Feature

Maintenance of Susceptibility to Mosquito Insecticides

by David Dame

Introduction

Around the globe insecticide resistance has occurred in over 500 species of economically important insects, and is not uncommon in mosquitoes. In Florida, mosquito resistance to organic insecticides occurred relatively soon after they were first introduced in the 1950's. These early compounds, the chlorinated hydrocarbons DDT and dieldrin, were used only briefly before their effectiveness against some mosquito species began to wane. Currently, Florida harbors regional or localized pockets of mosquito populations resistant to one or more of the chemicals currently registered for their control - malathion, fenthion, naled, methoprene.

The Phenomenon of Insecticide Resistance

Many circumstances can lead to the condition which we describe as insecticide resistance. Chemical resistance occurs in response to sublethal exposure in which some of the members of the exposed insect population survive, thereby initiating the process of selection. With continued exposure, selection pressure may eventually lead to full blown resistance. The phenomenon is fairly well understood from both the genetic and physiological perspectives for most of the chemicals used to control mosquitoes. All of the currently used mosquito control chemicals are subject to eliciting a physiological or behavioral response in the target species, with the possible exception of *Bti (Bacillus thuringiensis israeliensis).* Unfortunately, there is no easy method to predict which species might become resistant or to which chemical.

Initial exposure is not always related to mosquito control activities. Many examples exist of mosquito population resistance caused by exposure to agricultural chemicals. The most common physiological factor leading to the development of resistance is the occurrence within the insect population of detoxifying agents — esterases, oxidases and transferases. Suspicions of chemical resistance can be confirmed by properly planned laboratory bioassay of representative samples of the suspect insect population. And recent innovations by CDC have made it possible, and practical, to determine the type of detoxifying enzyme involved by rapid, microplate analysis of single specimens. However, when resistance is caused by failure of the toxicant to reach the sensitive sites in the insect or by behavioral response, identifying the cause may be more difficult.

The Resistance Process

The development of population resistance usually occurs in stages. Early exposures fail to kill those few members of the population which have the proper physiological characteristics to detoxify the insecticide. These individuals are usually in the minority and they may not be the most hardy members of the population. But repeated exposures favor their selection. When these individuals (with resistance alleles) mate with susceptible individuals (having only alleles for susceptibility), their offspring (heterozygous, and now with a mixture of susceptible and resistant alleles) are likely to exhibit an intermediate level of susceptibility to the insecticide. Gradually, through further selection brought about by repeated exposure to the original insecticide, or other insecticides with a similar mode of action, the population becomes predominantly the heterogygous mixed resistance allele type and eventually the fully resistance type - with only resistance alleles.

The rapidity of the onset of resistance is governed by many factors. For example, those species which have many generations per year are more likely to become resistant in a shorter time span than those with fewer generations per year, given equal rates of exposure and similar genetic makeup. Insect populations that do not extend beyond the perimeter of the treatment zones are more prone to resistance development than are those for which a considerable portion of their numbers are distributed beyond the treatment zone and thus are not exposed to the selection pressure. Some species are more tolerant to a specific insecticide than others, a factor which may reduce reliance on that insecticide. For example, because some *Aedes* and *Culex* species are significantly more tolerant than other species to the current ratio of resmethrin isomers in Scourge, one would expect selection pressure to be minimal because of reduced application of this material against the tolerant species. Seasonal,

nutritional and physiological factors may also play a role in exacerbating or inhibiting the rate of growth of resistant genome in mosquito populations.

Recognizing that resistance is related to conditions which arise within the insect population, we must also realize that inadequate insecticide application, poor formulation, bad timing, adverse weather conditions, and a host of other complications are not to be considered as evidence of the presence of resistance. Only by specialized testing, usually in a controlled laboratory setting, is it possible to document changes in susceptibility. Application failures must be followed up with specific studies to determine the cause of the failure.

Maintenance of Susceptibility

Which brings us to the question and the primary objective of this discussion: how can we manage the use of insecticides in order to maintain susceptibility in mosquito populations? Actually, there are several important, practical ways, to enhance the probability of maintaining susceptibility. The most obvious, and surest, approach is to depend on alternatives to insecticides as much as possible. Utilization of effective methods of source reduction and biological control options, if available, rather than dependence on pesticides reduces exposure and leads to extended susceptibility.

But when the use of an insecticide is deemed unavoidable, what approaches can be used to forestall the development of resistance? The following guidelines should be considered:

First, use materials to control the immature stages which have a different mode of action than those materials that are used for adult control. This approach will both reduce selection pressure and help eliminate newly selected genomes that might occur in the alternate life stage. Dr. Andrew Rogers advocated this approach for Florida more than three decades ago. As a result Florida mosquito control agencies have experienced relatively few resistance events.

Second, alternate classes of insecticides to ensure that selection for a single mode of action is not continuous. For example, if methoprene is used for larval control for one or two seasons, switch to *Bti* for a season (or at least for several consecutive applications).

Third, treat only as much area as absolutely necessary. Adjacent infested areas that are not treated provide a refuge that serves as a continual natural source of susceptible mosquitoes which might dilute the impact of selection within the treatment zone. Often insects selected by a particular stress, insecticidal in this case, have a reduced fitness in terms of survival. Thus, insects immigrating from unexposed refuges may have a fitness advantage that could translate into a reproductive advantage. The net effect in such a situation could be both the dilution of resistance (the selected characteristic) and a competitive advantage for the susceptible genome.

To maintain susceptibility successfully, mosquito control agencies must continually monitor the status of their mosquito populations by controlled exposures in the laboratory. Recent studies have revealed that resistance often is very localized. One corner of a county may have developing resistance whereas nearby mosquitoes remain completely susceptible. Thus, collection sites for mosquitoes to be tested as part of the surveillance program need to be carefully selected. Since mosquitoes have been shown to become resistant even though the material in question has not been used for mosquito control, sampling should take this phenomenon into account. Areas of intense agriculture usage are particularly suspect as they have been implicated throughout the globe. Regardless of the cause of selection, awareness of current status is very important. Field observations, in the absence of supporting laboratory based data, usually can not be considered indicative of decreased susceptibility because of the many factors that can reduce the effectiveness of outdoor exposures. Awareness is best achieved by laboratory testing to establish baseline data and subsequent sampling to conduct similar tests at regular intervals for comparison with the baseline and/or naive strains that are known to be susceptible.

It is not possible to predict which species may experience reduced susceptibility as a result of repeated exposure and continued selection. Nevertheless, when this does occur it may be possible to overcome the resistance by adding materials which counteract the insects ability to detoxify the insecticide. For example, piperonyl butoxide (PBO) interferes with the oxidases that mosquitoes use to detoxify pyrethroid insecticides. Addition of PBO in the proper ratio to the spray mix may counteract the decreased susceptibility in such instances. However, since the enzyme system is involved, it is not uncommon for loss of susceptibility to a specific mode of action to be transferred to other insecticides with similar modes of action. Cross resistance, as this phenomenon is called, predisposes the population to a rapid development of resistance to the subsequently used materials that have a similar mode of action.

At a minimum if at all possible, annual surveillance events should be scheduled. Some locations might prefer to conduct susceptibility tests at the beginning of the season and again at the end. Laboratory assessment of field-captured mosquitoes with the CDC bottle and microplate analysis system is relatively quick and foolproof when

conducted by well-trained and experienced personnel. The CDC methodology is much more practical and less time consuming than earlier methods of rearing test populations for exposure in the laboratory. But a word of caution can be offered. Whatever methodology is utilized, both the testing and the interpretation of the results require training and experience. Experience is such an influencing factor in this endeavor that "going it alone" may not be the best approach for the smaller districts. With the techniques now available, it would make sense in most circumstances to pool resources and set up diagnostic services at regional locations. Everyone would benefit from the standardization (in both testing and interpretation) that would result, and the information collected would have the greatest impact.

A Note of Caution

Relatively few mosquito control agencies currently alternate chemicals with differing modes of action. This is disturbing - and risky. All programs should seriously consider this option to preserve the effectiveness of the available insecticides as long as possible. Without such rotation most insecticides could cause selection, even through intermittent usage, if given enough time. Because of cross-resistance and scarcity of registered materials there are already severe limitations on our ability to establish schemes for alternating insecticide; and industry is not developing new molecular structures specifically for mosquito and vector control, primarily because the return on investment is insufficient to justify such activities. It is important to be mindful that there is a need for as many classes of insecticides as possible in order to apply the alternating exposure principle. And it is imperative that we practice good stewardship through proper insecticide management, rather than lose the battle because of the development of avoidable insecticide resistance.

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From the Editor

Do you have an interesting story you would like to share so others may benefit from your experience?

We are looking for interesting technical or field-related articles about mosquitoes, mosquito control and related topics. The articles do not have to be "scientific" in nature and are usually not too long - usually a page or two.

A considerable amount of applied research, equipment modifications, application technique alterations, along with other operational advancements are being conducted at mosquito programs, universities and military installations throughout the world. Much of this information is publishable but perhaps not in a refereed journal. We encourage you to consider publishing in Wing Beats.

Remember, if we don't receive any interesting stories, we have nothing to publish. Articles may be sent directly to me via e-mail or regular mail in either WordPerfect or MS Word. Contact information is listed on the top of page 3.

Respectfully,

Dennis Moore
Editor-in-Chief