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INSECTICIDE RESISTANCE : CAN IT BE AVOIDED ?

by

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Summary - **The genetic background related to resistance against insecticides has abundantly been demonstrated during the last decades, hence slowing down the development of resistance has to be adapted accordingly.**

Currently, assays are designed to monitor the mechanisms of resistance in individual insects. Remarks on the interpretation of results obtained with the available bioassays are given together with some recommendations to control resistance.

According to the WHO insecticide resistance is "the heritable ability in a strain of insects to tolerate doses of toxin which otherwise would prove lethal to the majority of individuals in a normal population of the same species".

Currently, most researchers agree that this ability is the result of the selection of individuals with a heritable capacity to withstand the toxicant rather than the action of the insecticide on an individual insect. Hence, development of resistance is dependent on the genetic variability already present in the population. There are several indications to support this so-called pre-adaptive nature of resistance, amongst those, the recent observation on benzimidazole resistance in nematodes (13). Benzimidazoles are anthelmintics preventing the formation of microtubules by binding tubulin of nematodes. In nematodes, resistance to these products seems to be correlated with the presence of a type of tubulin, less readily binding to benzimidazoles. Remarkably, this tubulin could also be demonstrated in some individuals of a susceptible population, isolated in the laboratory at a time before benzimidazoles were introduced to the market.

In our laboratory, another indication of the pre-adaptive nature of resistance was observed in a strain of houseflies (*Musca domestica*).

The laboratory selection of this field strain, indicated as the "Loedim-strain" is given in Table 1. This strain had an altered acetylcholinesterase (ACHE), with a reduced rate of reaction versus some organophosphate and carbamate inhibitors. The rate of inhibition of ACHE by a given insecticide can be calculated and is proportional to the inhibition constant Ki (16).

As shown in Table 1 this insecticide selection resulted in a decreasing susceptibility of the acetylcholinesterases in the offspring of the survivors. As mutations are far too exceptional in a small laboratory-colony to be at the basis of this rapidly decreasing susceptibility, this phenomenon in the "Loedim" strain has

to be attributed to the presence of several isoenzymes of ACHE with different susceptibility to inhibitors and the selection by the insecticide of those flies, possessing the least susceptible of these enzymes.

Table 1. The susceptibility of acetylcholinesterase(s) in housefly-strain Loedim during a selection procedure*

Method of selection	Dose of dimethoate	Percentage survivors	Inhibition constant (Ki) of ACHE to paraoxon
Topical application	0.88 µg/fly	35	-
	0.176 µg/fly	2	22×10^{4} M $^{-1}$ min $^{-1}$
	0.176 µg/fly	20	-
Dip in insecticidal solution for 3'	5 mg/100 ml	80	6.5×10^{4} M $^{-1}$ min $^{-1}$
	10 mg/100 ml	60	3.3×10^{4} M $^{-1}$ min $^{-1}$

* Selection was achieved in the laboratory by submitting successive generations of surviving houseflies to dimethoate.

Apparently, in most insect-vectors gene(s) for resistance are present, hence, this genetic nature of resistance implies that resistance will be selected each time the insecticide is used, unless the dose is too low to kill homozygous susceptible individuals or high enough to kill equally homozygous resistant individuals.

The use of insecticides in sufficiently high doses, as a preventive measure for development of resistance is impracticable because the dose, sufficient to kill even the most resistant individuals, is unknown, furthermore there are economical and environmental considerations.

Even in the absence of chemical control, resistance development in vectors seems unavoidable in view of the extensive use of phytopharmaceuticals in agriculture. It is a world-wide observation that mosquito resistance is most severe in places where crops are treated frequently with insecticides (5). Therefore control strategies have to be worked out to counteract or at least to delay the development of resistance.

Optimally, the rate of development of resistance in a vector population can be delayed by decreasing the selection pressure, whereby the following general recommendations can be considered to achieve this latter aim :

- Reducing the frequency of treatments : a good understanding of the ecology and bionomics of the vector, together with a profound study of the epidemiology of the transmitted disease, is imperative for the development of an adapted strategy allowing treatments to be kept at a minimum. The successful control of the cattle tick *Boophilus microplus* in Australia, before large-scale immunizations against *Babesia* were feasible, illustrates this. The control of the vector of *Babesia* was aimed at maintaining tick infestations at a level, too low to cause direct damage, but

sufficiently high to confer lifelong immunity against babesiosis in young calves without clinical disease (17).

- Combining chemical with non-chemical control measures : not only will this help to reduce the number of treatments but, because of the equal impact on susceptible and resistant individuals by non-chemical control, resistance will be delayed. Furthermore, the use of selective insecticides, killing the vector only and not their natural predators, will sustain existing biological control. This selectivity might be based either on the properties of the compound itself or on a particular application (e.g. target screens against tsetse flies).

- Applying short-acting compounds rather than residual insecticides: For about half a century, short-acting pyrethrins and pyrethroids are successfully used in Denmark against houseflies. In contrast, residual sprays of pyrethroids are forbidden for housefly control in Denmark. In many European countries however, where the use of such residual sprays is free, strong resistance against long-acting pyrethroids appeared already after a few years, impeding the use of short-acting pyrethroids.

- Reducing the proportion of the population being exposed to selective dosages. This is a reason why houseflies maintain their susceptibility for an insecticide longer when this is formulated as a stomach poison, applied randomly in the stable, than when used as a residual spray, applied all-over the stable. The second reason lies in the amount of insecticide, that a fly can take, this being more important for stomach poison than for others.

- Avoiding the selection of different stages of the vector by the same insecticide, resulting in multiple selections during a single generation.

Apart from selection pressure mechanisms, the insecticide i.a. dose, type and sequence of different products, plays an important role in the rate of resistance development. Furthermore, it influences the cross-resistance pattern and the type of resistance mechanism involved.

- It is obvious that high doses should be preferred above low doses. For economical and environmental reasons however very high doses can only be used in bait formulations with a very selective action against the target population.

- Particular resistance-mechanisms, with a large range of cross-resistance, seem to be selected preferentially by some insecticides. E.g. dimethoate, a polar organophosphate, penetrates easily through the cuticula of the housefly, submerging the detoxication enzymes. However, as compared with other organophosphates, dimethoate is a weak acetylcholinesterase inhibitor and selects therefore preferentially an altered acetylcholinesterase, less inhibited not only by dimethoate but equally by many other organophosphates and carbamates (15). Hence, the order of application of insecticides is extremely important to limit cross-resistance.

- Using insecticide cocktails appears to be interesting only, when the products have almost the same duration of activity. Furthermore, the resistance against each individual product of the mixture must not depend on two dominant or linked genes and no important cross-resistance should exist between the two products (6, 10, 14).

The absence of mutual resistance mechanisms is not guaranteed when the insecticides involved have different modes of action since some enzymes are able to metabolise insecticides belonging to different groups. According to Beach *et al.* (4), the high esterase levels in *Anopheles albimanus* of Guatemala does not only provoke resistance versus fenitrothion - the selecting agent - but also against the pyrethroid deltamethrine.

- Occasionally, insecticide rotation, using two unrelated insecticides, gives interesting and encouraging results, obviously when a negative cross-resistance between the two insecticides exists. Negative cross-resistance between the organophosphate temephos and the pyrethroid permethrin was demonstrated in a Californian strain of *Culex quinquefasciatus* (9) and in *Simulium damnosum* larvae (11). However, extrapolations on the effects of mixtures and rotations of insecticides are hazardous and require extensive trials against different populations of the species involved since populations with widely differing histories of control and biotype do not always respond to selection with a given material in the same way.

- According to Brown (2), long-term sequential selection by insecticides, where the change of product is made some generations after its introduction, is preferable rather than waiting until resistance develops before making the switch. Changing products, before resistance appears, has the effect of denying the target population of developing fitness alleles to counteract the reduction of fitness which characterizes the incipient stage of resistance development.

In order to design resistance countering strategies the following requirements are imperative :

- A better understanding of the mechanisms of resistance, their genetic background and the cross-resistance patterns they induce. This will allow the planning of sequential use patterns for the available chemical groups.

- A profound awareness about the population dynamics and ecology of some pests, as well as the degree of control to be considered necessary and acceptable, in order to obtain maximal effect of the control measures with the least selection pressure.

- The development of methods for detecting resistance genes at low frequency. Standard susceptibility-tests might probably be sufficient to confirm whether failure of control is a consequence of resistance, but these tests are inadequate to detect resistance in its early stages when resistant individuals are still very rare but when, on the other hand, the opportunity for effective corrective action still exists. In addition these bioassays do not provide any information on the mechanism of resistance. Therefore resistance should preferably be monitored by either biochemical, immunological or biomolecular techniques aimed at detecting resistance mechanisms in individual insects (3).

Some biochemical methods for detecting specific resistance mechanisms in individual mosquitoes have already been developed and evaluated in the field (1). These assays are performed in microplates on diluted homogenate of single mosquitoes.

Generally speaking, there are two types of assays, one based on the demonstration of increased insecticide metabolism, the other one on detecting the target-site insensitivity.

The former assays detect resistance mechanisms by an increased colour response in resistant insects, caused by the increased metabolism of an alternative substrate.

Resistance by a target site insensitivity such as altered acetylcholinesterase is demonstrated by submitting a particular concentration of an organophosphate or carbamate, sufficient to inhibit all the ACHE of a susceptible individual. When this happens, no colour development occurs, when on the contrary altered ACHE is present, colour development is seen in an assay for acetylcholinesterase.

The value of these micro-assays is beyond any doubt, but the interpretation of the results warrants some caution :

- When a microplate assay shows an increased metabolism of a substrate by an enzyme, it does not necessarily mean that the insecticide is also metabolised by the same enzyme. The increased metabolism of the substrate may be due to alleles of genes which in this particular population are linked to the resistance gene. This linkage may be absent in other populations. Besides, in some resistant strains of houseflies a mutation of alleles, leading to an insecticide-metabolising activity of an esterase with a concomitant loss of activity to other esters, was demonstrated (12). Such phenomenon will never be detected by the microassay.

- Currently, a microassay for detecting target site insensitivity is available for the detection of altered acetylcholinesterase only. In this test the choice of the inhibitor is of a primary importance, whereas several forms of altered ACHE with different susceptibility to the various insecticides seem to occur : e.g. in southern England the acetylcholinesterases of housefly strains, possessing this resistance mechanism, were shown to be as susceptible to the organophosphate azamethiphos as those of susceptible reference strains (8). However, this observation did not correspond with similar resistant strains in Belgium, where the altered ACHE was also less inhibited by azamethiphos (7).

In conclusion, insecticide resistance is inevitable, but it appears to be possible to delay the onset and to decrease the rate of development. This implies, however, the design, implementation and monitoring of useful control strategies, which require a working knowledge of many disciplines such as biochemistry, epidemiology, environmental biology and entomology, so a multidisciplinary approach by co-operative teams is necessary.

Résumé : Ces dernières décades, la base génétique de la résistance a été établie de manière incontestable. Les mesures déstinées à retarder le développement de la résistance doivent être adaptées conformément.

De nos jours des tests ont été mis au point pour démontrer des mécanismes de résistance à partir d'un seul insecte. Des remarques sur l'interprétation des

résultats obtenus avec ces tests ainsi que quelques recommandations afin de contrôler la résistance sont proposées.

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