JOINT FAO/IAEA DIVISION
OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

INTERNATIONAL ATOMIC ENERGY AGENCY
FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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FAO/IAEA Consultants Group Meeting on

The Potential for Tsetse Flies to Develop Resistance to Insecticides

15 - 19 November 1993
Vienna, Austria

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EXECUTIVE SUMMARY

Chemical insecticides are playing an increasingly important role in control of tsetse flies (*Glossina* spp), vectors of human and animal trypanosomiasis in large regions of Africa. Although insecticide resistance has not yet been reported in tsetse, there is no cause for complacency regarding its occurrence in the future. As new reports of insecticide resistance in other disease vectors and agronomic pests continue to accumulate at a rapid rate, it is increasingly clear that no comprehensive approach to tsetse control can afford to ignore the potential resistance problem, as the loss of insecticides from the limited set of options for control would be disastrous. It is likely that one or more of the pyrethroid resistance mechanisms already known from several other species of Diptera will manifest itself in tsetse, in response to the increased selection engendered by the wider adoption of deltamethrin-treated targets in tsetse control at the local level and in eradication efforts. Also, selection for behavioural avoidance of traps and targets could result in decreased control efficiency, although the mechanisms that might cause such behavioural resistance are poorly understood at present.

There is thus an increasingly urgent need for information on the potential for resistance development in tsetse, on accurate and feasible methods for detection, monitoring, and characterization of resistance, on properties of resistant strains, and on appropriate tactics for resistance prevention and management. Because of the extraordinary difficulties in rearing posed by tsetse life history, it is essential that these research efforts get underway immediately. The Consultants Group on the Possibility of Development of Insecticide Resistance in Tsetse has accordingly prepared this report with a consideration of the present state of knowledge, a discussion of the essential elements of a resistance research program, and specific recommendations.

A summary of the recommendations in the Consultants Report is as follows:

- Collection of information on the scope and intensity of insecticide use in past as well as future planned control projects, and dissemination of information (to planners, field operatives, and training manuals) on the possibility and consequences of resistance development in tsetse.
- Collection of baseline information on current levels of insecticide susceptibility in tsetse, focusing initially on pyrethroids, and incorporating all relevant approaches including bioassay (especially development of discriminating doses), activity assays for detoxifying enzymes, isolation and characterization of genes potentially encoding insecticide resistance, and neurophysiological measurements of nervous system sensitivity to pyrethroids.
- Development of laboratory-selected pyrethroid-resistant strains of tsetse.
- Consideration and evaluation of the resistance-delaying or resistance-management benefits of alternative strategies of tsetse control or eradication, especially with regard to inclusion of other compounds to reduce the dependency on pyrethroids.

Each recommendation is discussed in more detail in the Report. The Consultants Group urges its wide dissemination and consideration by all involved in programs of tsetse control and eradication, and by agencies and funding bodies with an interest in science in the service of sustainable development in Africa.

1. INTRODUCTION

The ability of pest populations to develop resistance to pesticides presents increasingly greater difficulties in the protection of man, animals and crops from pest attack. Of more than 500 species of insects that have evolved populations resistant to one or more classes of insecticides, at least 177 are Diptera, and many are pests of man and animals and vectors of disease, including malaria, filariasis, yellow fever, dengue and others. Several of these insects are now able to resist insecticides in each of the major chemical classes, namely organochlorines (DDT and cyclodienes), organophosphates, carbamates and pyrethroids.

A major constraint towards achieving appropriate levels of animal production in Sub-Saharan Africa is the presence of tsetse fly (*Glossina* spp.) and the disease it transmits to domestic animals and man. Thirtyseven African countries are tsetse infested and the majority have rated the need for control of Animal African Trypanosomiasis (AAT) as a high priority in their development programmes. The main techniques to combat AAT have been the control of tsetse fly populations through the use of insecticides on cattle or on baited artificial "targets" and traps, and the administration of trypanocidal drugs to livestock. The use of drugs in an *ad hoc* and uncontrolled manner has led to the development of drug resistance in the trypanosome parasites.

The control of the tsetse fly has come to rely increasingly on pyrethroid insecticides because of their outstanding efficacy. Pyrethroids are currently used in Africa on livestock as "pour-on" treatments for the control of tsetse, ticks and other ectoparasites. They are also used in homes on "coils" and similar devices for pest fumigation and on bed nets against mosquitoes. Additionally, pyrethroids are finding increasing use in agriculture, especially on cotton and other crops.

Under such increased use of insecticides there is serious concern regarding the prospects of development of resistance to pyrethroids by *Glossina*. Such resistance, occurring where the disease organism is also resistant to drugs, would have dire consequences. In view of these concerns, a Consultants' Group was convened in Vienna, from 15 to 19 November 1993 and charged with the following responsibilities:

a) To advise on the potential of tsetse flies to develop resistance to the insecticides and attractants presently used.

b) To suggest procedures for detecting, monitoring and characterizing any resistance that does arise.

c) To recommend broad measures for the prevention and management of resistance.

2. POTENTIAL FOR RESISTANCE DEVELOPMENT

Insecticide resistance is a pre-adaptive trait conferred by alleles arising through recurrent mutation in individual insects. Since these alleles confer no obvious advantage to their carriers in the absence of insecticide exposure, they are likely to remain at undetectably low frequencies in untreated populations. Under selection with insecticides, however, the proportion of resistant individuals can increase rapidly and, if unchecked, generate populations that are no longer controlled adequately with the selecting agent or related toxins. Hence when assessing resistance risks pertaining to tsetse flies, two distinct questions must be addressed: (i) what is the likelihood of individual tsetse flies already possessing genes for resistance, to pyrethroids in particular?; and (ii) what are the prospects of such genes being selected to frequencies that impair control of field populations?

2.1 Do Insecticide-resistant Tsetse Flies Already Exist?

Pyrethroid resistance is now widespread amongst insect pests, and the mechanisms responsible are becoming increasingly well understood at the genetic, biochemical and molecular levels. Four types of mechanism have been implicated so far:

a) Reduced cuticular penetration, whereby physical and/or biochemical modifications of the cuticle restrict or delay the transport of insecticide into the haemolymph. This is generally considered a minor resistance factor per se, but may considerably enhance the effect of metabolic or target-site insensitivity mechanisms.

b) Qualitative or quantitative changes in non-specific esterases, improving their capability to cleave or sequester pyrethroid molecules.

c) Enhanced detoxification of pyrethroids by mono-oxygenases, especially the Cytochrome P_{450}s.

d) Nerve insensitivity is most likely due to structural changes in an axonic sodium channel protein, the target site of pyrethroids and DDT. In houseflies this mechanism is referred to as knockdown resistance or Kdr. It appears invariably to confer cross-resistance to DDT. Kdr appears to be particularly effective against certain pyrethroid esters such as deltamethrin, although it confers substantial resistance to all commercially available pyrethroids.

All four types of mechanism have been documented, singly or in combination, in several species of Diptera. They also occur in more distantly-related agricultural and public-health insect pests. Equivalent mutants undoubtedly occur in *Glossina* spp., providing the raw material on which selection can operate. They may, however, occur more rarely than in other Diptera due to the comparatively low size of tsetse populations.

Nerve insensitivity appears particularly characteristic of Diptera, having been identified with reasonable certainty in houseflies, horn-flies and mosquitoes. It perhaps poses the greatest threat to tsetse control centered on the use of deltamethrin. It would nonetheless be very unwise to underestimate the potential role of metabolic factors in any pyrethroid resistance that might arise.

2.2 Will Resistance Be Selected in Tsetse Populations?

Whether and how quickly such pre-existing genes are selected by insecticides to damaging frequencies is still impossible to forecast accurately. From theoretical and experimental studies it is known that the effectiveness of selection depends on a large array of factors relating to the genetic and ecological properties of pest populations, and the nature of insecticide applications. Genetic factors including the frequency, potency and dominance of resistance genes are still unknown for tsetse flies. They can, however, be investigated before resistance becomes well established using approaches outlined and advocated later in this report. Ecological characteristics of *Glossina* spp. have received much attention from a pest control standpoint, but some with a crucial bearing on resistance development such as dispersal rates, and the proportion of insects exposed to control treatments require further clarification. Treatment parameters are of course fully controllable and well defined; these at present provide the only reliable basis for anticipating the selection pressure imposed by different control practices.

The diversity of these practices against different species and in different areas precludes any generalization of resistance risks over the tsetse belt as a whole. Where insecticide usage is still very localized and/or intermittent, the threat of resistance is undoubtedly still minimal. However, our unanimous view is that recent trends in tsetse control do give cause for concern by creating conditions conducive to the selection of resistant populations in some areas. This is based on four lines of reasoning:

a) The overall use of insecticides in impregnated targets or as pour-on treatments for livestock, whether as a control measure per se or as a precursor to eradication attempts using the Sterile Insect Technique (SIT), has increased markedly for some species in certain regions. This trend seems set to continue for the foreseeable future.

b) Insecticidal control of tsetse is now almost entirely reliant on a single chemical - deltamethrin - with a proven ability to discriminate very effectively between susceptible and resistant (especially nerve insensitivity) genotypes in other insects. This will probably be the case in tsetse as well.

c) Some recent reports suggest that levels of tsetse control (population suppression) being achieved with deltamethrin are extremely high (>99%), leading (temporarily at least) to the virtual elimination of susceptible genotypes from substantial areas.

d) Ongoing attempts to increase the persistence of deposits on cloth targets using UV absorbers, oils to improve rain-fastness, etc., appear destined to extend the efficacy of deltamethrin, and hence the period over which selection for resistance could potentially occur. Whether or not this development will promote resistance is still uncertain, since by reducing the occurrence of aged deposits it could help maintain insecticide concentrations that even resistant genotypes (heterozygotes especially) are capable of surviving. Resolving this issue requires detailed experimentation, ideally with access to a pyrethroid-resistant population generated through laboratory selection if necessary (see later).

Collectively, these developments emphasize that the risk of resistance evolving in *Glossina* spp. can no longer be ignored, and that measures to address this threat should be an important consideration in all future control operations. A first step is unquestionably to establish and implement techniques to obtain baseline response data for relevant insecticides, and for monitoring changes in tolerance of the most intensively-exposed species. Approaches to achieving this within the constraints imposed by tsetse biology are covered in the following section.

2.3 Physiological vs. Behavioural Resistance

An assumption made throughout this report is that any resistance mechanism(s) selected in tsetse flies will be of a conventional "physiological" nature, i.e. involving biochemical or physiological processes that improve an insect's ability to withstand uptake of insecticides. This is justified in that such resistance accounts for the great majority of cases of insects adapting to and resisting control by insecticides, and is the easiest to investigate using standard bioassay, biochemical and genetic techniques. We recognize, however, that the deployment of insecticides in conjunction with attractants - particularly on impregnated targets - also provides scope for the evolution of behavioural traits enabling insects to avoid or reduce contact with insecticide deposits. Cues promoting such avoidance behaviour could be the shape or colour of targets, various odour attractants (octanol, phenols derived from bovine urine etc.), components of the insecticide formulation or even repellent or irritant properties of the toxin itself. This is a controversial subject, and one requiring specialized techniques dependent on the species involved and unfamiliar to most resistance researchers. Biologists involved in evaluating these attractants would be best placed to advise on the likelihood of behavioural resistance, and on appropriate techniques for documenting its occurrence.

2.4 Recommendations

1. Tsetse workers, field operatives, and agencies supporting control programmes must be alerted to the possibility of resistance developing, particularly in areas where the use of insecticides is most intensive.

2. Basic information on the development and possible impact of resistance should be included in manuals and training courses for biologists and field operatives in the relevant countries.

3. Available information on the scale of insecticide use must be collated to identify possible resistance "hotspots".

4. Specialists in tsetse behaviour should be consulted regarding the possibility of behavioural resistance, and approaches for investigating its occurrence. Any reports, however anecdotal, of tsetse flies becoming less attracted to targets deserve attention in this respect and should be followed up whenever possible.

3. DETECTION, MONITORING AND CHARACTERIZATION OF RESISTANCE

Many methods for resistance detection, monitoring and characterization have been extensively developed and applied to a range of insect pests over the last decade. Some of these methods allow the generation of large amounts of information from small numbers of insects and may be suited for development for use in tsetse. However it should be stressed that if the objective of the programme is early detection of resistance when the genes are rare, there is no substitute for analyzing a large number of insects no matter what type of methodology is used.

Very little baseline data for resistance detection in tsetse is available using any methodology. Before a resistance monitoring or management strategy can be implemented, methodologies must be developed for the insect species concerned and baselines for each methodology for a known susceptible strain need to be determined. These baselines can and should be developed before resistance becomes a field problem, and because of the limited availability of insects for testing in tsetse, an effort should be made initially to identify and develop detection methods that give the maximum amount of useful information with the minimum number of insects. For resistance monitoring it is also important (if comparisons of changes in resistance status are to be made over time) that standard susceptible strains of the different species be established and maintained for use as baselines. These strains should be co-tested as controls in all subsequent determinations of resistance status with all the methodologies.

Priority in starting to develop these methodologies should be firstly as tools for resistance detection and monitoring, then as a system for the characterization of resistance mechanisms.

The biology of tsetse, specifically the reproductive physiology may also be a complication in a number of methodologies, including bioassays and biochemical assays, if field collected insects, which may have been exposed to insecticides, are used. In a number of insects, effects of prior insecticide exposure are avoided by using the F1 generation from the field collected material. With tsetse, where insecticide residues may be transferred from the mother to the larvae this approach may not be practical. The extent of residue transfer, and the longevity of the residues within the parent and larvae in tsetse should be determined.

3.0.1 Recommendations

a) Susceptible reference strains should be established for major tsetse species and used to obtain baseline data for all methodologies that are exploited.

b) The level of insecticide transfer between mother and larvae should be determined after treatment, along with the duration of the residual insecticidal effect in both mother and offspring.

3.1 Bioassays

The principle bioassay approach taken to date with tsetse has been to generate log-dosage probit-mortality (ldp) lines using topical application of insecticide. This approach requires large numbers of insects to generate accurate lines, and is a relatively insensitive tool for resistance detection and monitoring. A single discriminating dose test for each insecticide would be more appropriate, particularly with the constraint on insect numbers from field collections. The discriminating dose should initially be generated from ldp lines for reference strains and slightly exceed that which kills 100% of the susceptible insects under defined temperature and humidity conditions. Further ldp lines need only be run thereafter as required, (for example to check the exact changes in LD50 and LD90 when resistance has developed, to generate resistance ratios). As there are changes in insecticide tolerance with age, sex and physiological state of the insects, a separate discriminating dose will need to be determined for males and females, and a uniform age and physiological state should be used throughout. Once accurate discriminating doses are determined these can be used for future screening. Due to the heavy reliance on pyrethroids (particularly deltamethrin) with the impregnated target system of tsetse control, effort in setting the doses should concentrate primarily on two or three pyrethroids and DDT (the latter being included to assist with the diagnosis of nerve insensitivity). Further compounds can then be included at a later date as needed. Topical application is practical when analyzing small numbers of insects, but requires an accurate applicator, which may be a constraint in many African field sites. A tarsal contact toxicity test, as used in mosquitoes, which may be less accurate, but has a lower equipment requirement, may therefore be preferable, for detection and monitoring of resistance in field populations.

Bioassays utilizing a synergist/insecticide mixture, or synergist pre-treatment can be used to get initial indications of underlying pyrethroid resistance mechanisms. For example, mono-oxygenase involvement in pyrethroid resistance can be implicated by applying piperonyl butoxide (PB) in conjunction with the insecticide, while esterase involvement can be implicated by using S,S,S-tributylphosphorotrithioate (TBTP) in a similar manner. Nerve insensitivity can be implicated by exploiting the cross-resistance between pyrethroids and DDT, and by showing no effect on DDT resistance with both FDMC (fluorinated dimethyl carbinol) and PB pre-treatment. These synergists block all other commonly occurring mechanisms of DDT resistance. It is important when using synergist combinations that the susceptible strains are also tested with the same treatment regime, as there is likely to be some synergistic effect even in the susceptibles, that must be discounted when analyzing possible resistant strains.

3.1.1 Recommendations

a) Discriminating doses must be set for a number of pyrethroids and DDT using either topical application and/or a contact bioassay system for both male and female tsetse flies of standard insecticide susceptible colonies.

b) Baselines should be set for these susceptible strains for the synergist/insecticide combinations specified above, so that these can subsequently be used to give a first indication of the resistance mechanism.

3.2 Biochemical Assays

A range of biochemical assays are currently available for resistance detection and have been used under both field and laboratory conditions for a range of insects. When primarily concerned with detection of pyrethroid resistance we need to be able to detect all the possible resistance mechanisms already detailed earlier (in Section 2). Hence, although accurate and sensitive assays for the detection of altered acetylcholinesterase and elevated glutathione S-transferase exist, they are irrelevant for pyrethroids, and need to be adapted for tsetse only if a broader range of insecticides (including the organochlorines and organophosphates) is to be covered in resistance studies. Any biochemical assay adopted should work at the level of the individual insect to give the maximum likelihood of resistance detection at an early stage. The biochemical assays currently in use have the advantage over bioassays of being applicable on live and frozen insects, which allows material to be collected and analyzed at a subsequent date in a central laboratory facility. The biochemical assays may be affected by the sex, age and physiological state of the insect, and the influence of such factors should be determined in standard susceptible strains.

a) *Esterases*

Detection of quantitative increases in esterase activity is straightforward using artificial substrates in either a filter paper assay or a microtitre plate assay. These assays, however, detect only an increase in overall esterase activity and a positive result still needs to be linked to pyrethroid resistance. Elevated esterase activity in the aphid (*Myzus persicae*), due to the E4 or FE4 enzymes, confers both pyrethroid and organophosphate resistance, while that in *Culex* mosquitoes conferred by either the "A" and/or "B" series enzymes gives organophosphate resistance only. Furthermore this approach will probably not detect resistance associated with esterases which are qualitatively but not quantitatively changed, such as occur in houseflies and anopheline mosquitoes. The latter must be detected using electrophoretic methods, or implicated through synergist and metabolism studies. Electrophoretic methods are applicable on whole or fragments of individual insects and would be practical for small numbers of insects. A range of esterases involved in resistance have already been characterized electrophoretically in several insect species. Where homology occurs between these esterases and the tsetse esterases, the development of detection systems could be accelerated by the use of information and material already available on these esterases. Antisera are available for the "A" and "B" esterases of *Culex* and the E4 esterase of aphids. The antisera can be used as a field-based assay to quantify specific esterase levels by a microtitre plate assay. The cross-reactivity of esterases within tsetse species to these antisera should be determined, with a view to using them in a similar assay. Alternatively, one approach to produce antisera specific to tsetse esterases could be through gene expression.

b) *Oxidases*

The possibility of using an enzyme-based microtitre plate assay for the quantification of mono-oxygenase activity exists. However, the efficacy of this will depend on the normal baseline activity in the tsetse fly, which is currently unknown. A simpler test for a crude measure of mono-oxygenase activity is the bioassay with synergist (PB) pretreatment.

c) *Nerve Insensitivity*

No simple biochemical method exists for detection of this type of resistance mechanism, and neurophysiological or molecular approaches are required for its direct detection.

3.2.1 Recommendations

a) The baseline esterase activity levels in individuals from standard tsetse strains should be determined biochemically and the electrophoretic patterns in these and other field populations should be checked to determine the current level of isozyme variation within and between strains, so that new resistance-associated variants can be rapidly detected.

b) Western blots of tsetse homogenate against antisera to known resistance-associated esterases should be done. A strong cross-reactivity would suggest that cloning the relevant tsetse esterases by homology would be practical.

c) Baseline mono-oxygenase levels in individuals of the standard susceptible strains should be determined. If the levels are sufficiently high in the susceptible insects, attempts could then be made to develop a microtitre plate-based assay to detect activity levels in single insects.

3.3 Molecular Studies

Several genes responsible for insecticide resistance mechanisms have been already cloned from various Diptera. They include:

a) genes encoding proteins which act as insecticide targets such as:
- acetylcholinesterase from *Drosophila melanogaster*, housefly and *A. stephensi*;
- sodium channel genes from *D. melanogaster*;
- chloride channel pore integral to the GABA receptor from *D. melanogaster* and *A. aegypti*.

b) genes encoding detoxifying enzymes such as:
- esterase genes from *C. pipiens* mosquitoes;
- glutathione transferases from houseflies and mosquitoes;
- mono-oxygenases from houseflies.

Many other genes are in the process of being characterized and will become progressively available.

Many regulatory and structural DNA sequences are probably sufficiently conserved within Diptera so that genes already cloned and sequenced may be valuable in isolating similar genes from the tsetse genome. A number of genes potentially responsible for insecticide resistance in tsetse fly could be isolated in advance, using current information from other Diptera. Furthermore, when a gene has been isolated from one tsetse species, it should be relatively easy to isolate the corresponding genes in other related species.

3.3.1 Recommendations

a) Genes potentially responsible for insecticide resistance in tsetse fly could be isolated:
- from tsetse genomic DNA and cDNA libraries screened by heterologous hybridizations using as probes genes previously cloned from other species;
- directly, from polymerase chain reaction (PCR)-amplified tsetse genomic DNA and RNA, using primers designed from relatively conserved homologous sequences.

Genes possibly involved in pyrethroid resistance should receive priority.

The tsetse genes cloned in this way should be sequenced in order to identify the coding and regulatory sequences which may be involved in. The isolated genes may also be useful as probes to define a number of characteristics in tsetse flies from natural populations. These include:
- the RFLP polymorphisms of each locus, or eventually of specific gene regions, such as those encoding insecticide target sites;
- the level of DNA amplification by a quantitative dot-blot hybridization assay; and
- the level of mRNA expression, by a dot-blot assay, in order to detect more efficient transcription of resistance genes, resulting from a gene amplification event or from enhanced transcription.

b) Broad genetic studies of the tsetse genome will yield tools that can facilitate future identification of resistance mechanisms. This work would aim to improve isolation strategies of genes which have potential for conferring insecticide resistance, once they have been identified by biochemical approaches. To aid in this type of genetic approach for tsetse fly we need:
- the construction of a genetic map, through determination of linkage groups each having several markers;
- use of RAPD analysis;
- availability of molecular markers such as microsatellites; and
- a polytene chromosome map.

3.4 Neurophysiological Methods

These methods are applicable for use on individual insects and can detect unambiguously the presence of a nerve insensitivity mechanism. However, differentiation between resistant allelic forms, for example kdr and super-kdr in houseflies, is more challenging with this method. This approach for tsetse could be developed for either larvae or adults, and different nerves e.g. the ventral nerve cord of the larvae or the dorsal flight muscle of the adult, could be used. With this method it is practical to analyze only small numbers of insects. The disadvantage of this approach is the complexity of the equipment required plus a high level of skill and manual dexterity in isolating and infusing the nerve preparations. A more simplistic "single leg" assay which does not require nerve dissection has been used in *Aedes* mosquitoes. The appropriateness of this assay for tsetse could be determined, although in the assay development phase this would need to be compared to more traditional nerve preparations. This method is not advocated as a field monitoring tool, and is currently practical in only a small number of laboratories worldwide, but should be used to validate full scale molecular characterization of suspected resistant variants of the sodium channel protein in tsetse. In the long term, a molecular approach to field monitoring for Kdr-type resistance may be both practical and accurate.

3.4.1 Recommendations

a) Baseline levels of nerve sensitivity could be determined on nerve preparations of larval and/or adult tsetse species of standard susceptible strains. However, because of the complexity of these techniques it is recommended that neurophysiological studies should not be attempted until there is some indication that nerve insensitivity is at least partially implicated in pyrethroid resistance in tsetse.

3.5 Priorities

a) *Detection and Monitoring*. This requires the development of a good discriminating dose assay which will detect resistance no matter what the mechanism. Bioassay is the simplest and quickest approach and should be developed initially. The second priority would be the development and validation of biochemical and/or molecular screening assays. The biochemical and molecular assays will take much longer to develop but can potentially yield a greater amount of information per insect.

b) *Characterization*. This has a lower priority than the detection and monitoring assays. In some respects it is impossible to characterize resistance mechanisms until resistance has been detected and selected (see Section 4). However, it is possible to put some basic information in place even when resistant strains are not available. For example, comparison of esterases in susceptible tsetse with those associated with resistance in other species could be done rapidly and inexpensively. If and when resistance does occur, the quickest and simplest method of getting an initial indication of the mechanism involved is by the use of synergist/insecticide combinations. Results from this will then suggest the mechanism to be emphasized and the methods to be used for its characterization. The molecular, genetic and biochemical approaches detailed above are those primarily involved in resistance characterization.

4. SELECTION OF RESISTANT STRAINS

Availability of laboratory-selected insecticide-resistant strains is essential for biochemical, physiological, and behavioral studies of resistance mechanisms. Such strains have played a crucial role in detection, monitoring, and characterization of resistance in other species, and their current absence in tsetse is a significant impediment. Although the process of laboratory selection may not mimic perfectly the results of field selection, it is better to have some information on resistance well before it appears as a problem in the field than to have no information at all.

Resistant strains are important in providing an early indication of what may be physiologically and evolutionarily attainable by the insect. Physiological and biochemical studies using them will reveal which of the many possible mechanisms actually occur and how potent they are. Genetic studies will enable measurement of the degree of dominance at individual resistance loci, and assist in differentiation of multiple mechanisms and identification of the genes responsible and how they interact. In some cases molecular approaches based on these strains will enable development of rapid and specific DNA-based diagnostic tests for measuring frequencies of specific resistance alleles in the field.

Selected strains are often helpful in providing estimates of how resistance may develop in the field under different pest control scenarios. This can be accomplished using computer models of the selection process based in part on parameters obtained from the resistant strain, or re-selection experiments starting with mixtures of resistant and susceptible strains allowing the initial frequencies of resistant alleles to be known.

It is, therefore, important that laboratory-selected insecticide-resistant strains of tsetse be developed promptly. Priority should be given to developing those species-insecticide combinations most relevant to large, sustained control programmes that may be planned for the future. A deltamethrin-resistant strain of *G. palpalis* originating from Zimbabwe might be a reasonable first attempt; experience in developing this strain will be useful for other compounds and other species of *Glossina*.

Efforts must be made to ensure that a sufficiently large amount of genetic variability is present at the start of the selection experiment. Ideally, strains should be founded from large numbers of tsetse from areas with substantial previous insecticide exposure, as this is where resistance genes are most likely to be found. If possible, genetic variation should be quantified using allozyme analysis, minisatellite probes, or RAPDs.

An unselected line from the same initial insect collection should be maintained as a control population for the selection experiment itself and for subsequent studies of the resistant strain. The selection regime should be carefully planned in advance to take into account the long generation time and low fecundity of tsetse and to avoid inbreeding. The method of application should ideally resemble the manner in which flies will encounter the insecticide it in a field control situation.

Progress of the selection experiment (before, during, and after) should be monitored using a standardized bioassay method that can be compared to field monitoring results as discussed earlier. Genetic variability and strain fitness components should also be monitored during this process.

Throughout this work and subsequently, precautions should be taken to prevent reintroduction of the strains into the natural habitat. Ideally, they should be developed and studied in parts of the world where tsetse populations cannot be sustained in the field. This resource should be made available, with relevant information on its selection to interested parties at minimal cost to encourage its utilization. These strains should be analyzed employing genetic tools being used in tsetse for genome mapping and characterization of genetic traits useful for the SIT.

4.1. Recommendations

a) Laboratory-selected deltamethrin resistant strains of tsetse should be developed. The choice of species, and its site collection should be influenced both by previous insecticide use in the area and future control plans for the species.

5. PREVENTION AND MANAGEMENT OF RESISTANCE

The exact design of tsetse control operations is influenced by economic and biological considerations. Where practical, both factors should be considered, and those making economic judgements on the extent of pesticide usage should be aware of the biological consequences of these decisions, including resistance.

It is evident from the above that for the effective management of resistance it is important to have a clear understanding of the genetics, biology, ecology and population dynamics of the insect. The lack of adequate information, however, does not preclude the consideration of some basic measures for preventing or delaying the onset of insecticide resistance.

There is a consensus of opinion that the rate of selection of pre-existing resistance genes to damaging frequencies is contingent on the selection pressure applied. This in turn is determined by the proportion of the population that is exposed to the insecticide, and the percentage of these that are killed by the treatment. The frequency with which insecticide treatments are repeated, the decay rate of insecticide deposit, the presence of unexposed insects and their entry into the treated area to dilute the effects of the treatment are also important influences on the selection process.

Three pest control strategies are listed below in order of decreasing risk of pyrethroid resistance:

a) Where suppression rather than eradication is the objective, it would be advisable to maintain the selection pressure as low as can be tolerated. Under these conditions, it is conceivable that the status quo will be maintained and resistance will not arise. However, the risk of resistance is still present as long as pyrethroids are the major source of mortality.

b) Where eradication based solely on targets is the desired goal, greater emphasis must be placed on the use of targets. The targets should be maintained at high pyrethroid concentrations which do not allow the survival of resistant individuals. In such cases, the joint use of pyrethroid and juvenoids may offer the prospect of sterilizing resistant individuals surviving the pyrethroid. If a sufficiently large area is treated in this manner, it is likely that the population can be eradicated or reduced to levels from which a future recovery will depend on the arrival of susceptible migrants from longer distance. At this time, resumption of the same tactic may be considered. However, the practicality of using juvenoids at sterilizing doses with impregnated targets in this type of strategy needs evaluation. Complete reliance on pyrethroids for eradication would increase the resistance risk if eradication is not 100% successful.

c) Where eradication with SIT is the objective, less emphasis will be placed on the use of targets. Targets will be used for a period of 2-12 months to reduce the native population prior to the release of sterile males. Thus, there would be little chance for resistance to evolve.

In the three situations described above, it is essential that data be collected periodically to assess changes in population density and behaviour and to determine whether an alternative strategy is desirable.

5.1 Recommendations

a) That pyriproxyfen and other juvenoids be evaluated as potential partners of deltamethrin on impregnated targets,

b) That IAEA/FAO seek funds to commission a major review of factors pertaining to tsetse ecology and control with a direct bearing on anticipated resistance risks and to design possible management strategies.

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