POTENTIAL FOR RESISTANCE TO PYRIPROXYFEN: A PROMISING NEW MOSQUITO LARVICIDE

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ABSTRACT. An organophosphorus-resistant strain of *Culex quinquefasciatus* was pressured with pyriproxyfen for 17 generations. Egg viability began declining in the F_7 generation and became lower as the selection process continued; by the F_{17} generation egg viability was too low to proceed further. Susceptibility tests on larvae of the F_5, F_{10}, F_{15}, and F_{17} generations showed no indication of increased tolerance to pyriproxyfen.

INTRODUCTION

Pyriproxyfen, 2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy] pyridine, is also known as S-31183 and by the trademark names Nylar® and Sumilarv®. This compound acts as a juvenile hormone mimic; it does not produce direct larval toxicity but disrupts the normal process of insect development, which results in pupal mortality or in the production of abnormal adults. Pyriproxyfen is highly active against a variety of insects of public health importance including cockroaches (Chow and Yang 1990), fleas (Palma and Meola 1990), the tsetse fly (Langley et al. 1990) and mosquitoes (Estrada and Mulla 1986, Mulla et al. 1986, Schaefer et al. 1988).

Mulligan and Schaefer (1990) showed that pyriproxyfen could be used to treat animal waste lagoons for *Culex quinquefasciatus* Say larvae and that single treatments provided up to 2 months' control. The active ingredient readily leaves the water and adsorbs onto organic matter, where it then decays at an exponential rate while also providing biological activity (Schaefer et al. 1991). Such highly polluted habitats are a well known source for the breeding of *Cx. quinquefasciatus* and closely related *Culex* species all over the world. The very large populations frequently encountered in such habitats often require regular insecticide applications. Large populations and high selection pressure over relatively long time periods lead to insecticide resistance, especially when only one agent, or closely related chemical control agents, are utilized. For example, Schaefer and Dupras (1970) showed that when sewage lagoons were treated with chlorpyriphos, the active ingredient adsorbed onto organic debris and the biological activity against *Cx. quinquefasciatus* larvae lasted for 4–8 weeks. However, repeated treatments of these sources led to loss of effectiveness of chlorpyrifos and related compounds due to insecticide resistance (Stewart 1975).

Another consideration is the potential for cross-resistance of strains which have already been selected for insecticide resistance. For example, when a new benzamide larvicide which had a high degree of efficacy against mosquitoes was used to pressure an organophosphorus-resistant (OP-R) strain of *Cx. quinquefasciatus*, cross-resistance became apparent after only 4 generations of selection (Schaefer et al. 1981).

Information on how quickly insecticide-resistant strains might develop tolerance to pyriproxyfen was sought. Insecticide pressure experiments using pyriproxyfen were initiated in 1987 and were continued through 1990 and are summarized in this report.

MATERIALS AND METHODS

Insecticide: A technical standard of pyriproxyfen (99.6%) was provided by the McGlaughlin, Gormley, King Company, Minneapolis, MN.

Mosquito colonies: An OP-R strain of *Cx. quinquefasciatus* was obtained from underground pipelines in Fresno, CA, in 1985 and colonized; they were occasionally pressured with fenthion. A susceptible (OP-S) strain of the same species, which has been maintained at this location for ca. 20 years, was used for initial comparisons of susceptibility.

Insecticide susceptibility tests: For larval susceptibility tests to pyriproxyfen, 25 4th-instar larvae were placed in 250 ml tap water in Pyrex® storage dishes (80 × 100 mm). A series of 10 concentrations (range 0.001–0.000002 ppm) was obtained by applying 10 µl from acetone solutions to the water. Each concentration was made in duplicate for a test. Three separate tests were conducted, each on a different date, and the lot were combined in a single probit analysis. Test containers were held at 27°C at a photoperiod of 14:10 (L:D). Mortality counts were made after adult emergence was complete; the data were adjusted for control mortality (Abbott 1925) and

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analyzed by probit analysis (SAS Institute 1985).

Just prior to initiation of pressuring of the OP-R strain with pyriproxyfen, the susceptibility to malathion, parathion, fenthion and chlorpyrifos was determined, as previously described (Wilder and Schaefer 1972). For comparison, the same compounds were also run against a susceptible strain.

Pressure technique: Pressuring of the OP-R strain of *Cx. quinquefasciatus* was initiated in September 1987. Mid- to late 4th-instar larvae were placed in a glass aquarium containing 4 liters of water treated with 0.00001 ppm pyriproxyfen. Pupae were removed as age cohorts at various time intervals and reared as separate groups. For those groups in which the combined final mortality was 50% or greater, the surviving adults were added to the next generation adult cage.

Because of reduced egg production and viability (see Discussion), the method was changed beginning in the F_{11} generation. Thereafter, lots of 50 late, 4th-instar larvae were placed in 250 ml water in Pyrex storage dishes and treated with pyriproxyfen at 0.00002, 0.00004 and 0.00007 ppm. Adults successfully emerging from treatments where mortality exceeded 50% were added to the cage of the next generation.

RESULTS AND DISCUSSION

Susceptibility of laboratory strains of Culex quinquefasciatus *to pyriproxyfen:* A comparison of the susceptibility of OP-S and OP-R laboratory strains to pyriproxyfen and 4 organophosphorus larvicides is summarized in Table 1. The OP-R strain was most tolerant to fenthion as would be expected since this compound had been used to maintain pressure to this colony. There was no apparent difference in susceptibility to pyriproxyfen based on the LC_{50s}, but there was a possibility of a small increase in tolerance of the OP-R strain based on the LC_{95s}.

Table 2. A summary of larval numbers and mortality during pressuring[a] with pyriproxyfen.

Generation	No. exposed	No. survivors	Mortality (%)
Parent	1,162	619	47
F_1	1,996	824	59
F_2	2,013	862	57
F_3	953	212	78
F_4	2,782	530	81
F_5	1,226	495	60
F_6	1,950	622	68
F_7	2,714	693	74
F_8	2,198	523	76
F_9	492	55	88
F_{10}	850	193	77
F_{11}	889	252	72
F_{12}	384	72	81
F_{13}	600	156	74
F_{14}	483	162	66
F_{15}	750	117	84
F_{16}	183	80	56

[a] Pressure dose: parent through F_{10} generations, 0.00001 ppm; F_{11} through F_{16} generations, 0.00002 to 0.00007 ppm.

Results from pressuring multiple generations: Table 2 summarizes the numbers of larvae exposed and the resulting mortality for each generation. Beginning in the F_7 generation, egg production and viability became reduced and adults from unpressured larvae were returned back to the cage of the same generation to maintain the colony. As sufficient numbers of viable eggs were obtained, the pressure sequence was continued. The problem of obtaining viable eggs became worse, and by the F_{11} generation the method had to be changed (as noted under Methods) as too few larvae could be obtained for pressuring at a given time. However, in the F_{17} generation, egg viability became too low to continue. Enough larvae were obtained to run single susceptibility test, but attempts to obtain sufficient larvae for additional tests failed.

Table 1. Susceptibility of larvae of *Culex quinquefasciatus* laboratory strains prior to selection with pyriproxyfen (in ppm).

	Strain				Resistance ratio	
	OP-S		OP-R			
Insecticide	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}[a]	LC_{95}[b]
Chlorpyrifos	0.0019	0.0027	0.0070	0.030	3.7	11.1
Fenthion	0.0033	0.0057	0.021	0.097	6.4	17.0
Malathion	0.052	0.089	0.14	0.43	2.6	4.8
Parathion	0.0035	0.0058	0.0087	0.030	2.5	5.2
Pyriproxyfen	0.000018	0.00016	0.000022	0.00042	1.2	2.6

[a] LC_{50} OP-R/LC_{50} OP-S.
[b] LC_{95} OP-R/LC_{95} OP-S.

As pressuring with pyriproxyfen continued, the survivors became increasingly homogeneous, as is apparent from the slope values (Table 3). This process resulted in the selection for reduced egg viability, as described above, but there were no indications of increased tolerance to pyriproxyfen.

Interpretation of results: This study is encouraging with respect to the response of the genetic pool of one OP-R strain. However, there is no question that mosquitoes have the potential for development of resistance to pyriproxyfen, or any other new control agent, if large numbers are exposed to high selection pressure over enough time. The only practical method for prolonging the life expectancy of a new, promising chemical agent is for judicious use. In habitats having continual breeding of large numbers of mosquitoes, we feel that it is prudent that pyriproxyfen not be used as a sole means of control but rather that its use be alternated with applications of control agent(s) having a different mode(s) of action.

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Table 3. Susceptibility of the OP-R strain of *Culex quinquefasciatus* before and after pressuring with pyriproxyfen (in ppm).

Generation	LC_{50}	LC_{95}	Slope	Date tested
Parent	0.000022	0.00042	1.29	Jun. 1987
F_5	0.000015	0.000099	1.98	Mar. 1988
F_{10}	0.000032	0.00031	1.68	Apr. 1989
F_{15}	0.000015	0.000049	3.18	Mar. 1990
F_{17}[a]	0.000018	0.000055	3.33	Sep. 1990

[a] Single test; all others in triplicate.