Chemicals of the future

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In nature, many organisms produce specific chemical substances that influence their own behavior, growth, survival, and population biology and those of other organisms. In some cases, the chemical interactions result in attraction, repulsion, growth aberrations, and even death of the organisms involved. The chemicals and their interactions are the subject of a new, rapidly expanding area of ecology, and these substances have been termed ecological chemicals.

Some of the ecological chemicals could be used to manipulate insect behavior and development in pest management programs. These chemicals offer several advantages as insect control agents: they are produced abundantly in nature by plants and animals; they can be used in moderate and restrained quantities without upsetting the environment; they leave no residues in the environment; and they are usually specific or group-specific, rarely affecting other organisms not involved in the interactions.

In our recent research, we have studied the ecological chemicals with significant biological activity on mosquitoes and we are exploring their control potential.

Oviposition attractants and repellents

Female mosquitoes are selective in choosing oviposition sites. Some choose polluted water with abundant organic matter; others show a preference for clear water.

In our research, various organic infusions induced different ovipositional responses in the females of various mosquito species. For example, standard laboratory animal feed, through microbial action, produced chemicals that inhibited oviposition. The chemicals were identified as simple lower carboxylic acids. Studies of other carboxylic acids showed that homologous caprylic and capric acids were more active and less odoriferous than the lower acids. Caprylic acid exhibited outstanding oviposition repellency at a 0.6 to 6 ppm concentration against *Culex quinquefasciatus.* In the field, this acid significantly suppressed the oviposition of *Culex* mosquitoes at 15 and 50 ppm for two to four weeks. Sensory physiology studies showed that chemoreceptors in mosquito antennae governed the perception of the oviposition repellents.

Microbial fermentation of chicken manure, on the other hand, produced chemical substances that encouraged oviposition in *Culex* mosquitoes. The manure became active from 9 to 26 days after the start of fermentation at ambient temperature. Stored at -20° C, it remained active for 10 weeks or more. Our field studies showed that the fermenting manure significantly attracted field populations of *Cx. tarsalis* and *Cx. peus.*

Isolated in semi-pure form, the oviposition attractants performed well against *Cx. quinquefasciatus* in laboratory tests. We are now attempting to purify and chemically identify the oviposition attractants. The feasibility of exploiting the oviposition-modifying substances for mosquito control is also being considered.

Photo by Max Clover

University scientists investigate the effectiveness of an oviposition repellent in preventing gravid mosquitoes from laying eggs. Sheet-metal cylinders dispense the repellent in experimental ponds.

Plant kairomones

During our studies on chironomid midges in southern California freshwater lakes, we found that larval density of pestiferous midges was inversely proportional to the density of the aquatic weed Eurasian watermilfoil (*Myriophyllum spicatum*) growing in the bottom of the lakes. Chemical or physical factors produced by the aquatic weed were suspected of interfering with the abundance of the midges. Extracts prepared from the watermilfoil caused significant midge larval mortality. More importantly, the extracts also were toxic to mosquito larvae.

While bioassaying larval toxicity of the watermilfoil extracts, we noted that many adult mosquitoes that escaped from the holding cages or emerged from the test dishes were attracted to test dishes containing an aqueous suspension of the extracts, and that most of the adult mosquitoes drowned in the water. We then conducted a series of experiments to determine the attractiveness of the watermilfoil extracts, and found they were indeed highly attractive to both sexes of the adult mosquito *Cx. quinquefasciatus.*

Apparently, the extracts contained bioactive substances combining attractiveness and toxicity. This unusual biological characteristic possibly could be used to attract and kill mosquitoes. We now are attempting to isolate and identify the bioactive substances in the weed extracts, after which their use as mosquito control agents will be fully explored. It is conceivable that specific mosquito control agents can be derived from this aquatic weed.

Algae and algal toxins

Unicellular and filamentous algae, such as *Rizoclonium hieroglyphicum* and *Chlorella ellipsoidea*, are important cohabitants of mosquito larvae. They are toxic to mosquito larvae and delay larval development. In studying the chemical interactions between mosquito larvae and their associated fauna and flora in flower vases in a large urban cemetery, we found that mosquito larvae did not breed in vases containing the alga *C. ellipsoidea.*

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.