RESISTANCE PROBLEMS WITH CHLORINATED HYDROCARBONS IN THE NORTHERN STATES[1]

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The objective of this communication is to review the status and problems of resistance to chlorinated hydrocarbons in the northern states extending from Delaware to Maine. This general area is confronted with upland mosquito control problems as well as those occurring in salt marsh areas which extend along the coastline intermittently from Delaware to Massachusetts. Generally in the highly populated areas, mosquito control is well-organized. In upstate New York, New Hampshire, Vermont, and Maine only slight if any control practices are exercised.

Two years ago (Sutherland, D. J., 1964), this area was surveyed for the types of insecticides, their relative amounts, and extent of usage in mosquito control activities. Briefly, as of that time, DDT was the most widely used insecticide, with other chlorinated hydrocarbons, dieldrin and BHC, used on a much smaller basis in a few areas. In some states, organophosphates such as Baytex and Abate are employed on a limited basis, while in Delaware, dibrom is used widely for adult control. Of course, fuel oil alone remains a dependable tool for all mosquito control agencies.

With the continued use of DDT and other chlorinated hydrocarbons since 1946, it was almost inevitable that the spectre of mosquito resistance to insecticides would haunt the Northeast, particularly when resistance was encountered in other parts of the country and the world. The appearance of resistant *Aedes sollicitans* and *Aedes taeniorhynchus* in Florida in 1947 and subsequent years (Deonier and Gilbert, 1950; Keller and McDuffie, 1952) to many served as a warning that resistance and all its problems were imminent.

In 1951 came the first report of reduced effectiveness of DDT after 4 years' use against salt marsh mosquitoes in Delaware (Darsie and Cannon, 1952). Subsequently in 1952 only failure of DDT was in evidence (MacCreary, Darsie and Cannon, 1953). These failures, however, were inconsistent in both years, and bad control was experienced with larvae from untreated areas as well as good to excellent control of larvae from areas heavily treated previously with DDT. In the third year, control with DDT was excellent and the rapid disappearance of DDT resistance was attributed to dilution as well as the disuse for one year of DDT as a general marsh spray (MacCreary and Darsie, 1954).

Benzene hexachloride replaced DDT in the aerial spray program of 1952 and 1953 (Darsie, Krause, and Beadle, 1957). This was accompanied by a dimunition of DDT resistance and DDT was again employed in 1954. Increased tolerance to DDT was reported at the end of the season (Darsie and Murphey, 1955). In 1956, assays of resistance revealed relatively low tolerances of *A. sollicitans* to DDT, although practical control with DDT was not attained (Darsie, Krause, and Beadle, 1957). Subsequently, after BHC was used two years, resistance to this insecticide in Delaware was reported (Darsie and Sutherland, D. W. S., 1959), and BHC gave poor control on the marsh while DDT was quite satisfactory. Additional assays to determine the extent of resistance to DDT and BHC were conducted in the early 1960's (Doll, Lake, and Darsie, 1961; Doll, Darsie and Lake,

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[1] Paper presented in Symposium: Insecticide resistance in mosquitoes, A.M.C.A. 22nd Annual Meeting, Mar. 6–9, 1966, Atlanta, Georgia. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers–The State University, Department of Entomology.

1962) and these results will be discussed later. As of 1962 no other northern states had reported resistance of salt-marsh mosquitoes (Brown, 1962), although New Jersey had a few suspect cases in 1961 (Sutherland, D. J. and Hagmann, 1962).

Culex pipiens populations on the other hand, up to 1962, were reported to be resistant to DDT in New Jersey (Burbutis and Davis, 1955; Brown, 1962); tolerant to DDT and lindane in Delaware (Sutherland, D. W. S. and Darsie, 1960), resistant to DDT and dieldrin in New York (Brown, 1962) and in Massachusetts (Armstrong, 1962; Brown, 1962).

It is unfortunate in scientific disciplines that a term, such as resistance, is put to use to describe a phenomenon long before the parameters are established, the factors affecting it are known, or the methods to determine it are agreed upon and consistently used. Until 1958, when the World Health Organization standardized methods for the assay of resistance (Brown, 1958), methods varied from researcher to researcher. Acetone as a dispersant was used by some (Keller and McDuffie, 1952) while xylene plus emulsifiers were used by others (Darsie and Murphey, 1955). Some used various amounts of acetone (Murphey and Darsie, 1956) while other researchers did not reveal techniques completely (Keller and McDuffie, 1951; Armstrong, 1955).

During this period the majority of researchers assayed resistance on the basis of 48 hour toxic response; the W. H. O. method currently is based on 24 hours. Since an LC_{50} for 24 hours is some 2 to 4x an LC_{50} for 48 hours, one might tend to compare 24 and 48 hour data on this basis. However, since pupation often occurs during 48-hour holding, subsequent correction for pupation yields an LC_{50} of a younger population than that used for a comparable 24-hour study, and comparison becomes more difficult and dangerous. Other factors concerning age of larvae, state of nutrition, temperature, and weakening of larvae during transportation to the assay site were sometimes not considered. In short, there were many reasons why figures from resistance assays from one researcher could not always validly be compared with those of another researcher.

For each researcher there was the problem of what is the base-line for susceptible populations. Often these base-lines were not available, and the researcher had to compare mosquitoes from a treated resistance-suspected area with a so-called untreated area, although the latter might not have been truly representative of a base-line. Sometimes the researcher has taken the greatest susceptibility (i.e. the lowest LC_{50}) as being the base-line against which other strains should be compared and labeled as susceptible, tolerant, or resistant (Darsie, Krause, and Beadle, 1957; Darsie and Sutherland, D. W. S., 1959). However, it is well known that susceptibility is seasonal (Keller, Labrecque, Chapman, and Davis, 1956), and the base-line of susceptibility is variable (Pal and Kalra, 1965).

In brief review of resistance in the northeast up to 1958, it can be said that there was some evidence that at least it was potentially there and conceivably could develop to strong resistance.

With such a potential existing, what then has been the picture of resistance to chlorinated hydrocarbons since 1958 and what are its problems?

The greatest problem confronting the Northeast has been to collect and properly interpret sufficient numbers of resistance assays. Although resistance surveying has been recommended by the A.M.C.A. Research and Development Committee (Brown, 1962), outside of Delaware and New Jersey few assays have been conducted and their results made known.

Problems associated with interpretation can be illustrated by recent data from New Jersey. New Jersey was strongly influenced by the potential of resistance apparent in mosquitoes in nearby Delaware, and in 1960 a program was established whereby resistance was assayed yearly, the ultimate objective being eventually to map the resistance-biomes or clines of New Jersey. Basic to the philosophy of the program was the recognition that to be meaningful the resistance survey would be a continuing project and that firm base lines of susceptibility had to be established over a period of years. Since its initiation the methods have been those of W.H.O. with DDT concentrations for larval assays being 0.2, 0.1, 0.075, 0.05, 0.025, 0.01, 0.0075, 0.005, 0.0025, and 0.0001 p.p.m. The following data are summarized from Sutherland, D. J. and Hagmann, 1962, 1963, 1966 and Sutherland, D. J. and Bast, 1966.

The results from DDT assays of *A. sollicitans* larvae are shown in Figure 1. Pluses (+) indicate populations from areas receiving medium to heavy treatment with DDT; dots (•) refer to populations from areas receiving little or no DDT. Most populations, irrespective of treatment history, have LC_{50}'s in the range of 0.01 to 0.05 p.p.m. Although a value for a susceptible strain of this species was recently given as LC_{50} 0.002 p.p.m. (Brown, 1962), it is believed that this figure is low for N. J. conditions. The susceptible level for *Aedes taeniorhynchus* has been reported to be 0.02 p.p.m. (Rogers and Rathburn, 1964). The DDT LC_{50} of a population of *Aedes sollicitans* from BCH-treated areas in Delaware in 1959 was 0.03 p.p.m.; in 1961 after 2 years of DDT treatment a population from the same area had an LC_{50} (24 hours) of approximately 0.11 p.p.m. (Doll, Darsie, and Lake, 1962). In New Jersey only in 1961 have populations with suspected resistance been found. Both (0.11

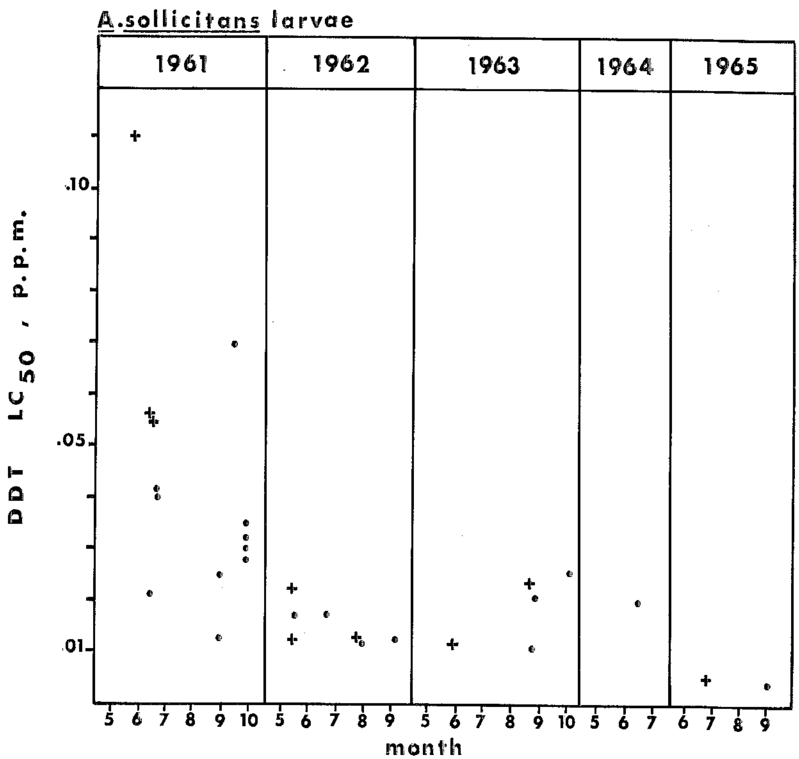

FIG. 1.—DDT LC_{50}'s of *Aedes sollicitans* larvae, 1961–1965.

and 0.07 p.p.m.) were from the two southern counties which received the greatest DDT treatment. In examining the dosage mortality curves of these populations in comparison to more susceptible populations (Figure 2), the flatness of some days after collection, generally there is a rise in LC_{50} up to the third day, probably as a result of mortality of weakened susceptibles during the holding period prior to assay. No data are available for *A. sollicitans* adults from else-

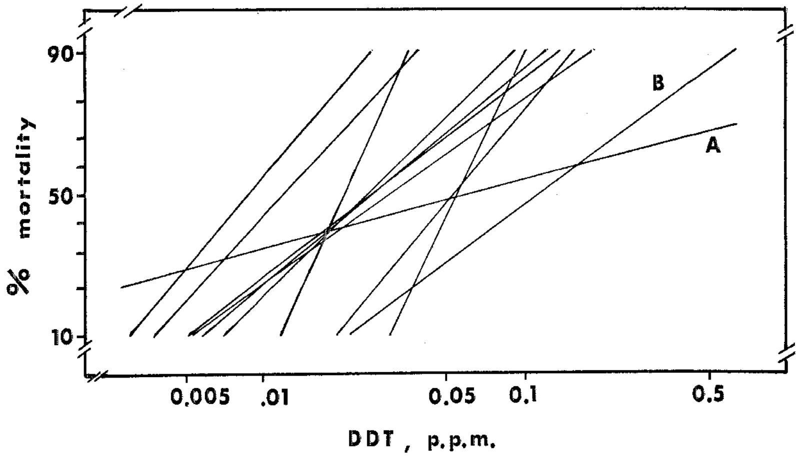

FIG. 2.—Dosage—mortality lines of *Aedes sollicitans* larvae to DDT, including 2 pre-resistant populations, A and B.

their curves, A and B, is remindful of the general flattening and shifting on the way to true resistance (Brown and Abedi, 1960). On this basis, five years later they are now termed pre-resistant.

Although there has been a general lack of resistance in *A. sollicitans* larvae, additional adult assays were conducted to determine if susceptibility extended to the adult stage. Figure 3 shows the LC_{50}'s in percent DDT, utilizing the W.H.O. method, pluses (+) indicating medium to heavy DDT treatment, dots (•) medium, and zeros (0) little or no treatment. The adults were maintained in humid atmosphere and exposed for 1 hour to DDT residues. Again, no apparent correlation exists between LC_{50} and history of DDT treatment. A possible explanation for LC_{50}'s higher than 3 percent is shown in Figure 4, the data being rearranged to show that in populations returned to the laboratory and tested

where. After cautiously comparing the *A. sollicitans* figures for mortality caused by 1 hour exposure to 4 percent DDT paper (range of 52–100 percent, average 72 percent) with those of *A. taeniorhynchus* susceptible adults (Mathis, 1961), there is as yet no evidence of DDT resistance in adult *A. sollicitans* in New Jersey.

An early season mosquito of increasing importance to some northern states is *Aedes stimulans;* LC_{50}'s of larval populations for 5 years are given in Figure 5, arrows indicating values less than the specified level. Populations came from areas receiving little if any DDT treatment. Assays were conducted both at field and laboratory temperatures and followed the negative-temperature coefficient of DDT. All populations have been susceptible, apparently more than 2x as susceptible as an Illinois population from an untreated area (0.007 p.p.m.) reported in 1963 (Hedeen, 1963). DDT

LC_{50}'s for adult *A. stimulans* from untreated areas have ranged from 1.9 to 4.8 percent for 1 hour exposure, generally comparable to *Aedes sollicitans.*

In five years of assaying, values for larvae of *Aedes sollicitans* and *stimulans* have dropped to their lowest points in the fifth year possibly in association with continued drought conditions. In contrast, however, values for *Culex pipiens* have increased as shown in Figure 6. Most populations fall between 0.005–0.04 p.p.m., in close agreement with values from Illinois (Hedeen and Allen, 1961; Hedeen, 1963). Two populations examined in 1965 have LC_{50} values over 0.1 p.p.m., are at least pre-resistant, and are currently in laboratory culture for studies. In Table 1 are values for *Culex pipiens* larvae from one 4-acre pond on Long Island, N. Y., during a 4-year period, (Courtesy of M. B. Fleming). The pond receives some sewer lines, is considered polluted, and has received intermittent DDT applications.

Table 1.—DDT LC_{50}'s of *C. pipiens* larvae, Fort Hamilton, N. Y.

Date	LC_{50}, p.p.m.
8/22/59	0.1
8/24/59	0.022
8/25/60	0.56
8/16/61	0.25
8/1/62	0.054
8/23/62	0.14
9/5/62	0.05
9/19/62	0.06

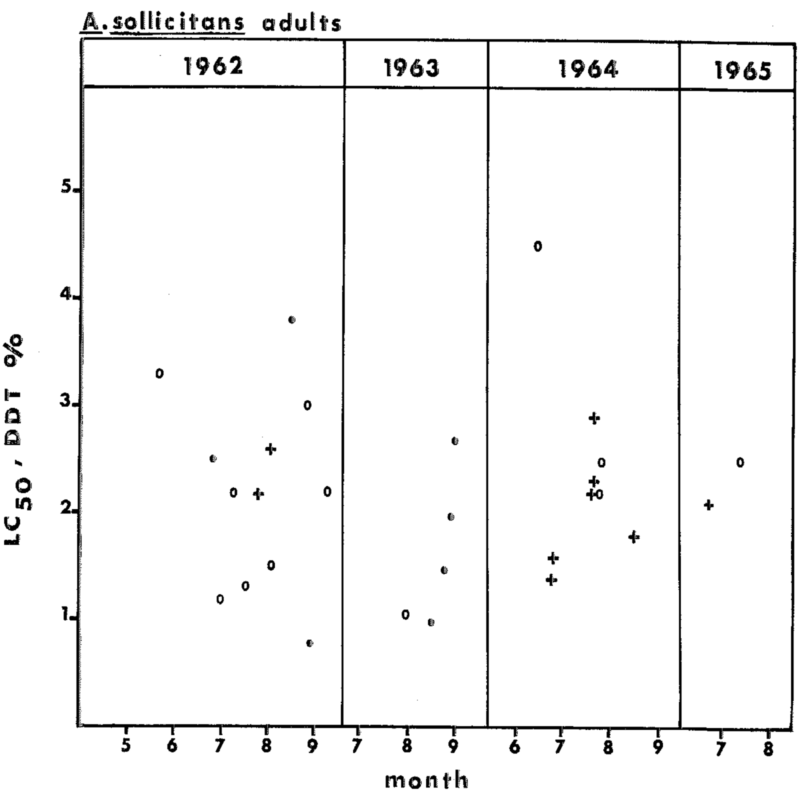

Fig. 3.—DDT LC_{50}'s of *Aedes sollicitans* adults, 1961–1965.

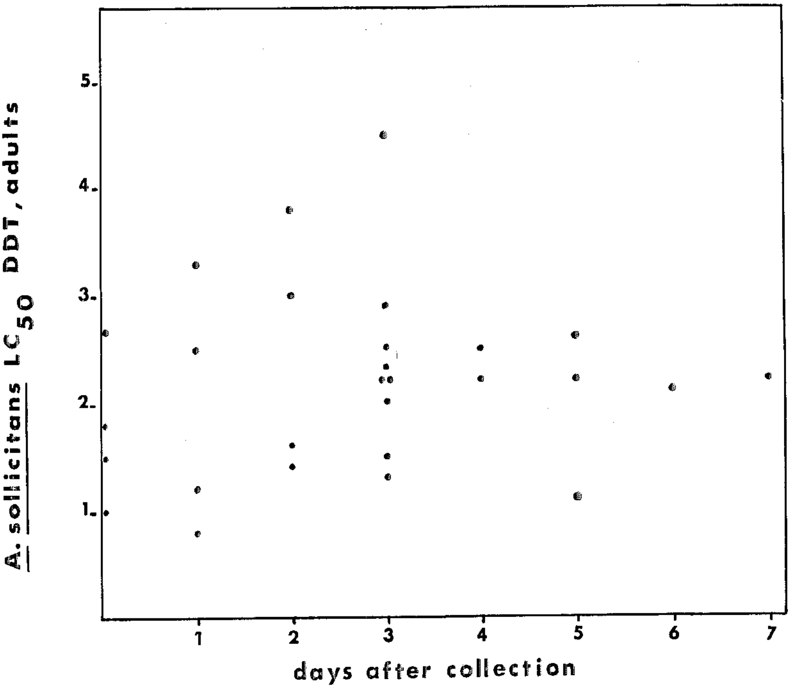

FIG. 4.—DDT LC_{50}'s of *Aedes sollicitans* adults according to day tested after collection.

TABLE 2.—DDT LC_{50}'s of various species of mosquito larvae, New Jersey.

Species; Instar	Date	LC_{50} (24 hours) p.p.m.
A. canadensis; 3, 4	4/16/62	0.0010
	4/21/65	0.00085
A. excrucians; 3	4/3/64	0.005 @ 12° C.
		0.002 @ 19° C.
A. excrucians and *A. canadensis*	4/11/63	0.004
	4/27/65	>0.005
A. cantator; 3	8/4/62	0.0071
3+	9/14/62	0.0035
3	4/16/64	0.004 Field
3	4/16/64	0.006 Lab
A. cantator and *A. vexans*	9/7/62	0.0066
A. vexans; 3	8/15/62	0.017
3+	9/14/62	0.021
	5/17/63	0.002
	5/23/63	0.0013
C. salinarius; 4	8/3/62	0.12
	6/18/64	0.005
C. salinarius and *C. pipiens;* 4	9/7/62	0.0035

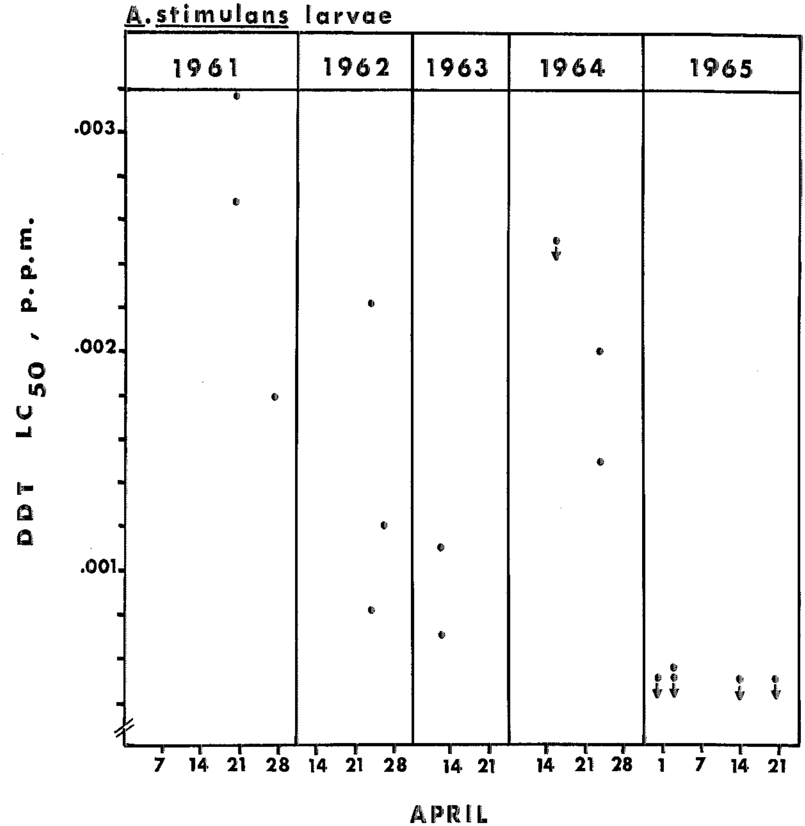

FIG. 5.—DDT LC_{50}'s of *Aedes stimulans* larvae, 1961–1965.

Values vary approximately 25 times, sometimes very rapidly as in 1959. The apparent sporadic resistance here is probably due to pollution. In order to correctly label genetic resistance as opposed to pseudo-resistance due to adsorption of toxicants by pollutants, assays in the future should include *C. pipiens* populations in habitat as well as in non-polluted water.

Shown in Table 2 are LC_{50}'s of other species assayed at various times in New Jersey. Of these, only one population of *Culex salinarius* with an LC_{50} of 0.12 p.p.m. appears resistant. This population was from a heavily treated area in 1962. The remaining species, including the variable *Aedes vexans,* appear to be within susceptible ranges. It is of interest that resistant *Aedes canadensis* and *Aedes cantator* have recently been reported in Canada (Pal and Kalra, 1965).

In brief, most of New Jersey species of mosquitoes appear to be susceptible to DDT and probably to other chlorinated hydrocarbons. In nearby Delaware, BHC and DDT have not been used for routine mosquito control since 1961, and the last check on DDT and BHC resistance took place in 1962 (Lake, 1965). In the absence of DDT, strains tolerant to hydrocarbons may have reverted by now. Currently there is no information on possible organophosphate resistance to such compounds which have replaced chlorinated hydrocarbons.

A question arises particularly about the difference in the status of DDT resistance of *A. sollicitans* in Delaware and New Jersey. What caused increased tolerances in Delaware while, with the exception of southern counties, general tolerance is absent from New Jersey? The answer may lie in the number of applications yearly to marshes in Delaware, sometimes in excess of 20 (MacCreary, Darsie, and Cannon, 1953). In New Jersey, after a preseason treatment on closed marshes, five to six adult airsprays are used. Later, *Culex salinarius* and *Culex pipiens* migrating onto the marsh are controlled by malathion, since these species are naturally tolerant of DDT. Undoubtedly some resistant populations do exist in New Jersey but they are few in number, and DDT remains an efficient material.

In other northern states, where mos-

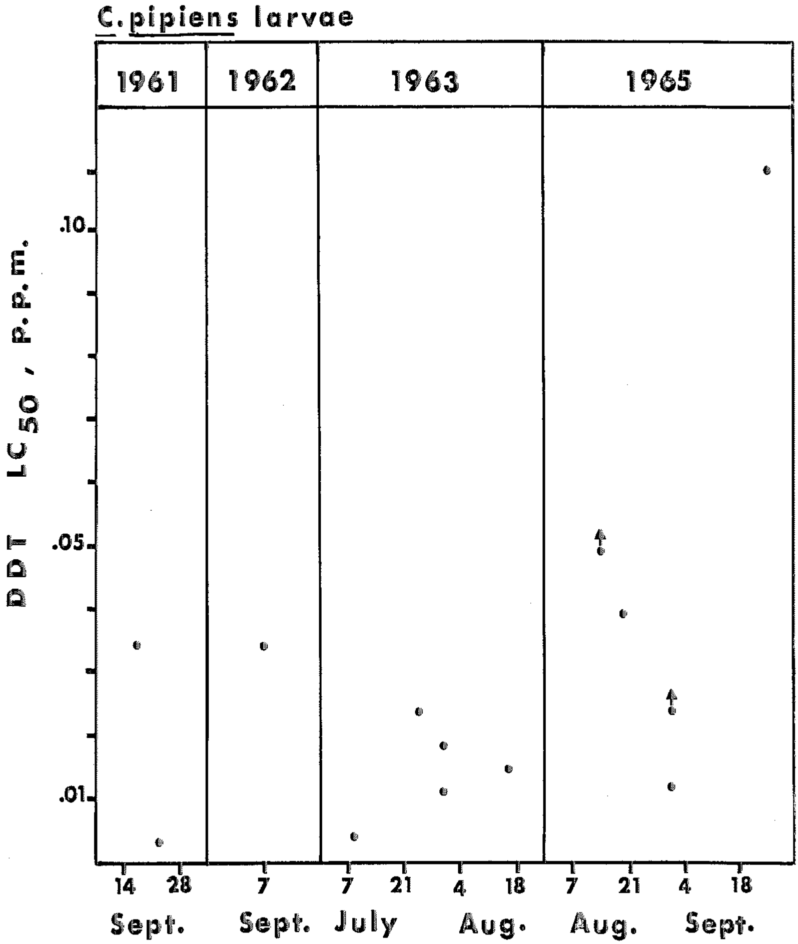

FIG. 6.—DDT LC_{50}'s of *Culex pipiens* larvae, 1961–1965.

quito control is highly organized, probably some resistant populations occur in areas of heavy insecticide treatments (e.g. Camp Drum, N. Y., Pal and Kalra, 1965). But the majority of the areas in New York, Vermont, New Hampshire, Maine, and possibly Massachusetts receive few if any insecticide applications for mosquito control. Resistance therefore is the exception rather than the rule.

Two factors which have been found to mimic resistance are (1) poor formulations and application and (2) pollution. Poor formulations include those granular formulations which are known to contain correct percentages of the toxicant but never release it completely from the granular carrier. Pollution has not only increased mosquito problems in some of the northern states, but also prevents the insecticides from performing properly. Even newer insecticides such as Abate and Baytex are sometimes ineffective under such conditions. Indeed, with man's increasing population, and widespread urban mosquito problems, pollution may well surpass resistance as a problem to mosquito control agencies in the northern states.

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