CHANGE IN RESISTANCE TO DDT IN LARVAE OF *CULEX TARSALIS* COQUILLETT AT OAKRIDGE, OREGON IN TEN YEARS

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Apparently the first failure of DDT as a mosquito larvicide in the Pacific Northwest occurred in 1955 in a log pond at Oakridge, Oregon after the compound had been used for two years for larval control. Poor control of larvae of *Culex tarsalis* Coquillett was noted late in 1955 and established beyond doubt in 1956 (Buehler 1955, 1956). Bioassays of larvae from this pond at the Corvallis laboratory in 1956 confirmed Buehler's observations and indicated that the larvae were about 1000 times more resistant at the LC_{50} level than larvae from a susceptible laboratory colony (Eddy *et al.*, 1958); resistance was so great that the LC_{90} was virtually unattainable.

The small community of Oakridge is surrounded on three sides by many miles of the Willamette National Forest and on the fourth side by a few miles of this same forest; thus it is somewhat isolated from cropland and from urban areas. Since the chief industry is timber processing, a large sawmill and the 55-acre log pond are located there. Because the pond is a prime source of pest mosquitoes, the mosquito control section of the Public Health Department of Lane County treats it regularly. However, DDT has not been used in the pond nor elsewhere in the community since 1956. This paper is a report of the studies made since 1956 of the continuing resistance to DDT in *C. tarsalis* at Oakridge.

MATERIALS AND METHODS. For our tests, we collected egg masses fom the log pond at Oakridge and placed them to hatch in separate aerated pint containers in rooms maintained at a constant temperature. The emerged larvae were left in these containers until they were large enough to be easily identified (late 2nd instar or slightly older). Then all larvae of *C. tarsalis* were pooled and reared in larger, aerated containers until they were tested as 4th instar larvae. Tests were made in 250 ml of distilled water in pint jars by adding the desired amount of DDT in acetone, no more than 0.5 ml and no less than 0.1 ml. Twenty-five larvae were used in each determination with two determinations per dose. Tests were made at a constant air temperature of about 80 ° F. Mortality was recorded after 24 hours.

For comparison, a similar test was made in 1959 with 4th instar larvae of *C. tarsalis* that were reared from egg masses collected from a log pond in Corvallis, Oregon. Larval control had not been attempted in or around Corvallis before 1959, so it was not unreasonable to assume that the tolerance of these Corvallis *C. tarsalis* to DDT was similar to that of the Oakridge larvae before 1953.

Probit regression lines were fitted by eye, and the standard errors and slopes were then computed by the methods of Finney (1964).

RESULTS. The results are presented in Table 1. Resistance to DDT dropped considerably at the LC_{50} level in the Oakridge larvae between 1956 and 1959. The extent of the decrease is difficult to evaluate, but we estimate it to be a minimum of about 18-fold. We were therefore surprised when no further marked change occurred between 1959 and 1961 and when the LC_{50} was found to be slightly higher in June 1966 than in May 1959. Moreover, resistance in July and August 1966 was even greater than in June and higher than in either 1959 or 1961.

In 1959, the Corvallis larvae were susceptible to DDT at both the LC_{50} and

TABLE 1.—Percentage mortality of 4th instar larvae of *C. tarsalis* collected as egg masses on indicated date at Oakridge and Corvallis and treated in the laboratory with DDT (two replicates; 25 larvae each/dose).

DDT (ppm)	Oakridge[1] Collections					Corvallis[2] collection
			1966			
	1959 May (12)[3]	1961 September (13)[3]	June (9)[3]	July (10)[3]	August (13)[3]	1959 May (10)[3]
0.003	..	..	..	..	..	0
.004	0	..	..	..	..	13
.005	..	..	..	..	..	51
.006	4	..	..	..	..	79
.007	..	..	..	..	..	92
.008	16	..	..	..	..	97
.01	18	..	0	4	0	100
.02	14	..	2	4	0	100
.04	22	..	4	4	6	100
.08	26	..	..	..	..	100
.10	27	32	4	6	10	..
.20	55	..	12	12	18	..
.40	66	..	..	..	..	..
.50	..	62	66	26	22	..
.80	74	..	..	..	..	..
1.00	81	84	90	38	44	..
2.00	94	62	98	78	82	..
4.00	92	90	..	..	..	..
5.00	..	..	96	80	86	..
6.00	..	86	..	..	..	..
8.00	96	100	..	..	..	..
10.00	..	..	100	94	90	..
LC-50(ppm)	0.2	0.3	0.31	1.0	1.0	0.0049
Standard error	±0.021	±0.056	±0.035	±0.15	±0.13	±0.00001
Slopes[4]	1.16	1.10	2.51	1.50	1.28	9.14

[1] Estimated LC_{50} was at least 3.7 ppm in 1956.
[2] Since Corvallis had no mosquito control district during or before 1959, these results are included to assist in evaluating the extent of the resistance of the larvae from Oakridge.
[3] Larvae pooled from number of masses given in parenthesis.
[4] In probits.

LC_{90} levels and were about 40- and 370-fold less tolerant at the two levels than the 1959 Oakridge larvae (Table 1).

DISCUSSION. A high order of resistance to DDT obviously persisted from 1956 through 1966 in the larvae of *C. tarsalis* at Oakridge. Since DDT was not used for mosquito control during that decade, the reason for this persistence was considered.

Because Oakridge is isolated by the surrounding forest, the immigration of *C. tarsalis* might be limited; then an essentially closed gene pool could exist at Oakridge that would insure an essentially homozygous population as far as DDT resistance was concerned in 1956 and thereafter. However, our data do not seem to support the assumption. The LC_{50} of DDT in 1956 was apparently at least 3.7 p.p.m. (Eddy *et al.*, 1958). In 1959, it was 0.2 p.p.m., a decline that seems large enough to eliminate any assumed homozygosity.

We believe the continued high resistance since 1959 indicates the existence of selection favoring the resistant genotype. The only such factors obvious to us are the larvicides used by Lane County since 1959 or DDT persisting in the Oakridge environment since 1956.

The larvicides used in the pond between 1956 and 1966 were malathion and fenthion (Baytex®). Fenthion was used

before our first egg collection in 1966 and between each of the subsequent collections, and resistance, as measured by our technique, definitely increased during the summer (Table 1). However we can only speculate that the increase was caused by fenthion, and not by vagaries in sampling, seasonal changes similar to those described by Keller *et al.*, (1956), or other causes.

Of course the eggs in the three collections (9, 10, and 13 masses, respectively) made in 1966 tests may have produced too few larvae to give a good estimate of the tolerance within the population. Still, this argument cannot explain why resistance to DDT has not decreased at Oakridge since 1959. Studies should be made to determine the precise mechanisms that are perpetuating the resistance.

SUMMARY. Larvae of *Culex tarsalis* Coquillett in Oakridge, Oregon were found by other investigators to be highly resistant to DDT in 1956. Our test with laboratory-reared 4th instar larvae collected as egg masses in Oakridge in 1959 indicated that the larvae still had an LC_{50} and an LC_{90} that were about 40- to about 370-fold, respectively, those of a susceptible population in Corvallis, though resistance at the LC_{50} level had declined at least 18-fold from the 1956 level. Subsequent tests at Oakridge in 1961 and in 1966 indicated that the level of resistance had apparently become somewhat stabilized near but not lower than the 1959 level.

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