In the laboratory, the alga caused mortality in the first-stage larvae of *Cx. quinquefasciatus* but did not kill significant numbers of fourth-stage larvae or pupae. Larval mortality depended on the density of the alga. These findings clearly demonstrate the possibility of using the alga or its bioactive chemicals as biological control agents against mosquitoes.

We also found that extracts of *C. ellipsoidea* and *R. hieroglyphicum* retarded the growth of and were toxic to *Cx. quinquefasciatus* larvae. The extracts also caused abnormal larval development, including contraction of the dead larvae, incomplete pupation, adherence of metathorax legs to pupal cases in the adults, and inability of the adults to fly. The findings strongly support the feasibility of developing these ecological chemicals for mosquito control.

Mosquito autoinhibitors

When a species is under pressure that threatens its survival, it may employ biological mechanisms to cope with the situation. Under extremely overpopulated conditions, older larvae of *Cx. quinquefasciatus* secrete adaptive autoinhibitors that exert toxic and growth-retarding effects on younger larvae. The younger larvae die, and the total mosquito population decreases. We consider this a self-regulating mechanism for adjusting the population.

In chemical tests, we determined the population-regulating autoinhibitors to be a mixture of branched-chain fatty acids and hydrocarbons. To procure more active compounds, we conducted intensive studies on the structure-activity relationship of the autoinhibitors. Several analogues and homologues of the autoinhibitor had a high degree of insecticidal activity against immature mosquitoes, showing a good potential as alternate larvicides and pupicides.

The most promising substances in this category are 3-methylnonadecanoic acid, *N,N*-dimethylhexadecanamide, and their homologues. The acids were highly toxic against first-stage larvae of *Cx. quinquefasciatus*, with LC_{50} and LC_{90} (concentrations killing 50 and 90 percent of the larvae, respectively) below 0.5 ppm, but not very toxic against the fourth-stage larvae and pupae. The amides, on the other hand, displayed a wide spectrum of toxicity against all stages of larvae and pupae. These compounds are now being assessed under field conditions to determine their efficacy in controlling mosquito larvae.

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Photo by Max Clover

Author George Georghiou points out remains of insecticide-resistant mosquitoes (dark spots) on gel plates. A dye in the liquid around the gel reacts with metabolites produced only by resistant mosquitoes.

Mosquito resistance to insecticides

George P. Georghiou

Resistance to insecticides—development of the ability to survive doses of insecticides that previously were lethal to the majority of individuals in a population—has become increasingly more common since the early 1950s and now affects the control of at least 414 species of arthropods (insects and mites). Resistance has had its greatest impact in the control of mosquitoes, because these pests have been subjected to intensive chemical applications ever since synthetic organic insecticides became available.

Resistant populations evolve through selection, or the survival and propagation of individuals that have genetic traits enabling them to remain insensitive to a toxic chemical or to metabolize it to harmless products. To date, 84 species of mosquitoes (44 *Anopheles*, 20 *Aedes*, 15 *Culex*, 2 *Psorophora*, 2 *Armigeres*, and 1 *Culiseta*) in various parts of the world have developed strains that can resist one or more types of insecticides. Included among these species are the principal vectors of malaria, yellow fever, encephalitis, and filariasis, as well as many "nuisance" species. Although control can still be achieved with some chemicals, the number of effective insecticides has declined dramatically in recent years. This is especially the case in agricultural environments where mosquitoes are also subjected to indirect selection by sprays intended for crop pests and have thus developed a wide spectrum of "multiresistance."

California's intensive agriculture, well-organized mosquito abatement services, and high level of affluence have been especially conducive to selection of resistant mosquitoes. Species affected include the pasture mosquitoes *Aedes nigromaculis* and *Ae. melanimon,* the encephalitis vector *Culex tarsalis,* and the house mosquitoes *Culex quinquefasciatus, Cx. pipiens,* and *Cx. peus.* Of these, *Ae. nigromaculis*, *Cx. tarsalis*, and *Cx. quinquefasciatus* now demonstrate multiresistance to all organochlorine and organophosphorus insecticides that have been employed for their control, including DDT, malathion, parathion, methyl parathion, fenthion, temephos, and chlorpyrifos.

The severity of the resistance problem has induced a comprehensive program of basic and applied research in this laboratory to: develop efficient, sensitive methods for detecting and monitoring resistance; search for and assess new chemicals against resistant strains; clarify the evolutionary dynamics of resistance; and develop techniques for its prevention or suppression.

Detection and monitoring

Populations of *Cx. quinquefasciatus*, *Cx. tarsalis*, and *Ae. nigromaculis* from localities in which resistance was suspected were established in the laboratory, and their chemical defense mechanisms were investigated in detail. Resistance was shown to be due mainly to the selection of esterases that detoxify a wide variety of organophosphorus compounds. Oxidative enzymes, which are important in resistant house flies, were not significant in these mosquitoes; thus carbamate insecticides (propoxur, and the like), which are normally affected by oxidases, continue to be relatively active against these populations.

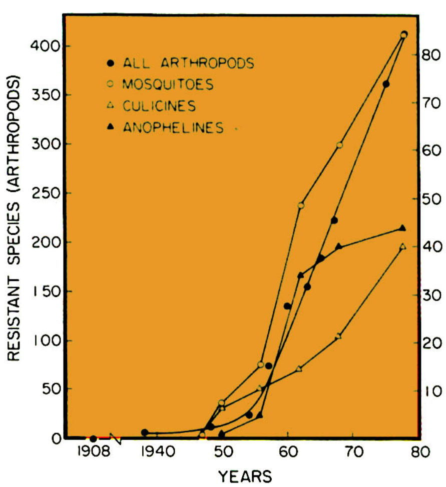
A corollary of this research has been the elaboration of diagnostic doses that can be used to detect resistance to common insecticides, thus eliminating the need for time-consuming full-scale bioassay tests. A further innovation is the development of a simple filter-paper test for detecting the presence of detoxifying esterases in individual mosquitoes. Such techniques are significant aids in the detection and monitoring of resistance at the field level.

New chemicals

Because of widespread resistance, new chemicals must be evaluated not only against susceptible mosquitoes but also against strains that represent specific resistance mechanisms. This laboratory maintains several strains of mosquitoes, each possessing one or more of the known mechanisms of resistance, such as DDT-dehydrochlorinase, DDT-nerve insensitivity, organophosphorus-degrading esterases, carbamate-degrading oxidases, pyrethroid-insensitivity, juvenile hormone resistance, and organophosphorus- and carbamate-insensitive cholinesterase. Every new insecticide received from universities, from industry, or by agreement through the World Health Organization is thoroughly tested against these strains to determine the impact of already existing resistance mechanisms on its effectiveness. Several hundred new chemicals have been subjected to this crucial test during the past decade.

Successful compounds are subsequently studied for their propensity to induce resistance. A representative field population is established in the laboratory, and successive generations are subjected to intensive selection by the new chemical. The resultant resistance is then characterized biochemically and genetically, providing vital information on the future of the chemical.

Such studies in recent years have yielded useful information on potential resistance to methoprene in *Cx. tarsalis*, resistance to pyrethroids in *Cx. quinquefasciatus*, insensitive cholinesterase in *Anopheles albimanus*, and organophosphorus resistance in *Cx. tarsalis* and *Cx. quinquefasciatus*. They have also revealed a limited response to selection by propoxur and diflubenzuron in *Cx. tarsalis*, and to selection by chlorphoxin in *An. albimanus*.



Chronology of appearance of resistance to insecticides in mosquitoes, in relation to resistance in all arthropods.

Counteraction of resistance

To remain effective, an insecticide must be used in ways that minimize selection pressure toward resistance. To achieve this goal it is necessary to develop means of quantifying the "propensity for resistance" in a given situation, and to devise sophisticated control techniques that keep selection below a permissible level. Progress has been achieved toward both objectives.

The genetic and biological parameters that influence the evolution of resistance have been identified and their contribution quantified by simple models on a computer. Population isolation, reproductive fitness of resistant individuals, and dominance of resistant genes were found to be strong determinants of the propensity for resistance. Likewise, significant operational parameters included the dosage of chemical applied, the size of the treated area, and the environmental persistence of the residue. This information was used in constructing a computer simulation model to predict population densities and evolution of resistance in *Cx. tarsalis.* This research and similar studies in other laboratories should eventually make it possible to forecast the course of resistance in insects.

Research on chemical approaches for countering resistance has focused on the principle of synergism, or utilizing interacting chemicals, and on the employment of chemicals in mixtures or in rotations. A thorough knowledge of the types of resistance already present in the target population is required, and new chemicals are needed with distinctly different modes of action and pathways of detoxication.

The principle of mixtures assumes that genes for resistance to each member chemical are so rare that they are not present together in the same mosquito. Thus, an individual capable of surviving one insecticide will be killed by the other insecticide in the mixture. The principle of rotation is based on the premise that resistance to a given chemical declines in the absence of selection pressure. Thus, when insecticides are used in rotation it is expected that resistance to each member chemical will decline during the time intervening between successive uses of the same chemical. Recent discoveries of compounds of disparate chemical structure and mode of action have prompted us to initiate long-term experiments aimed at testing these approaches to forestalling the development of resistance.

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Immunochemical methods to detect pesticide residues

For the safe, effective use of a pesticide, the residue of the pesticide in food, fiber, and the environment after application must be known. Gathering residue data on a new pesticide is a major expense in its development, and this expense may be prohibitive when registration of a pesticide is being considered for use on minor crops or as a mosquito larvicide. To insure the future availability of safe new compounds, especially for minor uses, the cost of residue analysis must be reduced.

Gas liquid chromatography has been the most commonly used residue analysis technique. More recently, high-performance liquid chromatography has proved useful, especially with nonvolatile or heat-unstable compounds, such as benzoylphenyl ureas and carbamates. However, these techniques often require extensive sample cleanup before the assay, which is costly both in time and money. These analytical techniques also have some deficiencies and—for various reasons—rapid, sensitive, reproducible, and economical residue technology is not presently available for some compounds including the benzoylphenyl ureas, juvenile hormone analogues and some pyrethroids.

Immunochemical methods of residue analysis may solve some of these problems. Immunochemical analysis is not new; it has been used successfully for many years in several fields including clinical chemistry. In fact, Rosalyn Yalow received the 1977 Nobel Prize in medicine for her pioneering work on immunochemical analysis. In many cases, the chemical structures now assayed by immunochemical methods are not fundamentally different from those of