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The use of chemosterilants for vector control (1)

by

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THE USE OF CHEMOSTERILANTS FOR VECTOR CONTROL

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1. INTRODUCTION .

Since about twenty years many arthropods of medical and veterinary importance have been kept under control by the use of residual insecticides, for the benefit of human health and welfare. But the mass spraying of insecticides, either for vector control or disease eradication programmes, as well as for agricultural and domestic uses, has selected insecticide-resistant populations of various vectors in many areas of the world. Year after year the number of resistant species and their distribution increases. Now, 60 to 70 species of medical and veterinary importance, belonging to 9 major families, are resistant to one or several chemical groups of residual insecticides, and their occurence is a very serious complicating factor for the organization and development of many public health programmes (W.H.O., 1963 - COZ & al., 1964 - PAL, 1964 - SHAN & STONE, 1964 - UNGUREANU, 1964). W.H.O. has sponsored a coordinate research programme for the development and screening of new insecticides, on a world basis; the results of which are very promising, but it should be anyway wiser to do not rely entirely on one type of vector control and to investigate other vector control possibilities than insecticides .

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Insecticides used for public health programmes are, as a rule, selective toxicants for insects, with a low or moderate mammalian toxicity. However, their use modifies more or less the environmental conditions and the greater the treated area, the greater the impact on the environment. Moreover, some of the most widely sprayed insecticides are chemically very stable and disappear only slowly from treated premises, soils, watercourses, and so on. Contamination risks of the environment bolongs to two major types : acute and immediate intoxications, easy to detect and to prevent, and long term accumulation of the most stable compounds; this last risk is less easily detected and nothing but time can clear the contaminated areas and people (DALE & QUINEY, 1963 - HAYES & al., 1963 - HAMON & al., 1965). Up to now this situation has not caused difficulties for human health, the sporadic damages being restricted to the most developed countries and being mainly induced in wildlife. However such a situation underlines the necessity to combine all available methods of vector control to get the best of each of them in an integrated control programme, and to use residual insecticides only when and where they offer the best prospects of success, so to avoid useless contamination of the environment. One of the new developing control procedures is based on the mass release of sterilized males.

2. THE THEORY OF VECTOR CONTROL BY MASS RELEASE OF STERILIZED MALES

In many vector species the female is inseminated only once for its whole life or, when inseminated several times, the first received batch of spermatozoa is the major one, or the only one, employed for egg fertilisation. There are however some exceptions (ROTH, 1948 - GOMA, 1963) .

If it is possible to release, in a stable population, as many sterile males as there are normal males, 50% of the virgin females will be inseminated by sterile males and either shall not lay eggs, or shall lay sterile eggs, with the remote possibility to induce parthenogenetic development. The next generation will be reduced to 50% of the initial generation, and, if the number of released sterile males is constant, 33% only of the resultant females shall give a progeny, and so on, along a decreaing exponential curve, until the disapearance of the species. The success will be achieved much quicker if, from the beginning, the sterile malos outnumb by several times the normal males (KNIPLING, 1955) .

It should be moro difficult to get the same results by release of sterile females as the males copulate many times during their first days or weeks of life and should be able anyway to insominate normal females. The mixed release of both sterile males and females slow down the process, a proportion of the sterile males being diverted from the normal to the sterile fomales .

Sterile males, to be competittive with normal ones, must have a normal expectation of life or, at least, must be sexually active in large numbers as long as the normal males. They must also inseminate females with a normal amount of spermatozoa and accessory gland secretions because the females with empty or partially empty spermathecae could agree to be inseminated a second time. Sterile males must be also potent inseminators. The difficulty to fulfill all these conditions is a secon reason for releasing a number of sterile males several times higher than the estimated number of normal males, the ratio of sterile to normal males being at least 10 to one. Finally sterile males must be evonly scattered among wild population. Difficulties increase with the natural density of the vector population, with species either with dormant eggs and/or with multiple inseminations, and with short flight-range species.

The first known method of male sterilization was based on gamma or X-ray irradiation of larvae or pupae, which implies laboratory mass-rearing of the vector to control. The first wide-scale scheme of vector control by mass release of sterilized males was carr: out against a pest of veterinary importance, the screw-worm Cochliomyia hominivorax which has a long flight-range (HIGHTOWER & al.,1965). The success of the screw-worm eradication programme, first in 1954 in Curaçao (BAUMHOVER & al., 1955) and then in 180,000 square kilometers of the southeastern United States during 1958 and 1959 through the release of more than two billions of sterile flies (KNIPLING, 1960), not only stimulated wide interest in insect control by male sterilization and in laboratory massrearing of posts of economic importance (FAY & al., 1963 - MORLAN & al., 1963), but also in control by stérilization with chemicals as well as by irradiation .

The sterilization by irradiation requires costly installations, not very casily handled in field conditions. Moreover many workers have stressed that irradiated males have a shorter expectation of life and are less competitive for matimg than normal males (SACCA, 1961, Musca domestica - RAMAKRISHNAN & al., 1962, Culex pipiens fatigans - HENNEBERY & McCOVERN, 1963, Drosophila melanogaster- STAHLER & TERZIAN, 1963, Aedes aegypti - GOMEZ-NUNEZ & al., 1962 & 1964, Rhodnius prolixus), the safety margin between the 100% sterilizing dose and the lethal one being often very narrow. Chemosterilants offer better prospects of development and practicability (SCHMIDT & al., 1964) and the screening of thousands of chemicals for sterilizing properties against insects begun some years ago in laboratories of the Agricultural Research Service of United States Department of Agriculture .

3. THE CHEMOSTERILANTS COMPOUNDS .

3.1. Chemical nature and properties .

Chemosterilants are chemicals capable of causing sexual sterility; they prevent reproduction in insects or other organisms. Insects chemosterilants may act in one of the three following principal ways :

- They may cause insects to fail to produce ova or sperm ;
- They may cause the death of sperm and ova after they have already be produced ;
- They may injure severely the genetic material in the sperm and ova and, although the sperm and ova remain alive and the sperm retain full motility, the zygotes, if formed, do not complete development into mature progeny .

The two first ways are not very efficient for vector control as normal females can be induced to mate later on with fertile males if their spermathecae are empty. The third type of action presents the greatest interest at the present time and is that shown by the so-called "radiomimetic" compounds .

The selection of insect chemosterilants has been guided, from the first beginning, by the relationship between compounds effective in cancer chemotherapy and mitotic agents (SMITH & al., 1964

FAHMY & FAHMY, 1964 - CHANC, TSAO & CHIANG, 1963). Other investigations have been carried out with compounds similar with normal insect metabolites which could replace these metabolites and stop cellular development. Then some guidelines have been established in selecting chemical and structural features characteristic for chemosterilants amongst all the candidate compounds investigated (RISTICH & al.,1965).

The chemosterilants can be classified into two main categories the antimetabolites and the alkylating agents, which are also radiomimetie. Some other agents, at the present time without practical importance, cannot be classified (MOUCHET & RAGEAU, 1963 - KENAGA, 1965). The antimetabolites, purine and pyrimidine antagonists, antifolics, are mainly active against female insects and their action may be of a temporary nature. The alkylating agents are mutagenic and damage the genetic material of ova and sperm but, generally, do not inhibate live spermatozoa formation; their action is of a more permanent nature. The most promizing alkylating agents are the derivatives of aziridine, some of them being used since several years in tho textile industry and in cancer therapy. The commonly investigated aziridine derivatives, for insect chemosterilization, are now the following (fig.1) :

- Tepa = Aphoxide = tris (1-asiridinyl) phosphine oxyde .
- Metepa = Metaphoxide = tris (2-methyl-1-aziridinyl) phosphine oxyde
- Thiotepa = tris (1-aziridinyl) phosphine sulfide .
- Apholate = 2, 2, 4, 4, 6, 6, hexa (1-aziridinyl) 2, 4, 6, triphospha 1, 3, 5, triazine .

The toxicological risks inherent in the mutagenic properties of alkylating agents restrict their study as insect sterilants to laboratory experiments and to very limited field trials under close supervision. However recent researches have shown that some compounds, BMPA (=hexamethylphosphoramide) and HNM (=HNM = Hemel = hexamethylmelamine), structurally similar respectively to Tepa and to Tretamine (= 2, 4, 6, tris (1-aziridinyl) S.triazine), but lacking alkylating properties and being of low toxicity to mammals, still retain the property of sterilizing insects (CHANG & al., 1964). We can hope that further investigations will discover insects chemosterilants more acceptable for practice than those hitherto available, and researches are progressing along this way (ASCHER, 1965 - FYE & al., 1965 - KENAGA, 1965 - PARISH & ARTHUR, 1965[a] - SHAW & RIVIELLO, 1962 - RISTICH & al.1965), but too often the most efficient new chemosterilants are also the most toxic for mammals .

Used as dry deposits Tepa and Metepa disappears rapidly by volatilisation, and they are also actively sorbed on porous surfaces (DAME & SCHMIDT, 1964). Thiotepa is probaly slightly less volatile and has been active against female Aedes aegypti during 23 days after application at the dosage of 400 mg/sq.meter on filter paper (BERTRAM, 1964).

In solution alkylating agents are slowly decomposed in inactive compounds. Tepa and Metepa are more stable in neutral or in slightly alkaline than in acid solutions. Decomposition rates vary directly with temperature, but even at 25°C, the half-life may attain 32 days for Tepa and 72 days for Metepa (BEROZA & BORKOVEC, 1964, in BERTRAM, 1964 a).

However DAME, WOODARD & FORD (1964) stress that, in field conditions, Tepa solutions loss their efficacy in three days .

Inside insects alkylating agents distribute themselves quite rapidly into all tissues, as shown by DAME & SCHMIDT (1964) with radiolabelled Metepa in mosquitoes. But the compounds are rapidly excreted and retain their sterilizing form no more than 6 to 8 hours after the end of the insect treatment (PLAPP & al., 1962) .

3.2. Biological action of chemosterilants in laboratory experiments.

In laboratory experiments chemosterilants have induced sterility in a great variety of organisme (CRESSMAN, 1963, acarina - BURDEN & SMITTLE, 1963, cockroaches - CHANG & CHIANG, 1963, moths - KEISER & al. 1965, fruit-flies) including mammals (GAINES & KIMBROUGH 1964) and their efficacy and limitations have been assessed on some of the major insects of medical importance (SMITH, 1964). Some compounds are much more toxic for some species than for other ones, and the order of efficacy of chemosterilants varies according to the species used and the application method employed in screening tests (COUCK & al., 1963 a) .

Chemosterilants can be applied to insects by various methods : dipping, dusting, topical application, tarsal contact with treated surfaces, aerosol administration or ingestion of treated bait (DAME, WOODARD & FORD, 1964 - DAME & SCHMIDT, 1964 - CRYSTAL, 1964 & 1965). Physical properties varying with the compounds standardized assessment techniques cannot be used for all candidate compounds ; however the majority of routine assessments are done by oral application (GOUCK & al., 1963 a - CHANG, TSAO & CHIANG, 1963) .

3.2.1. Biological action on vectors .

3.2.1.1. Males .

The sterility induced by apholate treatment of Aedes aegypti larvae is caused by chromosomes damaging, with dilatations, linkages, splitting and so on; tsetse have a normal size but produce less spermatozoa (RAI, 1965). BERTRAM (1963) has found active sperm in Thiotepa sterilized males of Ae.aegypti, mating and insemination being still possible; the sterility was complete in the days following the treatment but later on some of the inseminated females gave eggs with a hatch rate of 13%. Similar results have been obtained in Drosophila sterilized by Tretamine by FARMY & FARMY (1954) and in males in Musca domestica sterilized by Tepa (SACCA & al.1964 - SHEN CHIN CHANC, 1965). Such results are probably the consequence of the greater susceptibility to chemicals of post-meiotic stages (sperms, spermatids) than of earlier pre-meiotic stages in spermatogenesis. Il is possible that longer exposures to - or increased concentrations of - the toxicants could kill the primary germinal tissue and eliminate all possibilities of recevery .

Chemosterilized males are not always as vigourous and able to copulate than the normal males (DAME & SCHMIDT, 1964 - CRYSTAL, 1964) and the amount of sperm is sometimes insufficient, leaving the females amenable to further copulations with normal males and subsequent formation of fertile eggs (DAME, WOODARD & FORD, 1964). However, for practical application , chemosterilized males are generally (but not always) competitive with normal males (NURVOSH & Al., 1964 - LABRECQUE & al., 1962 a- SHEN CHI CHANG, 1965) and the sterile spermatozoa accomplish a high rate of fertilization giving unfertile eggs even when they are mixed to normal spermatozoa in the female spermatheca (LABRECQUES & al., 1962 a). Nevertheless in some species, like

Culex p. fatigans, the safety margin between the sterilizing and the lethal concentrations is very narrow for the majority of the most promising chemosterilants (MULLA, 1964).

3.2.1.2. Females .

If the vector control programme is based upon the release of laboratory-bred insects it is not worthwhile to release sterilized females, first because their impact on the spermatozoa availability of wild males will be almost nil, and secondly because such a procedure will increase the size of the biting section of population. But, as we shall see later, chemosterilants offer wider prospects to vector control operations than irradiation procedures and, under some restrictions, shall probably be used in the future directly against wild males and females .

When treated with identical amounts of chemosterilants, females are slightly less susceptible than males, but in cage experiments and in natural conditions with traited baits, the induced sterility is generally highor in females as they ingest more bait and so more chemosterilant than males (CRYSTAL, 1965 - SACCA & al.,1964).

Wild-caught females mosquitoes, already inseminated, are sterilized by resting on tepa or thiotepa treated surfaces, even when follicules are as developed as stage V, as chown by WEIDHAAS (1962) with A.quadrimaculatus, and by BERTRAN (1963) with A.melas. Similar results have been observed in laboratory conditions with Ae.aegypti, Ae. togoi and C. pipiens (BERTRAM, 1964a). The speed of action of aziridine derivatives on house and fruit-flies is slower than on mosquitoes, specially when females flies are old (SACCA & al., 1964 - CHANG & CHIANG, 1964 - KEISER & al., 1965).

In treated females the ovarian development is soon anarchic, each follicle having its own rato of development. The amount of laid eggs is often nearly normal during the first oviposition, but decrease sharply in the following ovipositions due to somatic deterioration of ovaricles, and there is no hatching. At least in mosquitoes, damages to the spermatozoa in the spermatecae of treated females seem to be the main reason for sterility of the first batch of eggs laid afte exposure; in comparative experiments with low dosages of chemosterilants the percentage of infertile eggs is far higher if females are inseminated before exposure than if they mate after exposure (BERTRAM, 1963). Oogenesis can also be inhibited from the beginning if females are exposed to chemosterilants scon after their emergence but such a susceptibility of the follicular epithelium is restricted to the first hours of life; later on, the effects of chemosterilants on ovogenesis are only conspicuous on a long term basis (BERTRAM, 1963 - CRYSTAL, 1963 - WEIDHAAS, 1962 - DAME & FORD, 1964 - CRYSTAL & LACHANCE, 1963). So, with very early applications, the number of depisted eggs is reduced almost to nil, whercas with treatment of older females the number of mature eggs is normal, but they do not hatch (CRYSTAL, 1965)

3.2.1.3. Larvae .

Insects can be also sterilized as adults following their treatment as larvae or pupae by alkylating agents, but the mortality is generally in creased at moulting and emergence periods. Tepa and thiotepa seem to be more promising than apholate for such usage. The treatment of natural breeding places cannot be considered, as the compounds are mutagenic, and should be applied at short intervals, but larval treatment could be a method of choice for mass sterilization of reared mosquitoes (DAME, WOODARD & FORD, 1964).

3.2.2. Biological action on transmitted parasites .

Chemosterilants can act along two ways on transmitted parasites during the sterilization of infested vectors. They can either kill the parasites, or cause mutations of the parasites into new st. of different pathogenicity. If females are treated before becoming infested, the action of chemosterilants is nil as the compounds disappear from treated insects in the hours following their absorption. Up to now investigations have been carried out only on malaria and filarial parasites .

3.2.2.2. Malaria parasites .

Several investigations have been carried out, all based on Ae. aegypti and Plasmodium gallinaceum .

ALTMAN (1963), reported substantial, but variable reduction of malariometric indices (by about 85%) by exposing the vector for 90 to 540mn to 100 mg/m2 of tepa, on glass, immediately before and afte the infective meal and also, in one occasion, when sporozoïtes had reached the glands.

Using different strains of the same mosquito and Plasmodium and thiotepa, BERTRAM, SRIVASTAVA & MSANGI (1964) have got on the whole similar results, but with an average reduction in malaria transmission of 25% only. The deposit used was 400 mg thiotepa/sq.metre. Contacts of the mosquitoes with the treated surface for 1 or 2 hours was almost inefficient, but better results were obtained with 3 hours of contact. The action of the chomosterilant seemed particularly important when the mosquitoes were exposed some hours only after the infectant blood-meal (when gametocytes become gametes), but less efficient later on. In the most favourable conditions only 9% of the chickens were infected by the treated mosquitoes, with 100% infections in the controls. However, a second period of important susceptibility of malaria parasites occured 48 hours after the infectant blood-meal, during the meiosis in carly cocysts (30% of chicken infected). When the transmission to chickens occured the transmitted Plasmodium was normal and could be further transmitted by *Ae. aegypti* without modification of its pathogenicity; when occuring the reduction of transmission is not only caused by the interruption or decrease of oocyst grouwth and sporozoïtes production, but also by the loss or effectiveness of the sporozoïtes, probably through genetic damages (BERTRAM, 1964 b.).

The vector in both these experiments has been more easily sterilized than the parasite .

3.2.2.3. Filarial parasites .

The only experiment dealing with the action of chomosterilants on filarial parasites have been carried out by BERTRAM (1964 a), with *Brugia patei* and *Aedes togoi* exposed for 1 to 3 hours to 400 mg thiotepa/sq.m deposits on glass.

Contact of 1 hour does not change the speed of development of filarial worms in the mosquito, but 2 hours and 3 hours"exposure slows down the development of the parasite which apparently never reaches the infective stage and dies in the thoracic muscles. It must be noted that the chemosterilant dose received by female mosquitoes during such experiments is far higher than the required one for sterilistion, and is sufficient to kill 95% of the males in the 24 hours following exposure .

3.2.3. Metabolism and biological action on mammals.

The mammalian and insect metabolism of chemosterilants has been recently investigated by using phosphorus 32 labelled Metepa (PLAPP & al., 1962) and Thiotepa (PARISH & ARTHUR, 1965 b) applied to laboratory rodents and to several species of economically important insects. Metepa is very rapidly excreted as such, and is also excreted after metabolization, one major breakdown product being probably phosphoric acid; degradation and excretion are complete within 48 hours of administration. In insects Thiotepa is apparently metabolized into Tepa, then excreted; in rodents Thiotepa is excreted partly after metabolization into Tepa, partly under the form of water-soluble hydrolitic products .

The toxic hazards of chemosterilants have been recently investigated and summarized by BARNES (1964), GAINES & KIMBROUGH (1964) and by HAYES (1964).

The present conclusions are of very temporary nature as new compounds are discovered every year, some of them not belonging to the previously investigated chemical groups. Moreover, minor chemical substitutions can transform a compound of low mammalian toxicity in another one far more toxic. And the two main categories into which are classified the chemosterilants do not correspond to specific physiological action, but to large chemical or biological groups, alkylting agents and antimetabolites. So, no extrapolation of the toxicologic investigations already carried out shall not be permissible to deal with new compounds (BARNES, 1964 - HAYES, 1964).

On a whole alkylating agents are far less carcinogenic than expected by the workers using them as tools in cancer research. Human beings in cancer therapy have supported 0,6mg Thiotepa/kg (in three successive doses of 0,2mg/kg) and Thiotepa can be tolerated with as high doses as 10-40 mg a week, with some variation in individual susceptibility. Tretamine has been used up to doses of 15-25 mg initially and then 2,5-5 mg weekly, but some patients can only support 1/10 of these amounts without bone marrow depression .

On rats, which are probably less susceptible than humans to some of the actions of alkylating agents, five daily doses of 0,4 kg of Thiotepa rendered males sterile for five weeks, and Tretamine given as doses of 0,05mg/kg over a period of 30 days gave the same results; such rats remained fully active and mobile, sexually active, and their spermatozoa have been observed to penetrate the ovum of the female, but without resulting progeny. These effects of Thiotepa and Trotamine on fertility are completely reversible (JACKSON, FOX & CRAIG, 1959 - BOCH & JACKSON, 1957).

Susceptibility of rats is very variable, and so are the damages to the spermatogenesis which are comprised between slight damage to the sperm and complete destruction of the seminiferoux epithelium, Thiotepa and Tretamine, although non persistent in the tissues, having some cumulative action (JACKSON, FOX & CRAIG, 1961).

During experimentations with rats, GAINES & KIMBROUGH(1964) have observed that Metepa and Apholate, in one administration in the diet, are about as toxic than DDT and that Tepa is about as toxic than dieldrine; Tepa and Metepa are also toxic if introduced by dermic applications; in such conditions the CL 50, in mg/kg is of 136 for Metepa and 37 for Tepa. When rats received daily small doses of Metepa in their diet (5mg/kg/day) the general condition of the animals was good, but a cumulative action of the chemosterilant caused atrophy of testis in 77 days (in 55 dyas when the daily dose was 10 mg/kg) - and in 197 days with 2,5mg/kg; low doses as 1,25 mg/kg/day were apparentl not harmful. The damage to testis were to some extent reversible, but this aspect has not yet been adequately studied. When the male sterility is not complete some reduction in the number of babies occurs, but their further development seems perfectly normal, without any effect of parent treatment on their potentialities and survival .

The chemosterilants which are now investigation have not shown ahy sign of carcinogenic activity. They exert on mammals about the same effects than on insects and, at low dosages, have a highly specific and localized action on the developing sperm of mammals. It is only at far higher dosages than these compounds depress the bone marrow activity (BARNES, 1964 - HAYES, 1964 .

HMPA has a steriliming action at concentrations 50 times higher than Tepa, but its CL 50 for rats is as high as 2600 to 6400 mg/kg against only 37mg/kg for Tepa. Besides, HMPA has no mutagenic and carcinogenic properties .

Tepa, Metepa, Thiotepa and Apholate are more toxic than the commonly used insecticides. They are quickly metabolized and excreted without apparent accumulation in the organism; however, they seem to exert a cumulative effect when routinely absorbed, and may sterilize human beings at dosages which are not harmful if only behaviour and survival are taken in account. So it seems unthinkable to use the compounds now available for indoor house-spraying or for extensive treatment of breeding places (BARNES, 1964 - HAYES, 1964).

3.2.4. Possibilities of resistance to chemosterilants.

HAZARD and al.(1964) have investigated the possibility to select a strain of Ae. aegypti resistant to Apholate sterilisation by larval exposure. In standardized rearing and treatment conditions the percentage of sterility has decreased from 96% to 46% in 4 generations with 5 ppm Apholate in breeding water, and from 100% to 72% in 6 generations with 15 ppm Apholate. So Ae.aegypti seems able to develop some resistance to Apholate, with a decrease of efficacity of 4 to 5 times .

3.3. Possible use of chemosterilants for insect control .

As availablennow the chemosterilants compounds can either replace sterilization by gamma radiations, on laboratory-bred vectors for subsequent release, or be used to sterilize wild populations.

Sterilization by chemicals would be simpler and cheaper than by gamma radiations and the facilities required would be more readily movable. Morsover, the males treated with chemosterilants are far more competitive than the irradiated ones (WEIDHAAS & SCHMIDT, 1963), but the general limitations of the methods would be the same than with sterility induced by irradiation. .

The great advantage of the chemosterilisants is their possible application to wild populations. If they could be safely applied to natural populations, control or even eradication would be realized by induced sterility without the necessity of rearing and releasing large numbers of insects. If wild males as well as wild females could be treated the decrease of natural populations would be very sharp, a large proportion of the normal males inseminating sterile females and many normal females being mated by sterile males

(KNIPLING, 1962). Up to now, the administration of chemosterilants in the adult food or on resting places seems to be the only practical method for field use and is dependant of the adjunction of powerful and selective attractants (LIU, 1962 - HOCKING, 1963 - STEINER & al., 1961 - LHOSTE, 1962 - KESER & al., 1956 - BROWN, WEST & LOCKLEY, 1961) which could conteract the repellent effect of some of the chemosterilants (SACCA & al., 1965) and attract an high proportion of the natural populations.

Others developments in the use of chemosterilants await the assessment or discovery of new compounds without toxic hazards for mammals .

4. FIELD EXPERIMENTS WITH CHEMOSTERILANTS .

Field experiments with chemosterilants have been restricted to a few small tests to explore possible methods of application and evaluation, mainly with house-flies, but some of the results of the field experiments carried out with gamma-irradiation sterilized males give also useful informations on the practical problems to solve .

4.1. Anopheles quadrimaculatus

A. quadrimaculatus males, from the laboratory colony of Orlando, have been sterilized either by irradiation, or by exposure to chemosterilants (contact with Tepa or oral administration of Apholate). The sterilized males were sexually potent and gave a high rate of fertilization of wild females in laboratory conditions, but they were unable to perform the same duties when released amongst the natural population of A. quadrimaculatus. When the experiment was duplicated, using as sterile males, the treated progeny of wild caught females, the released males succeeded to induce sterility in wild females. So it is probable that the initial failure was not due to chemosterilant-induced decrease in sexual competitiveness, but to changes of behaviour during colonization and to basic inability of years-long colonized mosquitoes to mate in field conditions with wild females of the same species(DAME, WOODARD, FORD & WEIDHAAS, 1964) .

4.2. Musca domestica .

The results of only four field experiments carried out in Florida and in Italy, each based on a different chemosterilant in cornmeal bait (with sugar, dry milk and dry egg added), have been yet published, as well as two cage-experiments simulating natural conditions .

HANSENS & GRANETT (1965) have compared, in two cages of about seven cubic metre each, the development of two colonies of house flies, with larval breeding medium always available, baits treated with 2% Apholate being introduced in one of the cages twice a week. The Apholate treated cage has produced, in two sets of experiments, 10 to 40 fold less house-flies than the control cage .

During a second set of experiments, carried out along the same lines that the above ones, HANSENS (1965) has investigated the relative effectiveness of Apholate under the form either of 2% treated baits, or of impregnated cords, and of both combined, with various rythms of application, against susceptible and insecticide-resistant house-flies. Treated baits, introduced at weekly intervals, gave more than 90% control and, in combination with impregnated cords, eradicated the caged populations. Cords impregnated with a syrup at 72% Apholate were less efficient, giving only 57% control. Insecticide-resistant house-flies were as easily amenable to control with Apholate as the susceptible ones .

LABRECQUE & al. (1962) have assessed the efficacy of baits with 0,5% Tepa, on garbage dumps in one of the Florida keys, with once a week application during nine successive weeks.. The island was relatively isolated and another dump, situated at about 50 km from the treated one, was used as control. The house-fly and secondary screw worm (Cochliomya macellaria F.) densities were estimated in the most infested areas, by the grid method. The sterility of females was investigated weekly on representative samples of the house-fly population. House fly populations were reduced from 47 per grid to 0 within 4 weeks and the proportion of egg masses (from females collected on dump) containing at least one viable egg was reduced from 100% to 10% within 5 weeks, and the percent hatch among all eggs laid was reduced to 1% within 5 weeks. After treatments were discontinued the percent viability rapidly returned to normal. During the entire period of the experiment viability of eggs from females from the control dump ranged from 65 to 99%. It was not possible in this experiment to assess what proportion of the females was sterilized by feeding on the bait and what proportion by mating with sterile males. Blow fly counts in the treated area was also reduced markedly toward the end of the test, but all captured females oviposited freely on fresh meat and all the egg mass were viable; previous experiments have shown in the past that blow flies are difficult to control with dry granular insecticide bait .

LABRECQUE & al.(1963) have carried out an experiment with 0.5% Metepa baits in a poultry farm near Orlando, in a non-isolated situation. Baits were distributed once a week during 9 weeks and then twice a week. The density of house flies was drastically reduced. The hatching rate of egg masses was respectively decreased 2,6 times, then 5 times. Male fertility was not seriously affected by the weekly application of the baits, but decreased to 22% of the normal when baits were distributed 5 times a week .

SACCA & STELLA (1964) have assessed the efficacy of syrup sprays, including 1% of malt extract as attractant, and from 0,0625 to 0,2% of Tepa, on garbage dumps of several italian towns. A high sterility rate was observed one hour after the application, even with the lowest concentration of Tepa; although males be theorically more susceptible to Tepa than females, the highest rate of sterility has been observed in females, probably because males are less attracted to garbage dumps and also because in females the male-induced sterility adds its action to the intrisic female sterility. The fertility-rate returned to normal in the days following the treatment, through migrations and natural population replacement, helped by the rapid Tepa hydrolysis. With one, and then two, weekly applications of the Tepa spray on the garbage dump, and the insecticide treatment of the neighbouring farms, the house fly population has been strongly depressed; but despite the insecticide application the fly population has quickly recovered after the cessation of the Tepa spraying.

a,4.3. Culex tarsalis .

LEWALLEN & al. (1965) have studied the efficiency of Apholate applied to all natural breeeding-places of Culex tarsalis in small oases isolated in a desert of San Diego County, California. Apholate was used at the concentration of 75 ppm, with three applications within 22 days. The natural C.tarsalis population was only temporarily depressed and returned to its pre-treatment level 20 days after the last Apholate application. The failure is attributed to the survival of wild females during the whole length of experiment .

4.4. Observations collected during some of the field-tests carried out withe radiation-sterilized males .

Four field experiments, three dealing with mosquitoes and one with blow flies, based on release of radiation-sterilized males are very interesting. KRISHNAMURTHY & al. (1963) have worked with Culex p.fatigans; in India. The results were not satisfactory, partly because the number of released males was not sufficient, partly because the human population did not agree to the release of any mosquito, even male and sterile .

MORLAN & al. (1962) have released sterile males of Ae.aegypti in two areas of Pensacola neighbourhood, Florida, of about 1 square kilometer. They used two nearby untreated areas as control. The expected ratio of sterile to normal males (released as pupae) was of 47 : 1 to 170.000 : 1 in the first area, and of 27:1 to 148:1 in the second area. Estimation of natural populations of Ae.aegypti was based on actual counts of larvae every second week; except for samples collected for identification, larvae were counted and returned to their original breeding places. Field collected eggs, obtained by lining formerly infested receptables with paper towels, were counted and submerged in a 24-hour-old mixture of 0,1 g brewer's yeast and 0,1 g ground dog chow in one liter of water; each hatching test was completed in the week following egg collection; larvae were identified and counted. In area 1 the releases have reduced, but failed to eradicate, natural populations of Ae.aegypti; in area 2 the releases of sterile males failed to reduce the natural population. The rate of egg hatching, on the average, was only 60 % of the normal in the first area, but was 112 % of the normal in the second area. Amongst t reasons for failure are the greater age of sterile males, released once a week and competitive only some days with the normal wild males emerging every day, and the possible limited dispersion of the sterile males from the limited distribution of irradiated pupae .

WEIDHAAS & al.(1962) have released 1.500 sterile males of A. quadrimaculatus per week and per sq. km, during 11 months, in ten different situations on islands of 5 sq. km. of the Lakes Okeechobee and Panasoffkee, Florida. Another area was used as a control. To determine if these sterile males caused any reduction of, or sterility in, natural populations, the number of adult A.quadrimaculatus in resting stations, and the viability of eggs from females collected from these resting stations were followed in the release and check areas. The releases did not conclusively induce any sterility in wild females but perhaps when the natural population of one island was in the seasonal decline. Failure was attributed to the lack of basic knowledge of the biology and behaviour of A. quadricumulatus (which is, "en passant", one of the best known american mosquitoes and in the difference in basic behaviour of colonized and wild mosquitoes of the same species .

DONNELLY (1964), has attempted to control the blow fly Lucilia sericata on one small island of the eastern coast of England. The treated area was of about 2,5 sq.km with an estimated population of 2.000 wild males of the blow fly. The releases were carried out 4 to 5 months a year, during 2 years, and the sterile males were overwhelming the wild ones. The failure of the experiment is partly attributed to density-dependant factors reestablishing the normal densities when the natural population was decreasing, and to a possible focal distribution of natural populations, the sterile males being less numerous than normal ones in such foci.

It could be also interesting to underline here that the melonfly, Dacus cucurbitae, has been recently eradicated from Rota, Mariana Islands, by release of about 260.000.000 of radio-sterilized males and females in ten months. The island has a surface of about 97 sq. km. Females of D. cucurbitae are known for mating several times during their life, have a long flight range, but congregate in host plantations. Releases were preceded by reduction of the natural population through malathion-treated baits (STEINER & al., 1965).

5. DISCUSSION AND CONCLUSIONS.

Chemosterilants constitute a new class of vector control agents which offer promising possibilities for the future, but need more studies before becoming operational.

According to the available published knowledge the best known compounds are not safe for field scale use, but in special conditions. They are not chemically very stable, which in some aspects has the advantage to avoid residual contamination of the environment, but decreases the possibilities of large scale use of these compounds. The safety margin between the sterilizing dosages, and the lethal one is not always very wide, although much more than with irradiation procedures. Sterile males are not always competitive with the normal males and sometimes the sterility in both sexes, may be of a temporary nature. New compounds shall perhaps offer better prospect, but they are still to be discovered and we must not forget that one of our most potent residual insecticides was one of the first discovered ones; it could be the same for chemosterilants.

If chemosterilants are to be used as substitute of irradiation, to treat laboratory-bred vectors for further release in natural populations, a good deal must be learned on ecology, behaviour, dispersal, population dynamics, densities and genetics of natural populations of all vectors concerned (BIRCH, 1963 - KNIPLING, 1963), and the methodology of mass colonization of competitive strains must be elaborated for the majority of pests and vectors.

If chemosterilants are to be used in an original approach, to induce directly sterility in wild populations they must probably be associated to powerful baits or attractants, and at the present time very few pests bu house flies can be controlled by efficient treated baits. Much more is to be learned about attractants if we want to exploit the full possibilities of chemosterilants (HOCKING, 1963). However, in some circumstances, treated traps with periodic release could offer an efficient way to deal with species difficult to colonize but occuring in nature at low densities like tse-tse fl

Chemosterilants, like other chemicals, are metabolized into inactive compounds inside insects, and we can expect field development of chemosterilant-resistan populations of vectors, as we observed for insecticides. There is already one laboratory observation on apholate resistance in Ae.aegypti.

Vector control activities shall probably be slowly modified by the development of chemosterilants, bu these agents will more supply additional possibilities of control than replace the alrdy available methodes and compounds. Their intelligent use requires thes the collection of basic informations on the vector populations and shall help to develop integrated control procedures .

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