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PRESENT POSSIBILITIES OF CONTROLLING ARTHROPODS OF SANITARY IMPORTANCE WITH REGARD TO ECOLOGICAL ASPECTS

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The manifestation of negative effects of the exclusive application of chemical control method for protection of man and his property from arthropod pests has led to restrictions or elimination from practical use of stable and persistent insecticides produced on a mass scale. Thereby the amount of readily available and low-cost insecticides decreased. The production of other insecticides is much lower and the increasingly high cost caused by the continually growing demand limits their use. Simultaneously, the problem of vector and other pest control also became increasingly serious because of the enhanced virulence of many diseases, as well as of the range of resistance in arthropods.

Resistance

The phenomenon of resistance in arthropods to insecticides, defines the reaction of a population under persistant selection pressure, thus it is worth knowing the present state of this process. The outlines of resistance problems began as early as 2 years after DDT was brought into practice to control human disease vectors such as *Musca domestica* L. and *Culex pipiens* L. The problem of resistance, the mechanisms of its appearance, the range and means of control have already been reviewed in this country [4, 22, 38, 49].

The present state of resistance in arthropods of sanitary importance has been reviewed by Pal and Brown [46] who have shown that resistant populations already appeared in some 108 species such as mosquitoes (*Anopheles* sp., *Culex* sp., *Aëdes* sp.), lice, fleas, gnats, triatomine bugs, houseflies, cockroaches, ticks. Large numbers of them are resistant to several insecticides: 91 species are resistant to dieldrin, 61 to DDT, 27 to organophosphorus compounds and carbamates. In tse-tse flies (*Glossina* sp.) and *Phlebotomus* sp. resistance has not been shown. Among malaria vectors 19 *Anophelinae* species (*A. culicifacies, A. stephensi, A. albimanus compl. gambiae* and others) are resistant to DDT and dieldrin and the populations resistant to the second insecticide show cross resistance to lindane. For control of resistant species in Central America DDT was replaced by malathion 5 times higher in price and in 1970 by the still more expensive propoxur. But resistance to these insecticides also appeared quickly in consequence of the application of malathion and carbaryl in agriculture. The following species are resistant to DDT and dieldrin: *A. sundaicus* and *A. aconitus* on Java, *A. stephensi* and *A. culicifacies* in India. In Kenya, fenitrothion has been applied as an alternative insecticide against resistant species. In Jordania *A. sergenti* has been controlled in breeding places with Abate and in 1974 already a 10-fold tolerance to this larvicide was noticed. Larvae of *A. maculipennis* in Romania and *A. sinensis* in South Korea and on Ryukyu islands have been controlled with malathion and fenthion.

Mosquitoes *Culex pipiens fatigans* are resistant to DDT, HCH and dieldrin. In California *Culex tarsalis* and also *Aëdes nigromaculis* are resistant to DDT, lindane, dieldrin, parathion, metholparathion, fenthion, chlorpyrifos and Abate. In some regions they ramained susceptible only to petrol destillate FLIT MLO, juvenile hormone methoprene and growth inhibitor Largon (TH 6040).

Lice, vectors of typhus, are resistant to DDT and lindane. Furthermore, in Egypt, resistance also appeared to malathion and carbaryl. *Xenopsylla cheopis* and *X. astia* are resistant to DDT, HCH and dieldrin.

The vector of Chagas' disease *Rhodnius prolixus* is resistant to DDT. Some tick species in Australia are resistant to all compounds applied against them.

Apart from resistance to chlorinated hydrocarbons the housefly is also resistant to dichlorvos, trichlorfon and dimethoate. Practically speaking, in Europe and the USA the houseflies are resistant to insecticides of all 3 groups of compounds.

The bed-bug *Cimex lectularius* is resistant to organophosphorus compounds: to malathion in Israel and to trichlorfon in the USSR.

The cockroach *Blattella germanica* L. in the USA is resistant to malathion and at the same time, shows cross resistance to propoxur. Moreover resistance has been discovered in some species to so-called insecticides of the third generation, such as chemosterilants (*M. domestica, Aë. aegypti*), hormonomimetic compounds (*C. tarsalis, M. domestica*) and even biological agents (*C. tarsalis* and *M. domestica* to *Bacillus thuringiensis*).

In Poland the workers in the Department of Sanitary Toxiology of the State Institute of Hygiene have been engaged in research on the resistance of insects [8, 19]. According to Styczyńska [56] the populations of housefly resistant to DDT already appeared in 1952, i.e. after 3 years of application. Afterwards as the result of introducing lindane preparations in hygiene and in agriculture, resistance to this compound has also been pronounced, achieving the level of 85 and in Warsaw of 100 in 1967. In consequence of discontinuance of lindane application in 1972, the resistance index in houseflies in the Warsaw territory decreased to 2. The cockroach *B. germanica* also showed high resistance to DDT. In 1973 it was necessary to increase the dose of lindane to control this insect 13 times.

In many countries, the level of susceptibility of a number of pests is still being checked but the amount of publications on this subject, which has no longer the character of scientific news, is decreasing considerably. As support for this statement may be the fact of submitting to our symposium only one communication concerning investigations on resistance. According to Sitar [53] in one region in Yugoslavia where malaria vector control is directed mainly against *Anopheles maculipennis messae* this species, although not yet showing a marked resistance level, is growing more and more tolerant to DDT. It is still susceptible to dieldrin and malathion.

Even this superficial review indicates that the problem of arthropod resistance is severe and the process will continue and increase. The WHO Expert Committee on Insecticides considered the possibility of control of resistant populations by:

1. use of alternative insecticides,
2. environmental control,
3. genetic control,
4. application of biological agents.

The best results, however, may be achieved by the application, depending upon the situation, of combinations of these methods [12].

Chemical control

Since the introduction of DDT chemical control became almost an exclusive method of control because of the availability of effective and low-cost pesticides and the simplicity of their application techniques.

The conventional insecticides were classified into 3 groups.

The first group includes chlorinated hydrocarbons including DDT, HCH, dieldrin, chlordan, heptachlor, methoxychlor, endosulfan. The first 3 compounds have been most widely used, however they are persistent and as such they can accumulate in the environment and also become concentrated in food chains. Their residues ensured continous selection pressure leading to a faster formation of resistant populations and to maintenance of some level of resistance long after cessation of chemical control. Today in many countries DDT has been totally withdrawn from application or its use is considerably limited. Even earlier restrictions were undertaken towards the application of cyclodiene insecticides, especially in agriculture. The use of HCH is also to some extent restricted. Recently, in Italy, its use in veterinary hygiene for control of ectoparasites such as mites and ticks has been forbildden [1]. This group of compounds is now represented by such insecticides as the low toxic and quickly decomposing methoxychlor and endosulfan which shows selective action towards some beneficial insect species.

The second group form the organophosphorus insecticides, readily biodegradable and causing only limited environmental contamination. They are also selecting factors leading to the formation of resistant populations. Among them malathion and fenitrothion are used as substitutes for DDT and dieldrin and as larvicides: fenthion, chlorpyrifos and Abate [12]. In Poland mainly trichlorfon and dichlorvos have been used.

The third group includes carbamate compounds also readily degradable. Propoxur has been used in residual house spraying against resistant populations of *Anopheles* sp. and also triatomine bugs while carbaryl against lice and fleas. These insecticides, and especially propoxur, also cause resistance e.g. in *A. albimanus*. In this country propoxur is recommended for cockroach control.

As a result of this state there is an urgent need for the development of new compounds, which should not accumulate in the environment, be non-toxic to mammals and be highly selective. The cost of new compounds also should be relatively low not greatly exceeding the prices of malathion or carbaryl. It is clear that with selectivity of action must be connected a greater choice of insecticides and their application in lower amounts causes higher cost of production.

In 1960 WHO established a programme for the evaluation and testing of new insecticides for public health purposes [65]. In 1960-1974 about 50 producers from 9 countries had supplied approximately 2000 candidate chemicals that were examined in 5 universities and institutes in 3 countries (USA, Australia, Israel), in 7 research laboratories (USA, Great Britain, Upper Volta) and in 6 field research units. The screening programme had 7 stages and the compounds are given numbers with the prefix OMS. From the number of compounds submitted 30 were selected as useful for vector control. Recently the number of candidate insecticides has declined from about 200 a year in 1963 to an average of 44 compounds for 1972-1974 [12, 65].

Basing on the chemical structure of DDT Holan used its steric model for rational design of some insecticides incorporating the toxophore and synergophore groupings and synthesized several new insecticides having the desired properties [28].

English research workers have chosen another way, and been successful in the development of some pyrethroids showing high effectiveness of biological action, low oral toxicity for mammals, readily degradable in the environment. They form the fourth group of insecticides including such compounds as resmethrin, bioresmethrin [14] and permethrin (NRDC-143) which is analogous with bioresmethrin but showing higher activity and considerably greater persistence [15].

Investigations made by Novak [44] have shown that using bioresmethrin in fumigation process the females of *C. pipiens* were eradicated within 2 hours. In a tightly closed room effectiveness of fumigation lasted for one work. Sztankay-Gulyas and Balajti [60] inform in their communication of a new synthetic pyrethroid elaborated in Hungary. It has a quick initial and longlasting action.

In Poland the search for new biologically active substances was successful in the development of 2 organophosphorus insecticides named bromfenvinphos and methylbromfenvinphos [2, 26]. They are predestinated mainly for Colorado beetle control, but their high activity and fast decomposition in organisms permit their use also against pests of sanitary importance. Thereby the high selectivity of methylbromfenvinphos should be underlined. An advantage of organophosphorus compounds is the price, which is much lower than that of the synthetic pyrethroids (1 kg costs $ USA 23-27).

Finally, the insect growth regulators belong to the fifth group.

The process of metamorphosis in insects is regulated by 3 hormones. The first of them is the activating hormone also called adenotropic which is produced by neurosecretory cells in the brain. It activates the prothoracic glands, thus stimulating the secretion of the moulting hormone, the second hormone of metamorphosis. Finally, the 3rd hormone is the juvenile hormone produced by the corpora allata. The moulting hormone influences the secretion of moulting fluid. The excess of this hormone, being a steroid called beta-ecdysone, acts toxically and causes a number of developmental anomalies including effects similar to the action of the juvenile hormone. Exogenic ecdysones inhibit the formation of the epicuticular wax layer in larvae [52]. The juvenile hormone appears in the hemolymph of imago and in eggs. The maximal concentration of it was observed in larval stages which require its steady presence. The metamorphosis of larva into pupa takes place at decreased concentration of the juvenile hormone, and pupa into imago in its complete absence.

The natural hormones are epoxides of homosesquiterpens esters. In nature a number of compounds occur showing an action similar to that of the juvenile hormone. Many compounds have been synthesized as differently substituted analogues of the identified hormone of *Hyalophora cecropia*, monocyclic and aromatic sesquiterpenoids, derivatives of farnesene acid and many others. These compounds are called hormonomimetics or growth regulators of insects. When applied in excess concentration or in abnormal time period of the insect developmental programme they cause various effects but always lethal. These may be: lack of reproduction, abnormal moulting, creation of intermediate forms between larva and pupa, between pupa and imago or between larva and imago.

They are also insecticides but with different characteristics and mode of action. They are not poisons but they interfere with the normal physiological processes connected with the development and metamorphosis. They have the following advantages:

1. they are more specific,
2. they can be applied using usually available equipment,
3. they are biodegradable and so far no negative influence on other organisms has been established.

However, the feature restricting their use is that the time of application must be strictly defined and is usually fairly short. Each developmental stage of an insect has its own susceptibility period: during embryogenesis — that is the period of blastokinesis [48]. The effects of action may often be observed with delay, e.g. larva can undergo normal metamorphosis into pupa and then the imago fails to hatch. But populations resistant to these compounds can appear [52].

Work on the synthesis of these compounds is being carried out in the USA, Czechoslovakia, Japan, Poland and several other countries. In 1970 approximately 30 compounds were known which were active in nano- and picogram amounts, exceeding the activity of natural *Cecropia* hormone. The group of insect growth regulators includes such preparations as methoprene, OMS-1804 and 'Altosid, a product of Zoecon Corp. registered for use in California. This preparation is used against flies attacking cattle. Its active substance is 11-methoxy-3,7,11-trimethyldodeco-2,4-diene acid showing an acute oral toxicity for rats of 50,000 mg/kg expressed as LD_{50}. Many compounds against mosquitoes, and also against *Monomorium pharaonis* L. [13], *Hyalomma dromedarii* Koch. [62] and others, are under investigation.

In Polish literature two publications appeared on the subject of the prospects of insect hormone application to control of pest species. These were papers by Styczyńska [55] and Boczek and Gwiazda [7]. Furthermore, Styczyńska [57] investigated the influence of Altosid on susceptible and resistant strains of the housefly ascertaining its good effectiveness in both strains. In other research Styczyńska et al. [58] used the derivatives of 2,3-methylene-farnesol and 2,3-methylene-farnesene acid synthesized in Poland. These compounds showed high activity against pupae of houseflies, but lower activity against housefly larvae and *Pyrrhocorus apterus*. They were not active against *B. germanica*. The insect growth regulators were also investigated by Stępień in the Agricultural Academy in Warsaw.

The only communication submitted to the symposium is that by Styczyńska [59] presenting the activity of 6 other esters of 2,3-methylene farnesene acid towards larvae and pupae of the housefly. The compounds examined were active and the range of the effective doses (ED_{50}) of the 2 most active compounds were: for larvae: 1×10^{-9} — 1×10^{-10} g, for pupae: 1×10^{-10} — 1×10^{-11} g. It is to be regreted that no information came from the Czechoslovak Academy of Sciences in Prague, the best centre in insect hormones working in this field already for several years.

The growth regulators are very expensive, their price is 10-100 times higher than that of conventional insecticides. This disadvantage is recompensed by their much higher activity. They require, however, continuation of research particularly in the field of application techniques and also their fate in the environment.

Other chemical substances

Particularly useful against resistant populations, are insecticide synergists [25]. Synergists are compounds non toxic per se but increasing the effectiveness of insecticides mainly by inhibition of the enzymatic complex detoxifying the xenobiotics. They act in those organisms in which the resistance mechanism depends on faster detoxication of poison by microsomal oxidases of mixed functions. Among the known synergists of this type one should mention piperonyl butoxide and sulfoxide, sesamex and other compounds with synergophore methylenedioxyphenyl groupings.

The detoxication of DDT follows another path i. e. through dehydrochlorination catalized by the dehydrochlorinase enzyme. Its non-active structural analogues, which may play the role of competitive substrates, serve as synergists for DDT. In the USA, a synergist WARF-Antiresistant in a preparation called "Pramex" has been introduced against strains of housefly resistant to DDT. But as has been shown in investigations [17] a strain of houseflies resistant to DDT and diazinon also became resistant to this synergist as early as in the fourth generation.

In Poland some synergists were developed in the Technical University in Szczecin, and their effectiveness was evaluated by Gwiazda [24] and Święch [61]. Several new compounds have shown synergistic action particularly with propoxur.

Another group of compounds which may be efficiently used to combat harmful arthropods are attractants. These are substances which after reaching the olfactory or other insect receptors cause an approaching reflex [20]. Attractants are also substances secreted by insects into the surroundings and acting on the individuals of the same species that responds to them with characteristic behaviour. This group of attractants is called pheromones. An object of particular interest became sex pheromones which show very high attractiveness because of the possibility of their selective use in the destruction of pest populations, in traps containing attractant with a chemosterilant, hormonomimetic compound or insecticide. In the last few years substantial information has been noted concerning new pheromones, especially in agricultural and stored products pests. Among the arthropods of sanitary importance the secretion of sex pheronomes has been ascertained by females: *Acarina* — *Amblyomma emaricanum* L., *A. maculatum* Koch., *Dermacentor variabilis* Say.; *Orthoptera* — *Blatta orientalis* L., *Blattella germanica* L., *Leucophea maderae* F., *Periplaneta americana* L., *P. brumea, P. australasiae; Diptera* — *Drosophila melanogaster* Meig., *Lucilia cuprina* Wied. In *Glossina morsitans orientalis* Vanderplanck pheromone was not detected [29]. The sex pheromone of the housefly has been identified as (Z)-9-tricozene present in the lipids of cuticle in females [30]. Its common name is "muscalure".

In some species sex pheromones are secreted by males. This fact was ascertained in *Amblyomma maculatum* Koch., *Cochliomyia hominivorax* Coquerel, *Drosophila melanogaster* Meig. and *Musca autumnalis* DeGeer.

A sex pheromone has also been found in *Monomorium pharaonis* [13].

Sex pheromones are mainly derivatives of hydrocarbons with long and little branched chains [41]. They are often a mixture of volatile compounds produced by several glands: anal, tergal and sternal [15]. The intensity of secretion depends on the age of the insect and has a day and night's cyclic character.

For practical purposes not only sex pheromones are applied, but also other pheromones such as tracer, aggregation or suppression pheromones may be used, as suggested by Berndt [3] basing on his own investigations on the physiology of *M. pharaonis.* Tracer and aggregation pheromones were identified in tse-tse flies, ticks and several mosquito species. Except pheromones also in use are feeding attractants and oviposition attractants (e. g. the house fly lays eggs readily in a medium emitting ammonia).

At present we have not much information on pheromones of vectors, little is also known about the attracting factors from the host side. Warmth, carbon dioxide and lactic acid stimulate the mosquito attack. The host substances attracting species of genera *Simulium, Phlebotomus, Glossina,* fleas, hematofagic bugs, mites and ticks are unknown. *Hippelates* can be attracted by the fermenting white of the hen egg.

The pheromones of plant pests have been the object of much greater interest. Natural and synthetic pheromones have been evaluated in field conditions, but unfortunately the results were not always positive. Some lack of success was caused by the ignorance of the biology of insects, their mating behaviour, their habits of mating such as height above the ground or the surface of growing plants where mating takes place, longevity, population density etc. Knowledge of the volatility and the extent of action of the substance investigated are also important.

In Poland reviews by Boczek [5], Boczek and Dąbrowski [6] and Lipa [40] have been published. Attractants are being investigated by: Dąbrowski at the Academy of Agriculture in Warsaw, Nawrot at the Institute of Plant Protection in Poznań, Kania at the Academy of Agriculture in Wrocław and Lupa at the Station of Quarantine and Plant Protection in Nowy Sącz.

The advantages of attractant application are the following:

1. high selectivity of action,
2. safe use without causing environmental contamination,
3. possibility of combating resistant populations,
4. low cost of application.

The opposite action is shown by the repellents, substances keeping insects away. They are applied for protection of man and domestic animals. As regards the direct contact of repellents with the skin, there are special requirements for their low toxicity, lack of irritating and alergen properties and also for long duration of protection and high effectiveness. These compounds [51] show high vapour pressure: esters (dimethyl phthalate against mosquitoes, n-butyl ester of succinic acid — Tabatrex and n-butyl ester of adipic acid repelling ticks *Dermacentor variabilis* and *Amblyomma americanum,* Indalon — n-butyl ester of 6,6-dimethyl-5,5-dihydro-4-pirono-2-carboxyl acid against mosquitoes, also dimethylcarbate, MGK 326 and others), alcohols (Rutgers 612 for protection of cattle against mosquitoes and flies), amides (m-Delphan-N, N-diethylamide of m-toluyl acid with a broad spectrum of action e. g. against *Ixodes persulcatus, Aëdes,* also *M. pharaonis* [43], Komarep — diethyl amide of benzoic acid against mosquitoes, Merck 790 — N, N-diethyl amide of n-caprylic acid against mosquitoes, flies and cockroaches, Heksamid B (USSR) — N-hexamethylenebenzamide repelling several species of insects and ticks, n-alkanosulphonamides with N, N-substituents *Aëdes aegypti* and *B. germanica* [42] and others).

The fact that the repellents are substances active only for a short period of time and that all exposed areas of the body must be treated, is highly unfavourable.

In Poland two repellents were developed: Komarep for protection of man and Tabawon for cattle. In Czechoslovakia investigations on repellents were conducted by Novak who together with Paulovova submitted to the symposium the paper on properties of perfumes as repellents against mosquitoes [45]. They stated that 5 oil substances used in the production of perfumes have distinct repellent properties against mosquitoes.

Genetic control

Genetic control method is any kind of action causing changes in genetic material of insects leading to decrease of reproductory potential and, as an end effect, to the reduction of a population. In natural populations there are some genetic mechanism which cause high mortality or restricted fertility of progeny. This includes cytoplasmic incompatibility [64]. It has been ascertained that crosses between populations of mosquitoes within the same species but from distant geographic areas give no offspring at all. The sterility is due to a cytoplasmic factor transmitted through the egg which kills the sperm before karyogamy. In *Culex pipiens* species complex at least 15 incompatible types have been isolated all over the world and are available for mass rearing and release in filariasis areas. The incompatibility factor was also shown in complex *Aëdes scutellaris, Aë. aegypti, Drosophila, Mormoniella* and in one species of *Chironomidae* (Clunio).

Another possibility is to produce sterile hybrids. Crosses between appropriate genetic types in *Anopheles gambiae* result in an F_1 with fertile females but sterile males with atrophied testes, but showing sexual activity normal or even enhanced by heterosis. The crosses between *Glossina swynnertoni* and *G. morsitans* yield few offspring and almost all of them sterile.

In natural populations there are many kinds of deleterious genes such as lethal genes, factors reducing viability and others which can be increased by inbreeding and introduced into natural populations. The pest populations can also be replaced by innocuous populations competing for the same ecological niches. Finally, through irradiation or chemosterilant application the induction of dominant lethal mutations in gametes can be achieved.

The most effective method is the sterile male method. The successful experiment of controlling screw-worm fly *Cochliomyia hominivorax* Coquerel in Curaçao island by the release of males previously sterilized by gamma irradiation was encouraging to set this method on a broader scale. At present a mass rearing factory was set up in Texas. The pupae are irradiated by ^{60}Co and then released from planes over the infested area. This treated area is now about 1500 miles long and 300 miles wide in such states as north Mexico, Texas, Arizona, New Mexico and California [27]. Using this technique 99% of effectiveness can be achieved, whereas after treatment with conventional insecticides the effectiveness is only 90%. This method gives good results only when during male sterilization their mating competitiveness is not seriously affected and when the number of released sterile males is higher than the number of males in natural population.

Sterile males can be produced in an easier way by using chemical compounds called chemosterilants. These compounds are divided into 3 groups [37]:

1. alkylating agents — analogues of bis-(2-chloroethyl)-amines and ethylene imine analogues such as tepa, metepa, thiotepa, tretamine, apholate,
2. antimetabolites — antagonists of folic acid, glutamate, purine, pyrimidine,
3. other compounds, such as hempa, hemel and o.

Chemosterilants given sexual sterility in a broad sense as inability to produce mature progeny [23]. The laboratory investigations have shown that a lot of mosquito species are susceptible to chemosterilization and that this technique has less affect on their vigor and mating activity. This method seems to be particularly useful against mosquitoes of *Culex* and *Anopheles* species. Chemosterilized males *Culex pipiens fatigans* have been released on a small island near Florida and in consequence the natural population was eliminated within 5-6 generations. In El Salvador excellent results have been obtained against an isolated population of *Anopheles albimanus* [47]. Studies have begun on the possibilities of genetic control of *A. gambiae, Culex tritaeniorhynchus, M. domestica* and also *Rhodnius* sp. and *Ornithodoros* sp.

In Poland chemosterilization was also investigated. The Technical University in Szczecin developed some new compounds of which one showed activity similar to that of apholate [16]. The activity of chemosterilants against houseflies was investigated by: Byrdy et al. [10], Gwiazda [21] and Krzemińska [33, 34, 35]. Krzemińska has submitted to this symposium a communication [36] concerning the influence of organotin fungicides on the reproductive ability of houseflies and German cockroach. These compounds sterilized females of both species, but had little effect on males.

From the experiments to date, on the introduction of genetic methods into practice, it may be concluded that these techniques are more useful in small areas. They must be checked in field conditions to be able to establish their usefulness. Prior to the commencement of experiments, it should be ascertained if the target species can be mass reared and if rearing conditions do not reduce its activity and sexual behaviour. Populations destinated for reduction should be geographically or ecologically isolated or should occur naturally in small numbers at some period during the year. If the target population is too large, it may be reduced by other control methods. The WHO Expert Committee recommended intensification of research in collaboration with FAO and International Atomic Energy Agency particularly with tse-tse flies and mosquitoes [12].

Biological control

This means the active utilization of living organisms for suppression of other, harmful organisms. This method is not new and is used to-day more and more for plant protection. In the field of sanitary entomology it has played, up to now, only a small role. A complete description of principles on the proper use of biological agents regulating the number of pests in natural populations may be found in books by Sandner [50] and Lipa [39]. Because of the lack of space, this survey must be limited to a shortened list of organisms that may be used against sanitary pests.

Viruses [50]

The major part of viruses isolated from insects are pathogens of plant pests, but several viruses of nuclear polyhedrosis and of cytoplasmic polyhedrosis have been isolated from mosquitoes. Now research has been undertaken on viruses against *Aëdes* and *Simulium.* Virologists assume that advantageous viruses for biological control may be found among nuclear polyhedrosis and granulosis viruses. Prior to the introduction of a new virus into an ecosystem its physical, chemical and biological features should be carefully established and particularly its variability, the methods of identification and also its influence on non-target organisms.

Rickettsia [50]

These are pathogens of insects particularly of the orders *Coleoptera, Lepidoptera, Diptera* and *Orthoptera.* Until now they have not been aplied in biological control because in some experiments with mammals some species produced pathogenic effects.

Bacteria [9, 50]

The number of species of entomopathogenic bacteria used in biological control is very restricted but thanks to the *Bacillus thuringiensis* group they play an important role in practice. The first technique of biological control was elaborated before the II World War to control the beetle *Popillia japonica* Newm. using 2 species of bacteria: *Bacillus popilliae* Dutky and *B. lentimorbus* Dutky which caused so-called milk disease in larvae. This control is still continued and suitable preparations are made from artificially infected larvae. For control of several harmful lepidopteran pests biopreparations containing *B. thuringiensis* on industrial scale are being produced. This species forms some dozens of strains belonging to 2 species: *B. thuringiensis* Berliner and *B. cereus* Fr. et Fr.

The virulescence of *B. thuringiensis* towards insects is connected with the morphology of the crystal endotoxin produced inside the bacteria cell. Strains producing crystals oval-shaped with non-sharp edges are more toxic for insects than strains producing irregular romboid shapes. *B. thuringiensis* have a thick cell membrane and, therefore, they can also exist outside the host organism. When the spores and crystals enter into the alimentary canal of insect, the alkaline medium in the gut causes the crystals to dissolve liberating the toxin which paralyse the gut muscles. *B. thuringiensis* does not seem to be harmful for man and plants [47]. It is also non-toxic for honeybee and other *Hymenoptera.*

Besides endotoxins some varieties produce thermostable **exotoxin** (e. g. *B. thuringiensis* Serotype 1). *B. thuringiensis var. alesti* **Serotype** 3 does not produce the exotoxin. It has been established that the exotoxin gives teratogenic effects in insects and is mutagenic for the Colorado beetle.

The preparations containing *B. thuringiensis* are also effective against *Haematobia irritans, Aë. aegypti* and *Aë. stimulans.* In the USA has been isolated from larvae *C. tarsalis* a virulent strain of *B. thuringiensis* (strain BA-068) that can also infect other species of mosquitoes [12].

B. thuringiensis also infects the cockroach *B. germanica* with lethal effects, as has been shown by Ulewicz and Nowicka [63] in their communication to this symposium. These authors were working on the causes of death of insects at the histological level. It was already known that the changes are localized in the middle gut of insects. The authors ascertained the degeneration of epithelium cells, the separation of the epithelium of the muscle layer, the muscle layer becomes loose and separated from the basic layer. All these results contribute to the knowledge of mechanisms of action of biopreparations containing *B. thuringiensis.*

Parasitic fungi

The possibilities of parasitizing mosquitoes by the following fungi: *Coelomomyces* sp., *Lagenidium gigantheum, L. culicidum* and *Metarrhizium anisopliae* are being investigated. The greatest number of pathogenic species belong to the genus *Coelomomyces.* These fungi kill the mosquito larvae by injuring the adipose tissue and the tissues essential for adult stage formation. Inside the body of insect they produce sporangia resistant to dessication and temperature and these forms are suitable to store and disseminate. This fungus once introduced into a habitat may remain there for many years. Most *Coelomomyces* species infect only mosquitoes but nothing is known about the effects on nontarget organisms. *Coelomomyces* and *Lagenidium* are aquatic organisms and do not produce mycotoxins. In Zambia larvae of *A. gambiae* are infected by *Coelomomyces indicus.* Experiments conducted with *L. culicidum* on the possibility of infection of organisms such as *Cyclops, Daphnia, Polychaeta, Chironomidae,* fish of the genus *Lebistes, Gambusia,* hen, quail and rat gave negative results.

Protozoa [12, 47]

The most promising pathogen of mosquitoes appears to be *Nosema stegomyiae* (*algerae*) (*Microsporidia*). *N. stegomyiae* reduced the population of *A. albimanus* by 70-80% causing mortality in all developmental stages of this insect. *Protozoa* of genus *Nosema* can be mass-cultured in living larvae. The spores were sprayed in larval habitats (e. g. 1000 spores per 1 ml water). They are host specific. Much research is needed on taxonomy and classification of *Microsporida.*

Nematodes [11]

Among many groups of nematodes pathogenic to insects mention should be made of 2 from which the species infesting sanitary insects are recruited:

(a) Genus *Neoaplectana* (*Steinernematidae*) and particularly *Neoaplectana carpocapsae,* strain DD-136, infesting many plant pests such as the codling moth larva, potato beetle larva, cabbage root maggot, European corn borer and others. Nematodes of this strain did not infest rabbits, fish and reptiles and the mammalian body temperature was lethal to them.

(b) *Mermithoidea.* There is a number of records of mermithid parasitism in *Culicidae, Simuliidae, Chironomidae.* The invasion invariably causes the death of the host: the second stage juveniles penetrate the cuticle to the body cavity where they grow and fill the hoemocoel. Some *Mermithidae* are specific, e. g. *Agamomermis culicis* for *Aëdes sollicitans, Paramermis canadensis* for *Aëdes vexans,* but usually they infest a wide range of hosts. *Reesimermis nielseni* is infective for mosquito species including many *Anophelinae.* This species can be mass-reared and therefore it is worth further research. Parasitic nematodes of *Simulium* are also known but because of failure to rear both the host and the parasite they are difficult to investigate.

In order to control mosquitoes of genus *Anopheles* the developmental stages of nematodes, such as eggs, pre- or post-parasital stages, obtained in mass-rearing, are scattered: from 250,000 mosquito larvae bred weekly, the pre-parasital stage of *Reesimermis nielseni* was obtained in a quantity sufficient for treating 12 ha with ca 1000 nematodes per 1 m^2 [47].

In Poland Skierska and Szadziewska [54] are investigating the infestation ability of larvae *Culex pipiens molestus* through nematodes *Neoaplectana carpocapsae* strain DD-136. Preliminary results, which are the object of a communication to this symposium, showed increased mortality of larvae due to the presence of nematodes.

Predacious insects [47]

Predacious mosquitoes from the genus *Toxorhynchites* live in the tropics. This genus contains about 6 species of large mosquitoes. Their larvae feed on larvae *Aë. aegypti, Aë. simpsoni and Aë. albopictus.* The best-known predator species is *Toxorhynchites brevipalpis.* The larva of this mosquito consume about 250 larvae *Aë. aegypti* and about 2 days before pupation it kills not consuming all other inhabitants of the container. Mass-rearing techniques have been developed, which yield 100,000 imagines per week, the life cycle duration being 20 days. Some species of *Hymenoptera* can control bugs of the genus *Triatoma* and probably also *Muscidae* and ticks.

Larvivorous fish [12, 47]

In certain habitats, fish of the genus *Gambusia* and *Poecilla* can be used for effective control of mosquitoes. *Gambusia affinis* was applied for mosquito control already before the I World War. Fish are excellent for combating the resistant mosquitoes. So far, the environmental effects of the introduction of these fish are unknown.

Larvicidal plants [47]

It has been observed that aquatic plants belonging to the family *Characeae* may kill mosquitoes thanks to exudation of larvicidal toxins. These algae are common in quiet waters with muddy or sandy bottoms where they grow from less than 2 cm to more than 2 m. The main genera are *Chara* and *Nitella*. *Chara* does not grow in highly polluted water and sometimes it may become a pest.

Also under examination is the application of carnivorous plants, e. g. *Utricularia vulgaris,* where — on a single plant bladder 220 cm long — 150,000 organisms were found. Throughout the world some 90 species of *Utricularia* were found growing.

The seeds of some plants of the family *Cruciferae,* when placed in water, exude a mucilage from the seedcoat and the mosquito larvae are trapped and perish from hunger. This has been established in the laboratory.

As has been shown, a lot of promising micro- and macroorganisms have been identified which are now mostly at the stage of laboratory investigations. The WHO Expert Committee recommended a preliminary scheme for screening and evaluating the efficacy and safety of biological agents for control of disease vectors, in 5 stages [12]. For biological control the following conditions must be fulfilled:

1. the agent must reduce the population being controlled,
2. it can ben neither toxic nor pathogenic for man and animals,
3. it must be cheap and readily available in large quantities.

Integrated programme of control

None of the above-mentioned methods of control should be used exclusively, particularly over a longer period of time. Each decision must be preceded by a very careful analysis of costs and profits, including also the negative influence to the environment. Always before the choice of a method is made, a thourough examination of the environment is necessary to ascertain if any improvement of the environment may contribute to the elimination of the sources for pathogens or pests. When such improvement is not possible an integrated programme of control should be applied with combined chemical, biological and other methods, according to the actual situation. The modern programme of control must be based on precise ecological studies and requires ecological supervision during and after application.

The three communications, sent from the USSR, can be taken as examples of ecologically treated chemical control. Konstantinov and Gorchakovskaya [31] have investigated the residues of DDT applied in the taiga to control *Ixodes persulcatus,* in the soil, the foliaceous bedding and in earthworms, grass and milk of cows, defining the level of environmental pollution quantitatively. For 20 years, Gorchakovskaya [18] has followed the effects of the application of DDT for tick control and for the beneficial organisms, drawing practical conclusions on the frequency and methods of control. Finally, Korotkova and Gorchakovskaya [32] conducted an analysis of changes in population densities of *I. persulcatus* after the application of DDT.

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AKTUALNE MOŻLIWOŚCI ZWALCZANIA STAWONOGÓW O ZNACZENIU SANITARNYM Z UWZGLĘDNIENIEM ASPEKTÓW EKOLOGICZNYCH

M. GWIAZDA

Na tle krótkiego przeglądu aktualnego stanu w dziedzinie oporności stawonogów o znaczeniu sanitarnym przedstawiono możliwości ich zwalczania. Omówiono środki chemiczne konwencjonalne, drogi poszukiwania nowych związków, jak i regulatory wzrostu owadów, synergetyki, atraktanty, repelenty, a także inne metody zwalczania, jak metody genetyczne, walkę biologiczną, wreszcie zintegrowany program zwalczania. W każdej grupie metod uwzględniono prace polskie oraz prace przedstawione na sympozjum.