Rendiconti
Accademia Nazionale delle Scienze detta dei XL.
Memorie di Scienze Fisiche e Naturali
98° (1979-80), Vol. IV, fasc. 3, pagg. 15-26.

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Natural products in the control of insect vectors in Chagas disease (**)

RIASSUNTO — I vettori della malattia di Chagas, Tripanosomiasi Americana, sono insetti che appartengono ai generi *Triatoma* e *Reduviidae*.

L'impiego di insetticidi convenzionali nella loro lotta o non è risultato efficace oppure ha provocato una serie di altri inconvenienti.

Sulla base delle conoscenze sulla fisiologia e il comportamento degli insetti risulta possibile ricorrere all'impiego di sostanze naturali.

Recenti esperienze mostrano la possibilità di un pratico impiego di sostanze naturali, quali ormoni di insetti, feromoni e sostanze da questi derivate, come pure di derivati sintetici sul modello delle piretrine.

SUMMARY. — The vectors of Chagas disease, the american trypanosomiasis, are insects belonging to the genera *Triatoma* and *Reduviidae*.

Their control has been proved difficult by means of conventional insecticides.

Consideration on insect physiology and behaviour indicate the possibility of the use of natural products.

Recent experiences have shown the effectiveness of the use of insect hormones and pheromones and of synthetic pyrethrin analogs.

INTRODUCTION

In the last century the research for the control of insects has been mainly focused on plants. This effort not only led to higher crop yields but also avoided their complete destruction. The use of chemicals has created several problems because of their ecological impact.

We must bear in mind that only few species of plants are cultivated in comparison to those present in nature and that this cultivation represents a

(*) Accademico dei XL.

(**) Conference held at the International Congress on Chagas disease, Rio de Janeiro 28-VII-1979.

ISSN-0370-3460

limited phase, far from the natural equilibria that protect plants in the primeval stage. The use of persistent substances with toxic effects such as chlorinated hydrocarbons, or highly toxic products like phosphoric ester has caused a great disturbance of ecological equilibria; and in some cases predators of noxious insects have disappeared, causing several difficulties for the protection of the crops, as in the case of the Cañete valley in Peru (Smith, 1968).

The persistance of these substances foreign to the natural environment causes also the contamination of the food chain all over the world, from the poles to the seas with the health effects that wa can not yet appreciate fully at the present time.

For this reason in the last years many efforts have been made in order to coordinate the use of chemical products with biological control in an integrated system. Beyond these classical control systems, recently some chemical products such as aziridines were proosed as chemosterilants of male insects (Scossiroli, 1968).

The treatment causes the sterilization of males and prevents fecundation thus reproduction, greatly reducing the number of insects of a species. Male sterilization was obtained also with ionizing radiations (Table 1). In this case males are treated and then released. The results are good for limited areas, mainly on small islands (male annihilation techniques).

The first insecticides used in insect control were pyrethrum, nicotine, rotenone, ryania root, all natural products which, together with lead arseniate and other inorganic products formed the first generation of pesticides.

TABLE 1 — *Insect control.*

Integrated	Chemical	Natural and synthetic products
	Biological	Predatory species. Viruses
	Physical	Ionizing radiations

A second generation is represented by chlorinated hydrocarbons, phosphoric esters, carbamates.

The toxicological and ecological implications of these products, mainly the high toxicity of phosphoric esters and the long term effects of chlorinated hydrocarbons, have put a limitation to their use in a number of countries.

The present policy is to ban the use of the most toxic because they are dangerous in a risk benefict evaluation.

Therefore in recent years research has been oriented towards the use of natural products.

Their activity is connected with non conventional mechanisms of action. Together with natural products we must consider in this group some synthetic derivatives prepared on the model of the natural products, the so called mimics. All these substances are now considered the third generation of pesticides. Now whereas in the protection of plants with insecticides there is a direct interaction of the plant with the chemical and with the host, if man or animal is involved a completely different approach must be considered.

In the case of plants there are a number of parameters such as the climate, the temperature and the nature of the plants, which must be taken into account. Moreover, must be considered the different possibilities of applying an insecticide: contact, ingestion and even systemic as well the different attack strategies which depends also on the presence of useful insects on the plants which act on the pollination, and those which maintain the biological equilibria (Table 2).

TABLE 2 — *Chemical Insect control*

Paralyzing Toxic	Active on enzyme systems
Chemosterilant	Active on reproduction
Hormonal	Interfere with insect horgonal cycles
Behaviooral	Sex attractants Trail substances Pheromones

I will not here consider this point, i.e. the control of insect on plants. This argument has formed the object of a number of research studies: among then I wish to recall the discussions of the Study week promoted by the Pontificial Academy of Sciences in 1977 for the replacement of the second generation pesticides with non harmful natural products (Marini-Bettòlo, 1977).

Natural products in the control of insects for the protection of man and animals

The second point: i.e. the control of injurious insects which are vectors of diseases, poses a number of new problems because the object of the protection is man or animals: which requires a completely different approach.

The scope of the present meeting is the control with insecticides of a group of *Arthropods*, belonging to the family of *Hemiptera* and in particular to the genera *Triatoma* and *Reduvidae*, which are the vectors of the Chagas disease, that is, of the american Trypanosomiasis due to *T. cruzi*.

To this purpose we shall take as a model what has been done in the control of other insects dangerous to man such as the mosquitos (Anopheles, Aedes, etc.) as well other insects which affect man and animals such as flies, fleas and bugs (Hoppe, 1974; Haugarter, 1973). We shall here recall the fact that Carlos Chagas identified the cycle, in the various stages, of the parasite and made clear the role of the insect (Triatoma on Reduvidae) better known in Brazil as *barbeiro*, and *vinchuca* in Argentina, proposed, in order to break this cycle, to control the insect by means of insecticides on the walls of the ranchos and when possible to rebuild the ranchos avoiding the use of straw and mud (*adobe*) as occurred mainly in the interior of Brazil and of many other Latin American countries.

I think it should here be pointed out that the strategy of the treatment of the house with insecticides had been proposed for the first time by Chagas for the control of the mosquitos vector of malaria. The insecticide used at that time was a natural product, pyrethrum (Chagas, 1979).

At present the spraying of the houses is performed with second generation insecticides, mainly chlorinated hydrocarbon (Torrealba, 1979). Also phosphoric esters are largely used notwithstanding their high toxicity. Pyrethrum is still used for treatments in the houses just because of its low toxicity on vertebrates. Let us examine if there are for the future new possibilities and new means for a new strategy of vector insect control.

Insect physiology

In order to better understand the mechanism of action and thus the principles on which the control of insects can be based it is important to recall some data about the physiology and the behavior of insects.

It is well known that insects have a nervous system very rich in cholinergic receptors, where the acetylcholine binds and where the enzyme cholinesterase acts to transform the latter to choline. A substance which inhibits the cholinesterase, like most of the phosphoric esters, causes rapidly the death of the insects because they are no longer able to hydrolize acetylcholine, which is highly toxic. This means that the mechanism is a chemical on a biomedical process of the nervous transmission in the insect and thus quite general.

Naturally, it is necessary to find particular systems to apply these substances

to the insect and avoid the poisoning for man, who is also sensitive to these substances possessing the same type of chemical system of neuromuscular transmission.

The mechanism of action of phosphoric esters is their capacity to phosphorylate the esterase and in particular cholinesterase.

The blocking of this system avoids, as above indicated, the hydrolysis of acetylcholine and thus causes the death of the insect.

The chlorinated hydrocarbons, and in particular DDT, do not have a clear mechanism which can explain their action.

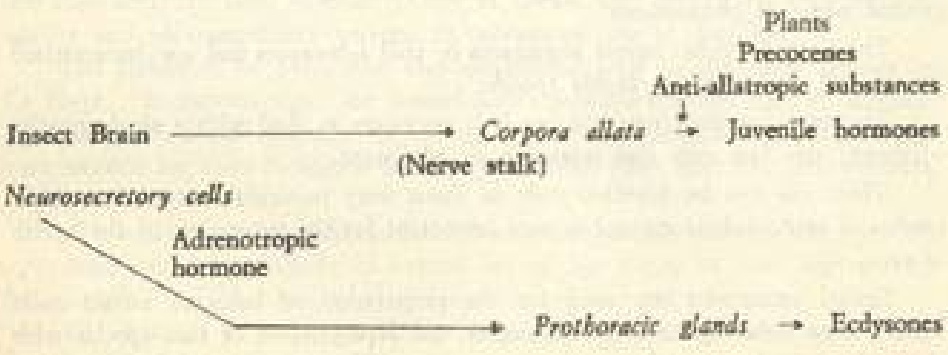
Being lypophilic substances, they may act at membrane level and on nervous tissues, thus blocking vital processes. We must here consider that these products like DDT are ineffective and have developed resistance (or are considered rather toxic for man as in Gama-hexane, and particularly Dieldrin) on the insects involved in the Chagas disease, according to the interesting work done in Venezuela (Torrealba, 1979). Also carbamates block esterase activity.

The resistance to chlorinated hydrocarbons genetically acquired by various species of insects poses a great number of problems in insect control and has lead to the use of increasing quantities of these products, with the resulting deterioration of the environment.

A particular aspect of insect physiology is the development from the larva into the pupal and later the adult stage.

In this mechanism the *Corpora allata* which are involved with the growth of the insect play an important role (Wigglesworth, 1936). In effect it was demonstrated that the *Corpora allata* may induce the development if stimulated by the brain, producing the Juvenile hormones (Bagley, 1972). These hormones may give rise to a number of modifications and alterations of the development in the insects, which in these conditions undergo an undue metamorphosis, which they do not survive (Table 3).

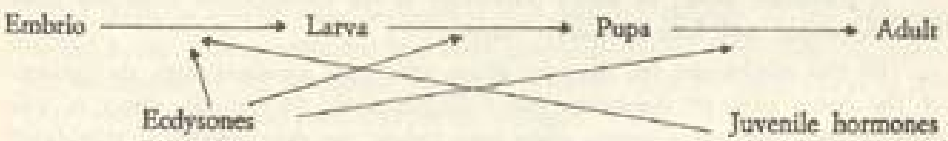
TABLE 3 — *Insect-hormones relationships.*



In recent years an anti-allatotropic factor, i.e., a substance which inhibits the action of *Corpora allata* in regulating the development, was found in a plant, *Argemone mexicana*, (Compositae). This natural product is a simple chromane which induces the capacity to enhance the rate of development of the insects (Bowers, 1977). Therefore, the name precocene was given to these substances by Bowers, who discovered this effect; but although it causes a precocious development, the resulting insects are dwarfs and not viable.

If the physiology of *Corpora allata* is fundamental for the knowledge of the mechanisms of development, and thus for the possibility to interfere with them for the purpose of controlling the insect, no less important is all the physiology of the moult. Ecdysones produced by the prothoracic gland under the stimulus of an adenotropic hormone from the brain are steroidal derivatives, regulate the moult in insects as well as in plants. An excess of ecdysone given to the insect not during the period of the moult induces an abnormal moult and thus the death of the insect (Karlson, 1977) (Table 4).

TABLE 4 — *Insect mutations and development. Influence of ecdysones and JH.*



These physiological mechanisms are common to a great number of families, groups and genera of anthropods and thus it is easily understandable how the use of both JH and MH is *quite general* for all insects.

Another approach is represented by the behaviour of the insects and mainly their relationships with insects of the same species or different species. The behaviour is generally regulated by natural products which are called by the general name of pheromones.

These can be either sexual attractants or trail substances and are characterized by the fact that they are *highly specific*.

Therefore for their practical use it is necessary to deal with a single species of insect; this fact may also represent an advantage.

Their use can be justified only in some very particular cases of a single species of insect whose control is very important for the protection of the health of man.

Sexual attractants are used for the preparation of baits to attract male insects, thus reducing, to a certain degree, the reproduction of that species with the male annihilation technique.

These are in general aliphatic derivatives: the quantity necessary for the attracting the males is very low (10 molecules per cm^3).

Another group of extreme importance is that of the trail substances because of their possible utilization in the control of vector insects as well as their alarm and defense substances. The latter may be used as repellents.

A research made by Pavan at the University of Pavia has shown that in seven hundred species of anthropods studied there are four hundred different natural products which act as repellents.

Among these we might mention some terpenes such as citral and citronolellol, present not only in plants — used for centuries as insect repellents — but also in ants, mainly in some Brazilian species of *Atta* (Gilbert, 1975), which show a strong activity in various species. Other substances, like iridomyrmecin and pederin, are produced by several insects and show high toxicity against other species. Along these lines the control of insect vectors of disease in man and animals is at present seeking a new approach through the use of natural products — or of synthetic substances based on the structure of natural ones — which do not interfere with the physiologycal processes of the insects.

Natural products in the control of insect vectors of disease of man and animals

Pyrethrum was the first natural product used in the control of insects for the protection of man.

Pyrethrum has high capacity to paralize insects and thus act on the central nervous system: according to the experiments carried out measuring the action potential of the gigantic axon of *Blatta*, its mechanism could be the blocking of the exchange at the nervous membrane level of Na^+ and K^+.

Its first use was reported in 1880, about a century ago. Since then its use has increased steadily and thus large areas are now cultivated with this plant outside of its original country, Dalmatia, and mainly in Ecuador and Kenya, which are at present the greatest producers and exporters. Its low toxicity for man and animals together with its high knockdown activity on arthropods, made this insecticide the most suitable for use in homes, also because its high chemical lability and photosensibility prevent its economical use in the field.

The chemistry of pyrethrum was established only in the early thirties by La Forge. In flowers there are several active principles which have in common an unusual chemical structure of cyclopropane and cyclopentane acids; which may account for their biological activity and at the same time for their chemical lability.

Also other natural products isolated from plants, mainly nicotine and rotenone, have been used in agriculture to control insects, but owing to their high toxicity for man they were not employed in homes for the control of insects.

The findings in insect physiology have made possible a new approach to the control of insect vector of diseases.

The physiological properties of the Juvenile hormones and of their synthetic analogs have shown very promising results. Altoside SR-10, which is a preparation of methoprene, i.e. isopropyl 2-E-4-E,11, methoxy, 3,7,11, trimethyl, 2,4, dodecaotreoate a JH-mimic, shows a low toxicity for man and is very effective in blocking the mosquitos both in land and on the water by interfering with their development (Siddal, 1977).

This fact is rather interesting, especially if we consider that many traditional insecticides, like DDT, have developed resistance in many species, as was demonstrated for various vectors of Malaria like *Anopheles* or *Aedes aegipti*.

Methoprene also has shown good activity on insect parasites to man and animals such as *Pediculus* and *Bovicola limbata*. On the basis of the fundamental knowledge regarding the mechanism of JH, it is possible to foresee the use of these products or any other type of insect and also on *Triatomae* and *Reduvidae*.

The same applies to the moulting hormones. Research at the International Center for Insect Physiology and Ecology in Nairobi has shown that substances with MH activity of the ecdysone type, may be of great interest in controlling cattle ticks, which are a real plague in East Africa.

In the same Center research on the physiology of the allatotropic system of *Glossinia morsitans*, the tsetse fly, indicates that both JH and MH may be employed in the control of this vector of *Trypanosoma gambiensis* (Chaudury, 1977).

New strategies for the control of insect vectors of diseases

On these basis a new approach and a new strategy can be envisaged for the control in Latin America of the *Triatomae* and *Reduvidae* species which are responsible for the transmission of the Chagas disease. It is obvious that any strategy now should be based on a perfect knowledge of the physiology and the behaviour of the vector.

General physiological principles make it easier to foresee a new strategy, but it should also be borne in mind that each species may show some particularity, that its habits and environment are different, and that this may modify the effects of the substance used for their control.

So far the control of *Reduvidae* and *Triatomae* was performed by spraying the houses with the second generation insecticides.

We may recall that experience has shown that DDT is very little or not at all effective. In Brazil good results were obtained with BHC, or better with the isomer Gamahexane. Venezuela's experience with Dieldrin has been good in regard to the control of insects dwellings, but a number of drawbacks have appeared both in the high toxicity for man of the Dieldrin and in the ecological modifications induced in the population of the *Triatomae* which are present in the surroundings of houses (Torrealba, 1977).

The use of phosphonic esters is always a danger for man for their high esterase inhibiting powers. Now the general mechanism of development of

arthropods, is bound to the complex hormonic system in the insect: cerebral neurosecretory glands, *Corpora allata*, prothoracic glands producing JH and MH, which act on three stages of the moult and in the juvenile development.

The present results with some species of insects indicate the possibility of a strategy also for controlling the *barbeiro*.

Although the above-described hormonal system is common to all arthropods, and thus the inhibition by natural products involved in this cycle is quite general, in the case of *Triatominae* and *Reduviidae* it should be taken into account that there is a very long period between the various stages and phases of development and moulting in these insects.

Therefore the treatment with hormones should be applied over a long period and be present mainly at the moment when the insect undergoes a transformation.

In order to act in due time with JH, which generally, owing their chemical structure, are not persistent, two different approaches have been proposed. The first is the synthesis of new compounds with JH activity but with long-lasting activity; the other is the use of particular preparations containing the JH or the JH-mimics in condition to release slowly the active substances.

This line was followed with success by Gilbert, Pinchin, Oliveira Filho and Figuereido, (Gilbert et al, 1978) who have developed a specific system for the control of *Triatomae*, using a dispersion of JH and a phosphoric ester in rubber, in PVC and in paints.

This preparation was active for a whole year slowly releasing the active substances. Since the period of latency of *Panstrongylus megistus* is between 110 and 140 days, it is possible by this means to block its development at the exact moment when it is most sensible to the modification induced by the hormones (Gilbert and Silva Neto, 1979).

More recently, as communicated during this Congress, the same technique was applied by Gilbert and coworkers with great success to the pyrethrum synthetic analogs decamethrin and permethrin (Gilbert, 1979). Precocenes can also be used to modify the *Corpora allata* function. Precocenes can in effect reverse the action of JH, inducing a precocious development and also acting on the sexual maturity of the insects.

As above stated, precocenes were found first in plants: they are not toxic for man and may perhaps be used successfully against *Triatomae*. The results obtained and communicated at this Congress by Furtado and coworkers in Pernambuco are most interesting and stimulating and should be extended to field experiments.

There are also important results which represent only a first experiment: further research is necessary both in laboratory and in the contry to establish the extent and the balance of the benefits that can be achieved by this new approach.

We must also bear in mind that the control of the insect vector is most necessary to speed up the eradication of the Chagas disease. The insect represent in effect one of the most sensible points of the cycle.

We must avoid the use of dangerous means like Dieldrin and phosphoric esters, which might create further problems, especially if particular precaution in their use it not always possible.

Thus a new and certainly promising avenue is open to research and from the results of basic research important developments can be foreseen. Another aproach requires new research on other aspects of the physiology of *Triatominae*. This applies to the study of pheromones, sex attractans, and the behavioral substances of these species. A knowledge of the chemistry of these processes may open new possibilities to the control of *Reduviidae* by the use of repellants, and particularly of pheromones for bait, as well as trail substances as confusing agents that may induce the change of behaviour in the *barbeiro* in attacking man.

The success of these products in the protection of crops indicates that also positive results for the protection of man can be obtained. No effort should be omitted in order to protect the rural population of Brazil and the Americas against these dangerous vectors of the Chagas disease.

References

Barley R. W. and Bauernfeind (1972) - *Insect Juvenile Hormones Chemistry and action*. Academic Press, New York.

Borkovec A. (1969) - *Chemosterilant in pest control*, « Accademia Nazionale dei Lincei », 128, 249.

Bowers W. S. (1977) - *Anti-juvenile hormones from plants*, da *Prodotti naturali et la protection des plantes*, « Academia Pontificia Scientiarum », *41*, 129-142.

Gilbert B., Oliveira Filho A.M. and Pinchin R. (1979a) - *Decamethrin An insecticide for the effective control of Chagas disease vectors*, International Congress of Chagas Disease, Summary, Rio de Janeiro.

Gilbert B., Silva Netto J. A., Oliveira Filho A. M. and Pinchin R. (1979b) - *The use of slow release insecticides and hormones for long term control of Chagas disease vectors*, International Congress of Chagas disease, Summary, Rio de Janeiro.

Gilbert B. (1979c) - *Communication on the new insecticides used in the control of vectors in Chagas disease*, International Congress of Chagas disease, Rio de Janeiro.

Haungartner W. and Masner P. (1973) - *Juvenile Hormone: Inhibition of Ecdysis in Larvae of « Blatella germanica »*, « Experientia », *29*, 1352.

Hoppe Th., Isler H. and Vogel W. (1977) - *Biological Activity of Juvenile hormone analogues against the larvae of Culex pipiens*, « Mosquito News », *34*, 293.

Karlson P. (1977) - *Chemistry and physiology of insect hormones and insect pheromones*. In: Natural products and the protection of plants, « Academia Pontificia Scientiarum », *41*, 17-28.

Kawoya J., Kunyiha R. W. and Chaudhury F. B. (1977) - *Studies on brain neurosecretory cell, CA and CC of tse tse fly*, « 5th ICIPE Annual Report », 52.

Lent H. and Wygodzinsky P. (1979) - *Revision of Triatominae (Hemiptera, Reduviidae) and their significance as vectors of Chagas disease*, « Bull. Am. Museum of Nat. Hist », *163*, 127.

Mango C. K. A. and Moreka L. (1977) - *Moulting hormones activity in the nymphal instars of the tick « Ornithodorus moubata »*, « V. ICIPE Annual Report ».

MARINI-BETTÒLO G.B. (1977) - *Natural products and the protection of plants*, « Academia Pontificia Scientiarum », Scripta Varia *41*, 5-14, 777-800.

MEINWALD J., PEREYWICH G.D., NAKANISHI K. and KUBO I. (1978) - *Chemical Ecology Studies from East Africa*, « Science », *199*, 1167.

PINCHIN R., OLIVEIRA FILHO A.M., FIGUEIREDO M.J. and MULLER C.A. (1978) - *Slow release J.H. formulations for Triatominae control Transactions*, « Roy. Soc. of Tropical medicine and hygiene », *72* (3).

SMITH R. (1969) - *The new and the old in pest control. Nuove prospettive di lotta contro gli insetti nocivi*, « Accademia Nazionale dei Lincei », *128*, 21.

SCORSBIROLI R. (1969) - *Selection of resistant plant and animal varieties*, « Accademia Nazionale dei Lincei », *128*, 255.

WIGGLESWORTH V.B. (1936) - *The function of corpus allatum in the growth and reproduction of Rhodnius prolixus*, « Quart. J. Micr. Sc. », *79*, 91.

ZUBERLUCH R.C. (1976) - *Phospholhers as insects growth regulators The Juvenile Hormones*, Ed. L.I. Gilbert, Plenum Press.