicoides*. Mosq. News 18:64–70.

Kay, B. H., K. J. Ferguson and R. N. C. Morgan. 1973. Control of salt-marsh mosquitoes with Abate® insecticide at Coomlabah Lakes, Queensland, Australia. Mosq. News 33:529–535.

Kline, D. L., R. H. Roberts and D. A. Focks. 1981. Extraction of larvae of the ceratopogonid biting midge, *Culicoides mississippiensis*, from salt marsh soils with a new agar technique. Mosq. News 41:94–98.

Labrecque, G. C. and R. L. Goulding. 1954. Tests with granulated BHC and dieldrin for controlling sand fly larve. Mosq. News 14:20–22.

Ray, A. A. 1982. SAS user's guide: Statistics, 1982 edition. SAS Institute, Inc. 584 pp.

Wall, W. J., Jr., and V. M. Marganian. 1971. Control of *Culicoides melleus* (Coq.) (Diptera: Ceratopogonidae) with granular organophosphorus pesticides, and the direct effect on other fauna. Mosq. News 31:209–214.

Wall, W. S. and O. W. Doane. 1965. Control of *Culicoides melleus* (Coq.) (Diptera: Heleidae) with granular insecticide and the effect on other fauna. Mosq. News 25: 191–196.